Chapter 5C: Restoration Strategies Science Plan

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SUMMARY

This chapter updates the status, progress, and key findings of ongoing and recently completed studies from the *Science Plan for the Everglades Stormwater Treatment Areas* (Science Plan). The South Florida Water Management District (SFWMD or District) developed the Science Plan in 2013 (SFWMD 2013) and updated it in 2018 (SFWMD 2018). It is a key component of the *Restoration Strategies Regional Water Quality Plan* (Restoration Strategies; SFWMD 2012a) as required by consent orders and permits granted by the Florida Department of Environmental Protection (FDEP; FDEP 2012a, b). The goal of Restoration Strategies is to achieve a stringent water quality based effluent limit (WQBEL) for total phosphorus (TP) concentration in discharge flow from the Everglades Stormwater Treatment Areas (STAs) to the Everglades Protection Area (EPA; SFWMD 2012b).

The Science Plan is a framework that develops and coordinates scientific research in support of the Restoration Strategies goal to achieve the WQBEL through identification of critical factors that influence TP reduction in the STAs, particularly those factors that are key drivers of performance in low TP environments (e.g., within the outflow region where TP concentrations are at or below 20 micrograms per liter [µg/L]). The ultimate purpose of this Science Plan is to support the design, operation, and management of STAs to achieve and sustain TP discharge concentrations that meet the WQBEL. The focus of these studies is specific to the Everglades STAs and does not encompass science related to source control technologies upstream of the STAs, which falls under a separate program (see Chapter 4 of this volume).

The Science Plan is framed around key questions regarding physical, chemical, and biological processes, as well as optimization of management in the STAs. Over the eight years that the Science Plan has been in effect, seven studies have been completed, 12 are ongoing, two are planned, and one is being merged into another study as a sub-study (**Table 5C-1**). Detailed updates of many of the studies have been reported in appendices of previous South Florida Environmental Reports (SFERs) including the Faunal Study (Evans et al. 2019, Barton et al. 2020b; see **Table 5C-1** for full study names), Phosphorus (P) Flux Study (King and Villapando 2020, Villapando and King 2018, 2019), PSTA Study (Zamorano et al. 2018), Cattail Study (Diaz and Vaughan 2019, Diaz 2020), Soil Management Study (Chimney 2017; Josan et al. 2019), and Water and P Budget Study (Zhao and Piccone 2018, 2019). Inflow, outflow, TP in, and TP out of the Periphyton-based Stormwater Treatment Area (PSTA) Cell continue to be monitored regardless of study completion in 2018 because annual performance of the cell has met the WQBEL every water year (May 1 to the subsequent April 30) since it came online in Water Year 2008 (WY2008) through WY2021.

The 2018 Five-year (2018–2023) Work Plan (Appendix A of SFWMD 2018) added eight studies to the original nine (SFWMD 2013). Five of these added studies are now underway. One study included in the 2018 Work Plan, Prescribed Burn Effects on Cattail Communities, is being incorporated into the Data Integration Study as a sub-study. The remaining two studies, Landscape and Advective Transport, are in the planning phase. Two studies not included in the 2013 or 2018 Science Plan, Data Integration and Ecotope, were added in 2020 and 2021, respectively.

Table 5C-1. List of all studies planned under the Science Plan (short title in parentheses).

Study Title/Description	Initiation and Status
Investigation of STA-3/4 Periphyton-based Stormwater Treatment Area Technology Performance, Design, and Operational Factors (PSTA Study) – assess the chemical, biological, design, and operational factors of the PSTA Cell that contribute to the superior performance of this technology.	2013 Completed in 2018 (inflow and outflow monitoring continue)
Evaluation of the Role of Rooted Floating Aquatic Vegetation in STAs (rFAV Study) – assess the ability of rooted floating aquatic vegetation (rFAV) to further enhance low-level P reduction performance of submerged aquatic vegetation (SAV) communities.	2016 Completed in 2018
Development of Operational Guidance for Flow Equalization Basin (FEB) and STA Regional Operation (Operation Study) – create tools and methodologies to provide operational guidance for FEBs and STAs.	2013 Completed in 2017
Influence of Canal Conveyance Features on STA and FEB Inflow and Outflow P Concentrations (**Canal Study**) – determine if and how conveyance through STA inflow or outflow canals alters TP concentrations or loads.	2013 Completed in 2017
Evaluation of Sampling Methods for TP (Sampling Study) – identify factors that may bias water quality monitoring results in order to improve sampling procedures for the STAs.	2013 Completed in 2017
Evaluation of P Sources, Forms, Flux and Transformation Processes in the STAs (P Flux Study) – improve understanding of the mechanisms and factors that affect P reduction in the STAs, particularly in the lower reaches of the treatment flow-ways.	2013 Completed in 2019
STA Water and P Budget Improvements (Water and P Budget Study) – improve annual estimations of STA water and P budgets of treatment cells to better understand and assess treatment performance of STAs.	2013 Completed in 2020
Evaluation of Inundation Depth and Duration Threshold for Cattail Sustainability (Cattail Study) – assess cattail health under different inundation depths and durations to identify thresholds for cattail sustainability in the Everglades STAs.	2013 Completed in 2021
Use of Soil Amendments and/or Management to Control P Flux (Soil Management Study) – investigate the benefits of soil amendment applications and/or soil management techniques to reduce internal loading of P in the STAs.	2013 Ongoing
Evaluation of Factors Contributing to the Formation of Floating Tussocks in the STAs (Tussock Study) – determine key factors that cause floating wetlands and cattail tussocks and the probability of their formation in STAs. Identify floating wetlands with unmanned aircraft systems.	2018 Ongoing
Investigation of the Effects of Abundant Faunal Species on P Cycling in the STAs (Faunal Study) – evaluate faunal processes and factors that affect P treatment performance of STAs at low TP concentrations.	2018 Ongoing
Improving Resilience of SAV in the STAs (SAV Resilience Study) – investigate the effects of operational and natural environmental conditions on SAV health in the STAs.	2018 Ongoing
Periphyton and Phytoplankton P Uptake and Release (Periphyton Study) – estimate P uptake and release rates from periphyton and phytoplankton in downstream STA treatment flow-ways to determine their influence on the P cycle and TP discharge from STAs.	2019 Ongoing
L-8 FEB Operational Guidance (L8-FEBOG Study) – provide guidance for FEB operations to moderate TP in discharge as potentially affected by stage, flow, and groundwater.	2019 Ongoing
Quantifying the Recalcitrance and Lability of P within STAs (Biomarker Study) – evaluate relationships between organic matter and P that capture the sources and potential turnover of P within the STAs.	2020 Ongoing
Data Integration and Analysis (Data Integration Study) – integrate STA and Science Plan data, research, and reports to support management decision making.	2020 Ongoing
P Reduction Dynamics in STA-1E, STA-2, STA-3/4, and STA-5/6 (P Dynamics Study) – evaluate biogeochemical factors and mechanisms influencing P reduction in underperforming flow-ways.	2020 Ongoing

Table 5C-1. Continued.

Study Title/Description	Initiation and Status
Assess Feasibility and Benefits of Consolidating Accrued Marl in the STAs' SAV Cells (Marl Study) – evaluate the technical feasibility of consolidating marl and determine if consolidation or improved aggregation of marl (an amalgam of calcium carbonate [$CaCO_3$] solids that include organic material as well as phosphorus) can lower the water column P concentration in the lower reaches of the Everglades STAs.	2021 Ongoing
P Removal Performance of Ecotopes in the STAs (Ecotope Study) – examine the P treatment performance of ecotopes commonly found in the STAs.	2021 Ongoing
Sustainable Landscape and Treatment in an STA (Landscape Study) – evaluate a range of constant and pulsing flows on mixing within the flumes at various degrees of plant biomass density.	Planning Stage
Effect of Vertical Advective Transport on TP Concentrations in the STAs (Advective Transport Study) – examine potential for advective transport from groundwater to affect TP concentrations in the discharge flow-way cells of the STAs.	Planning Stage
Prescribed Burn Effects on Cattail Communities (Prescribed Burning Study) – examine the effects of burning dense cattail stands on P treatment.	To be merged into Data Integration Study

PSTA STUDY

This completed study evaluated the chemical and biological factors contributing to consistently superior performance of the periphyton-assisted stormwater treatment area (PSTA) Cell. PSTA Study results are provided in previous years' SFERs with a detailed summary of the study findings presented in Appendix 5C-2 of the 2018 SFER – Volume I (Zamorano et al. 2018). One of the key factors controlling TP removal in the PSTA Cell was lack of organic soil. During construction, the muck was scraped and the subsurface limerock was exposed. Another key factor in the PSTA Cell's ability to produce ultra-low TP concentrations was low inflow TP concentrations and low TP loading rates. Monitoring of the inflow and outflow water quality (TP) and flow of this operational PSTA Cell continues. PSTA Cell performance calculations have been updated for WY2021 and are included in this chapter. For the 14 years of operation, this PSTA Cell has attained the WQBEL with annual outflow flow-weighted mean concentrations (FWMCs) of TP that are equal to or less than 13 µg/L.

Management Implications: The PSTA project demonstrates that the WQBEL can be attained if internal P loads are minimized using techniques such as soil removal. Removal or capping of phosphorus-laden soils, while not feasible for all locations, may be an appropriate management tool for some cells to achieve discharges at or below 13 ug/L.

CATTAIL STUDY

This completed study evaluated cattail (*Typha domingensis*) health under different inundation depths and durations to identify thresholds for sustainability in the STAs. Phase I of this study was completed in 2019 and observed stress in cattail regions of two STA cells when water levels were greater than 91 centimeters (cm; 2.99 feet), for over 100 consecutive days (Diaz 2018, Diaz and Vaughan 2019). Phase II of this study evaluated effects of water depths on cattail in the controlled environments of 15 STA-1 West (STA-1W) northern test cells. These 0.2-hectare (ha) test cells were refurbished, and cattail were planted and allowed to mature. Water depths in three randomly chosen test cells were set at 40 cm (1.31 feet, control), 61 cm (2.00 feet, shallow), 84 cm (2.75 feet, moderate), 104 cm (3.41 feet, deep), or 124 cm (4.07 feet, extremely deep) in July 2019 and communities were surveyed over the following 10 months. Cattail density, photosynthesis, leaf area index (LAI), and leaf elongation were measured at biweekly to monthly time periods to gauge stress. Initial results indicate that deep (104 cm or 3.4 feet) and extremely deep (124 cm or 4.07 feet) inundation treatments stressed cattail plants—observed from differences in density, photosynthesis, LAI, and leaf elongation—more than plants from the moderate (84 cm, 2.75 feet) and shallow (61 cm, 2.00 feet) depth treatments. These results are consistent with the Phase I in-situ study. Results are reported in Appendix 5C-1 of this volume (Diaz 2022).

Management Implications: Water depths greater than 84 cm should be minimized when possible to sustain healthy cattail communities in the STAs. While these communities can tolerate water levels deeper than 84 cm, the duration of deeper inundation should be kept short, as stress was observed (reduction in adult and juvenile density and increased leaf elongation) within 8 weeks.

SOIL MANAGEMENT STUDY

This ongoing study was intended to investigate methods to reduce internal P loading in the STAs including soil amendments, and P removal or burial. Chemical amendments that readily bond with dissolved P in soils may lower water column P concentration in constructed wetlands (Chimney 2015, 2017). However, due to uncertainties in treatment efficacy, high estimated installation costs and potential negative effects on STA operations and downstream marsh ecosystems, evaluation of soil amendments was discontinued. An existing deep tilling project, which buried high copper concentrations found in surface soils of STA-1W Expansion Area #1 was leveraged to evaluate the effects of P burial on STA performance. Soil inversion reduced P in surface soils and potential P flux to the water column (Josan et al. 2019). Soil sampling and water quality sampling undertaken after the STA-1W Expansion Area #1 cells were flooded found little difference between the inverted and non-inverted cells (DBE 2019b, 2020d). Flow-through operation of STA-1W Expansion Area #1 was delayed due to construction activities, which in turn delayed the initiation of this study's monitoring plan. Weekly monitoring of water quality at the inflow and outflow structures of these cells was initiated in WY2021 when normal flow-through operations began. Field surveys have been conducted to document the grow-in and species composition of submerged aquatic vegetation (SAV) within the STA-1W EA (DBE 2020c, d, 2021a).

Management Implications: When constructing new STAs on agricultural soils or refurbishing older STAs, capping high P surface soils by burial under lower P soils may reduce internal loads and improve STA P removal performance.

TUSSOCK STUDY

This ongoing study evaluates factors that may contribute to floating wetland community (tussock) formation in STAs and the effects of floating wetlands on STA treatment performance. A literature review (Clark 2019b) led to the development of a nomenclature scheme to describe these communities that vary based on species, size, and floating matter (Clark 2019c). Multispectral imaging sensors carried on unmanned aircraft systems (UAS) were effective in finding tussocks (Clark and Glodzik 2019). Thermographic sensors were less successful (Clark 2019a). Past agriculture uses, maximum annual water depths, and soil P are good indicators of potential tussock formation (Clark and Glodzik 2020). A buoyancy model to provide management recommendations to reduce the formation of tussocks in the STAs is being developed. Data to parameterize the model was collected in spring 2021. Refinement of UAS deployment and post-processing of multispectral data were completed in March 2021 (Clark and Glodzik 2021).

Management Implications: Tussock formation in STAs is a common and undesirable occurrence. These floating wetlands disrupt surrounding vegetation communities, increase turbidity, reduce light in the water column, and can block downstream flow structures, all resulting in decreased P removal by STAs. Regular use of drones to identify tussocks could support potential management activities such as planting desired vegetation, reducing water levels, or chemical or physical means to prevent cell-wide expansion in a timely manner and to minimize tussocks' negative effects. Buoyancy models and indicators of potential tussock formation will define areas prone to tussock formation.

FAUNAL STUDY

This ongoing study examines the role of fauna (specifically fish) on P cycling and STA P budgets through measurements of storage, excretion, and bioturbation. It began as a sub-study of the P Flux Study (Evans et al. 2018) and continues as a separate study. It evaluates the effect of fauna on STA TP cycling primarily through fish abundance surveys, bioturbation experiments, and excretion rate studies (Barton et al. 2020b). Small-bodied fish species recycled substantial amounts of P into the water through excretion, with most of this excretion in less bioavailable particulate forms (Barton et al. 2020b). Large-bodied fish contributed more P to the water column from bioturbation of floc and underlying sediments than from excretion (Barton et al. 2020c). The effect of large-bodied fish is dependent on biomass estimates from electrofishing surveys, which are underestimates (Barton and Trexler 2020). Electrofishing will be calibrated against known fish densities using the A-1 Flow Equalization Basin (FEB) to improve the estimates of faunal contribution to P budgets.

Management Implications: Fauna, specifically fish and invertebrates, are contributors to internal recycling of P to the water column of STAs through excretion and bioturbation, reducing the effectiveness of P removal. The impact of faunal activity on TP outflow concentrations will lead to better understanding of STA performance and potential management of fauna.

SAV RESILIENCE STUDY

This ongoing study investigates the effects of operational and environmental conditions on the health of submerged aquatic vegetation (SAV) in the STAs. A literature review and analysis of 18 years of semi-quantitative SAV surveys in the STAs indicate that water column and soil conditions can affect SAV resilience (DBE 2018). Soil type (mineral or organic), P loading, water depth, and herbivory were evaluated in field and mesocosm experiments (DBE 2019a, 2020a, b). Soil type did not affect biomass of SAV in mesocosm experiments, but plants grown in marl soils had lower concentrations of P and iron (Fe), copper (Cu), and Zinc (Zn) in their plant tissue. *Chara* spp. Density increased with increased P loads in mesocosm experiments, resulting in decreased dissolved oxygen and light at the soil surface. To determine if these oxygen and light conditions result in a collapse of the *Chara* spp. Density, this experiment continues. An additional set of mesocosms were added that included soils with higher TP concentrations. Preliminary results from soil drying studies suggest that dry conditions may support faster germination and growth of SAV seeds. An in-situ herbivory experiment in STA-2 Flow-way (FW) 3, begun in mid-2020, has demonstrated that fish added to enclosures preferentially reduced the biomass of *Naja guadalupensis* (DBE 2020b).

Management Implications: Maintaining healthy SAV in the outflow regions of STAs supports effective performance. Understanding the causes of SAV collapse events should provide opportunities for management to minimize negative effects.

PERIPHYTON STUDY

This ongoing study evaluates nutrient dynamics of periphyton and phytoplankton in downstream STA treatment FWs where TP concentrations are very low (≤ 20 µg/L). Phase I was a literature review of methods to measure nutrient uptake and release, growth, respiration, senescence, and death (Laughinghouse et al. 2019). Phase II will be a bench top and field study to measure bioavailability of dissolved organic P (DOP) and dissolved organic nitrogen (DON) in STA surface water inoculated with emergent aquatic vegetation (EAV) or SAV community periphyton. A laboratory bioavailability study was designed to determine the bioavailability of DOP by periphyton from SAV and EAV sites. This study will be carried out in summer 2021 and winter 2021 to 2022.

Management Implications: The periphyton community is an important but poorly understood component of STAs. It can play a significant role in sequestering P, but the conditions that promote optimal performance, specifically P loading, water flow, and water depth, are unknown. This study will improve our understanding of the periphyton community and its dynamics, which may provide options for managers to enhance P removal in the STAs.

L8-FEBOG STUDY

This ongoing study evaluates the relationships of L-8 Flow Equalization Basin (FEB) water quality to stage, surrounding groundwater, and the inflow and outflow structures of the FEB. This study will provide guidance for FEB operations to support management of STA-1 East (STA-1E) and STA-1W. Phase I of this study measured water quality (nutrients, ions, temperature, dissolved oxygen, and conductivity) monthly from L-8 FEB surface water, and quarterly from groundwater wells in 2019 (DBE 2020e). It was found that groundwater was not a source of TP to the FEB. The ongoing Phase II evaluates potential sediment contribution to P export from the FEB resulting from flow induced resuspension.

Management Implications: Sediment resuspension that occurs under high inflow events increases TP concentrations in the L8-FEB water column. This material slowly settles out. Thus, the timing of outflow events after high inflow events may affect the amount of TP in the discharge waters. Maintaining low TP concentrations in the FEB discharges will benefit STA-1E and STA-1W performance over the long term.

BIOMARKER STUDY

This ongoing study evaluates the relationships between organic matter (OM) and P to capture the sources and potential turnover of P within the STAs. This evaluation also will consider bioavailability of total DOP and nitrogen (N) in the surface water of well performing STAs. Water samples were taken from the major inflows to the STAs and a major outflow from Lake Okeechobee to the STAs. A workplan has been developed to measure faunal biomarkers from excretion as potential tracers of fish inputs of OM and P (UF 2021b). In addition, in situ litterbag studies will estimate the change over time of OM in fresh litter and the surrounding water.

Management Implications: Knowledge of the sources of organic P discharged from the STAs may result in potential strategies to manage those sources. This information can be used to determine if the P reduction efficiency can be improved in the STAs.

DATA INTEGRATION STUDY

This ongoing study collates and synthesizes research and data from studies of P removal in the STAs. Four independent substudies have been initiated. The first sub-study is a data mining evaluation of the relationships among water, soil, and vegetation in outflow regions of STAs. This sub-study has found lower TP concentrations in the water column of cells with dense SAV (DBE 2020g). The second sub-study is compiling and reviewing previous microbial research carried out in the STAs and other wetland portions of South Florida (DBE 2021c). The third substudy is developing a series of piecewise structural equation models to evaluate the water quality parameters that affect TP concentration in STA outflows. The fourth sub-study is developing two models: (1) a biogeochemical model of P and (2) a food web model (Comprehensive Aquatic Ecosystem Model or CASM). Both models will use the results from other STA studies and the other data integration sub-studies to guide development of interactions among the various model components. Results from these four sub-studies will be used to develop a guidance document to support management of the STAs to achieve the WQBEL.

Management Implications: This study compiles information gained through all Science Plan studies and will provide an overall assessment of P processes occurring within the STAs, providing indicators of processes that management should focus on to maintain and enhance P removal in the STAs. In addition, model development and enhancement using information from the Science Plan studies will provide robust models that can be used to predict the effect of management actions through simulations of scenarios.

P DYNAMICS STUDY

This ongoing study is a follow up of the P Flux study that evaluated P sources, forms, flux, and transformation processes in the STAs. Completed in 2019, the P Flux Study focused on understanding the biogeochemical factors and mechanisms affecting water column P in well-performing FWs. The P Dynamics Study will focus on under-performing FWs that have not met the TP concentration needed to meet the WQBEL. Causes of reduced P retention are likely a combination of less uptake and removal and increased P regeneration in comparison to well-performing FWs. To determine causes, analyses like those used in the P Flux Study will help identify major P forms/storages and chemical and biological processes related to P uptake and regeneration. This P Dynamics Study will evaluate water quality, soils, ultrafine particulates, and microbially-mediated processes in the STAs using three approaches. Two of the approaches focus on water quality: the first will sample FWs seasonally and the second will opportunistically sample the FWs at a more intensive spatial scale under target hydrologic conditions. These sampling approaches will be integrated with a quantitative assessment of vegetation and contemporaneous soil conditions, particularly in the backend region of the study FWs, to evaluate their potential role in treatment performance. The effects of P removal and P regeneration will be evaluated. The third approach will evaluate the biotic and abiotic mechanisms affecting P in the study FWs. These three approaches will provide important information from under-performing systems to compare with data from well-functioning systems to help improve our understanding of internal drivers that affect the performance of these STAs, especially in the lower reaches of the treatment trains.

Management Implications: Evaluation of under-performing STA FWs in comparison with well-performing FWs will indicate the components that contribute to this under performance. This information may lead to management actions to avoid under performance.

INTRODUCTION

The Everglades STAs are freshwater treatment wetlands constructed to reduce TP concentration in surface water runoff prior to discharge to the EPA (**Figure 5C-1**). The five STAs: STA-1E, STA-1W, STA-2, STA-3/4, and STA-5/6, are a major component of SFWMD's *Restoration Strategies Regional Water Quality Plan* (SFWMD 2012a). These STAs comprise an effective treatment area of approximately 24,700 ha (61,000 acres [ac]). This includes expansion areas of STA-1W (Expansion #1), STA-2, and STA-5/6. Two FEBs, A-1 FEB and L-8 FEB, have been constructed as components of Restoration Strategies and are operated to attenuate peak stormwater flows and improve inflow delivery rates to downstream STAs.

The STAs, located primarily on former agricultural lands, retain nutrients through plant and microbial uptake, particulate settling, chemical sorption, and ultimately accretion of this material to the soil layer. Over the period of record, which started in 1994, all STAs combined have reduced TP loads by 78% and achieved an average outflow TP concentration of 28 µg/L (see Chapter 5B, Table 5B-1 of this volume). Work initiated through the Science Plan, and reported in this SFER chapter, supports the development of strategies to attain the WQBEL at the STA outflows.

Two permits established the WQBEL for these STAs, a stringent limit of TP concentration in discharge waters: (1) a National Pollution Discharge Elimination System (NPDES) watershed permit (FDEP 2012b), and (2) an Everglades Forever Act (EFA) watershed permit (FDEP 2012a). Meeting the WQBEL assures that TP discharges from the STAs do not cause or contribute to exceedances of the 10 µg/L TP criterion (long-term geometric mean) within the EPA. The WQBEL includes two parts: (1) TP as an annual FWMC discharged from each STA shall not exceed 13 µg/L in more than 3 out of 5 water years on a rolling basis; and (2) the annual FWMC from each STA shall not exceed 19 µg/L in any water year. The WQBEL is separate from the 4-part test for the Everglades TP criterion (62-302.540(4)(d)1, Florida Administrative Code), which is discussed in Appendix 3A-6 of this volume.

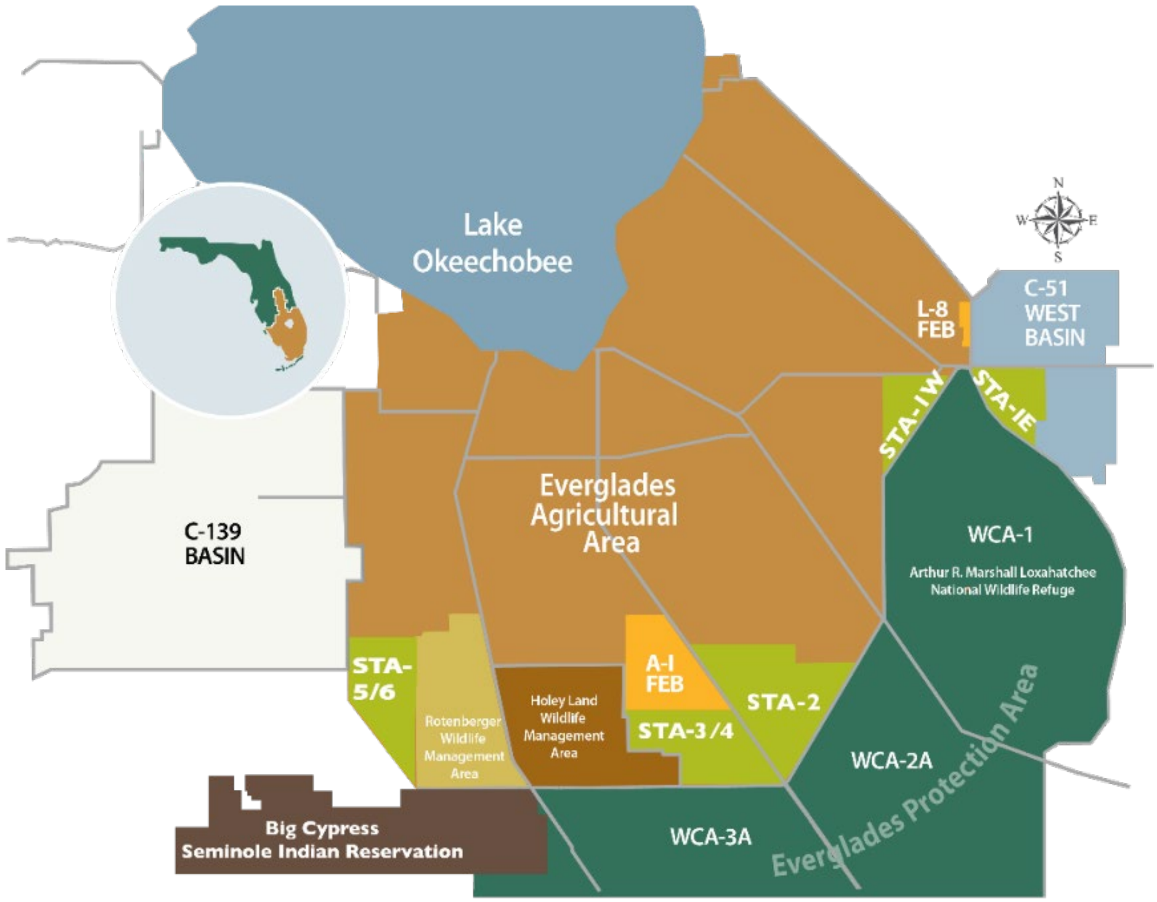

Figure 5C-1. Location of STAs (STA-1E, STA-1W, STA-2, STA-3/4, and STA-5/6) and FEBs (A-1 and L-8) in relation to the Everglades Agricultural Area, C-139 Basin, EPA, and other landscape features of South Florida. Maps of individual cells and FWs for all STAs can be found in Appendix 5B-1 of this volume.

Consent orders associated with the two permits for STA operation required SFWMD, in consultation with Restoration Strategies Technical Representatives from the United States Environmental Protection Agency, FDEP, United States Army Corps of Engineers, and United States Department of the Interior (Everglades National Park and United States Fish and Wildlife Service), to develop and implement the Science Plan (SFWMD 2013, 2018). The consent orders required the development of the Science Plan to evaluate mechanisms and factors that affect P reduction and treatment performance, particularly those that are key drivers to performance in low TP environments (i.e., < 20 µg/L), with the goal of attaining the WQBEL.

The original Science Plan was written in 2013 and defined nine studies that were initiated between 2013 and 2016 (SFWMD 2013). The 2018 update of the Science Plan added 10 potential new studies (Appendix A of SFWMD 2018). Three of these new studies—Tussock, Faunal, and SAV Resilience—began in 2018. Two new studies, Periphyton and L8-FEBOG, began in 2019 (**Table 5C-1**). In 2020, the Biomarker and P Dynamics studies were initiated, and another study not included in the 2018 Science Plan, Data Integration and Analysis, was added. Of the 3 remaining new studies proposed in the 2018 workplan, two, Landscape and Advective Transport, are in the planning phase, and one study, Prescribed Burning, will be merged into the Data Integration Study. Results from all studies will inform the design and management of the STAs to further improve STA performance.

In this SFER Chapter 5C update, the status of all current and recently completed studies are included with a few exceptions. The 2021 studies, Ecotope and Marl, have been recently initiated and no updates are included in this report. The PSTA Study was completed in 2018 (Zamorano et al. 2018), however, monitoring of the inflow and outflow structures continues. The annual performance criteria of the PSTA Cell is updated in this report.

RESEARCH QUESTIONS

Six key questions and 39 sub-questions were developed in workshops and meetings that reviewed existing knowledge and information gaps and were included in the original Science Plan (SFWMD 2013). The 2018 update of the Science Plan included a revised set of key questions and sub-questions (SFWMD 2018). The key questions and sub-questions were reviewed in numerous workshops and meetings to assess their continued relevance to support the development of strategies to meet the WQBEL, determine if they had been fully addressed, and consider if they could be answered through meaningful and cost-effective studies. Ongoing studies (**Table 5C-1**) currently address nine research sub-questions:

1. How should storage in the FEBs be managed throughout the year so water can be delivered to the STAs in a manner that allows them to achieve desired low outflow TP concentrations? (L8-FEBOG Study)
2. What key factors affect and what management strategies could improve system resilience of SAV communities? (SAV Resilience Study)
3. What key factors affect and what management strategies could improve system resilience of EAV communities? (Tussock and Cattail studies)
4. What is the role of vegetation in modifying P availability to low-P environments, including the transformation of refractory forms of P? (P Dynamics, Ecotope, and Periphyton studies)
5. What are the key physicochemical factors influencing P cycling in very low P environments? (P Dynamics and Periphyton studies)
6. Are there design or operational changes that can be implemented in the STAs to reduce particulate P (PP) and DOP in the water column? (Soil Management, P Dynamics, and Periphyton studies),
7. What is the treatment efficacy, long-term stability, and potential impacts of soil amendment management? (Soil Management and Marl studies)
8. What are the sources, forms, and transformation mechanisms controlling the residual P pools within the different STAs, and how do they compare to the natural system? (P Dynamics, Data Integration, Faunal, and Periphyton studies)
9. What are direct and indirect effects of wildlife communities at temporal and spatial scales on P cycling (e.g., are they net sinks or sources)? (Faunal and Biomarker studies)

RESEARCH STUDIES

Since the Science Plan was authorized, 19 studies have been initiated (**Table 5C-1**). As of WY2021, eight of these studies were completed. Two additional studies, Landscape and Advective Transport, proposed in the updated Science Plan (SFWMD 2018), are in the planning phase.

INVESTIGATION OF STA-3/4 PERIPHYTON-BASED STORMWATER TREATMENT AREA TECHNOLOGY PERFORMANCE, DESIGN, AND OPERATIONAL FACTORS (PSTA STUDY)

Tracey Piccone and Jacob Dombrowski

The purpose of the PSTA Study, completed two years ago, was to assess the chemistry, biology, design, and operations of the PSTA Cell in STA-3/4 that contribute to superior treatment performance of this technology (Zamorano et al. 2018). A key factor of the PSTA Cell's ability to produce ultra-low TP concentrations is lack of organic soil. During construction, the muck was scraped and the sub-surface limerock was exposed. Another key factor is low P loading rates. Monitoring of the PSTA Cell inflows and outflows and the associated TP concentrations have continued and the annual PSTA Cell performance calculations have been updated through WY2021. For the fourteen years of operation, outflow TP FWMCs ranged from 8 to 13 µg/L compared to inflow TP FWMCs that ranged from 9 to 27 µg/L.

ANNUAL PERFORMANCE

Annual P and hydrologic values for the PSTA Cell were determined for the WY2008–WY2021 period (**Table 5C-2**). Using measured water inflows and outflows and inflow and outflow TP concentrations obtained from surface water samples, TP loads were calculated and summed by water year. Annual inflow and outflow FWMCs were calculated from TP inflow and outflow loads divided by annual water inflow and outflow, respectively. Annual hydraulic loading rate (HLR), P loading rate (PLR), hydraulic residence time (HRT), and TP settling rate (k) were then calculated (see Piccone and Dombrowski 2020 for calculations). These annual values included the PSTA Cell's operational period for each water year. The operational period was defined as the span of time over which one or both PSTA Cell's inflow structures (G-390A and G-390B) were open. Days when both gates were closed due to protective measures for nesting birds, structure maintenance, or to preserve water during droughts were excluded.

For WY2021, the PSTA Cell produced an annual outflow TP FWMC of 8 µg/L (**Table 5C-2**). HLR, PLR, and k were within the same range as previous water years. The inflow TP FWMC, however, was at a record low (9 µg/L). Pump 2 at the outflow pump station, G-388, was taken out of service for repairs on February 24, 2021, and the inflow gate G-390B was closed on March 7, 2021, at which time the PSTA Cell was taken offline through the end of the water year. Over the period of record, inflow FWMC was 15 µg/L and outflow FWMC was 10 µg/L, a reduction in concentration of 33%. Based on superior performance, the PSTA technology is included in our adaptive management strategies toolbox.

Table 5C-2. Summary of annual hydraulic and treatment performance parameters in the STA-3/4 PSTA Cell during each operational period from WY2008 to WY2021. [a]

Water Year	HLR (cm/d)	HRT (d)	Water In (ha-m)	Water Out (ha-m) [b]	FWMC TP in (µg/L)	FWMC TP out (µg/L)	PLR (g/m²/yr)	k (m/yr)	Operational Period	Operational Period (d) [c]
WY2008	5.5	5.8	360	641b	27	12	0.24	14.2	06/05/2007–12/12/2017	161
WY2009	6.0	5.9	408	753	14	8	0.14	13.8	07/09/2008–12/23/2008	168
WY2010	6.2	6.2	866	1,243	20	10	0.42	27.4	05/26/2009–04/30/2010	340
WY2011	6.1	6.7	394	485	18	11	0.17	7.3	05/01/2010–12/072010	159
WY2012	8.6	4.4	919	1,185	17	12	0.39	12.5	07/19/2011–04/05/2012	262
WY2013	7.7	5.1	1,150	1,377	16	11	0.45	17.8	05/01/2012–04/30/2013	365
WY2014	3.3	16.7	497	468	24	13	0.29	10.0	05/01/2013–04/30/2014	365
WY2015	5.8	9.4	862	911	15	11	0.33	11.9	05/01//2014–04/30/2015	365
WY2016	6.9	7.3	1,023	1,285	11	9	0.27	11.6	05/01/2015–04/30/2016	366
WY2017	5.6	12.9	827	659	10	8	0.20	6.7	05/01/2016–04/30/2017	365
WY2018	5.0	10.5	748	1,102	10	9	0.19	3.7	05/01/2017–04/30/2018	365
WY2019	4.7	19.2	693	325	12	9	0.20	4.2	05/01/2018–04/30/2019	365
WY2020	6.0	11.3	754	778	11	9	0.20	4.4	05/01/2019–04/30/2020	310
WY2021	7.4	11.9	778	439	9	8	0.17	3.4	05/01/2020-03/72021	257
Mean	**6.0**	**9.5**	**734**	**832**	**15**	**10**	**0.3**	**10.6**		

a. Key to Units: µg/L – microgram(s) per liter; cm/d – centimeter(s) per day; d – days; g/m²/yr – gram(s) per square meter(s) per year; ha-m – hectare-meter(s); and m/y -meter(s) per year.

b. Outflow can exceed inflow as groundwater seepage and rain are not included in this table.

c. Both gates may be closed for a few days during the operational period. These days are excluded from the calculations.

EVALUATION OF INUNDATION DEPTH AND DURATION THRESHOLD FOR CATTAIL SUSTAINABILITY (CATTAIL STUDY)

Orlando Diaz

The purpose of the Cattail Study is to identify the inundation depth and duration threshold to sustain cattail (*Typha domingensis*) communities in the STAs. Dense cattail communities in the upper region of a treatment FW reduce PP in the water column and facilitate microbial P cycling through production of litter. Cattail growing in patches within a number of STA cells provide support to SAV communities, reducing turbulence and flow under severe wind events that could negatively affect SAV. Previous field observations and studies indicated that water depths exceeding certain criteria and maintained over long periods resulted in physiological stress, reducing growth, biomass, density, and anchorage capacity of cattail plants in STA treatment cells.

Phase I of this study surveyed many sites in STA-1W Cell 2A in 2015 and STA-3/4 Cell 2A from 2015 to 2018 (Diaz 2018, Diaz and Vaughan 2019; **Table 5C-3**). Prolonged water depths above 91 cm for over 100 consecutive days resulted in a decline of both cattail density and total belowground biomass, as well as increased leaf elongation in response to the high water.

Table 5C-3. Status and highlights for the Cattail Study.

Task	Status and Comments	Publications and Reports
In situ surveys – STA-1W Cell 2A	Year 1 wet season cattail monitoring was completed in October 2015. Evaluation of this cell was discontinued due to widespread decline of cattail conditions, including the presence of floating cattails.	Diaz 2018
In situ surveys – STA-3/4 Cell 2A	Cattail monitoring began in 2015 and was completed in early 2018. Results from the three monitoring seasons indicate significant stress to cattail when water depth was greater than 91 cm for more than 100 consecutive days.	Diaz and Vaughan 2019
Test cell study establishment	STA-1W Northern Test Cells, each covering an area of 0.2 hectare, were refurbished in 2016. Planting and seeding of cattail occurred between May and June 2018. Water levels were maintained between 10.2 to 15.2 cm (4 to 6 inches) above the soil surface for 2 to 3 months to encourage seedling growth. Water levels were maintained at about 40 cm (1.3 feet) from January to June 2019 to encourage and maintain the establishment and growth of cattail while minimizing terrestrial weed growth. Baseline sediment cores were taken.	Diaz 2020
Test cell study experiment	Test cells were flooded in July 2019 with a range of water depths to start the experimental treatments. Cattail communities were surveyed biweekly for three months and monthly until April 2020. Inflow and outflow water samples were collected at the same frequency. Biomass and soil samples were taken in June to July 2020. Data were analyzed and are presented in Appendix 5C-1 of this volume.	Diaz 2022

Phase II of this study evaluated the effects of various water depth and duration periods under more controlled conditions in 15 test cells constructed in the early 1990s within STA-1W (**Table 5C-3** and **Figure 5C-2**). In the later 1990s, full liners were installed in each test cell under a few feet of soil to hydrologically isolate them from each other. The test cells were refurbished in 2016 for this study. Cattail seeds, collected from STA-3/4, were distributed into these cells during the first week of May 2018 (Diaz 2020). Young cattail seedlings 12 cm (0.4 feet) to 15 cm (0.5 feet) in height (50 to 55 days old) were planted at a density of approximately 140 cattail plants per cell from mid-May 2018 through mid-June 2018 to improve uniformity of cattail community in the cells. Water levels were maintained between 10 cm (0.32 feet) to 15 cm (0.5 feet) above the soil surface for 2 to 3 months to encourage seedling growth. Water levels were maintained at about 40 cm (1.3 feet) from January to June 2019 to encourage growth and maintain the establishment of the young cattails while minimizing terrestrial weed growth. Cattail plants were mature prior to the initiation of the inundation depth treatments.

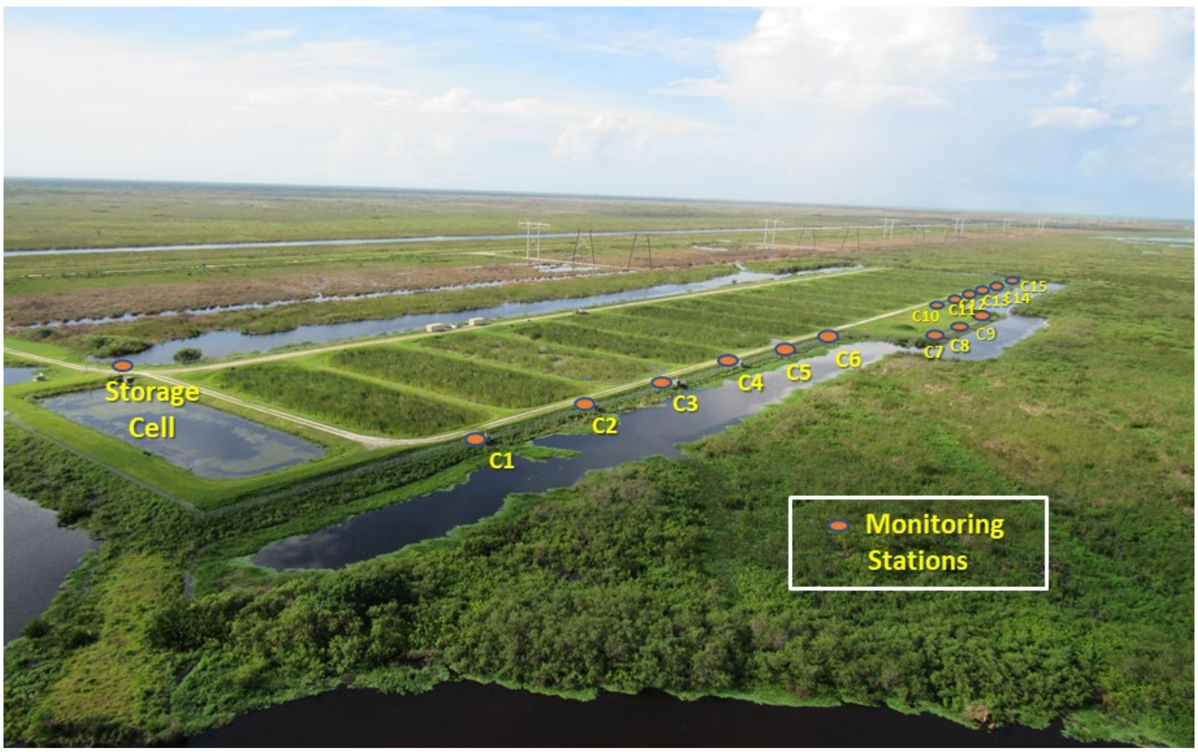

Figure 5C-2 Aerial view of STA-1W Northern Test Cells showing cattail communities and water quality monitoring stations during the study.

Five inundation depths treatments: 40 cm (1.31 feet, the control), 61 cm (2.00 feet, shallow), 84 cm (2.75 feet, moderate), 104 cm (3.41 feet, high), and 124 cm (4.07 feet, extremely high) above soil surface, were established on July 1, 2019. Inundation depths were replicated at random in sets of three treatment cells. Duration of inundation was continuous for 10 months. For the first three months, cattail vegetation was surveyed bi-weekly. These initial bi-weekly observations indicated that monthly field surveys were sufficient to capture the major phenological events due to treatments. Cattail density, photosynthesis, LAI, and leaf elongation were measured.

Water quality samples were collected bi-weekly for the first three months of the study and monthly for the remainder of the study. These water quality samples were collected from the storage cell outlet (representing inflow water) and at the outflow from each test cell. Baseline sediment samples were collected in January 2019 and a final set was collected in July 2020. Baseline biomass samples were collected in May–June 2019. A final biomass sampling was collected at the end of the study in May–June 2020.

During this study, outflow TP concentrations were significantly (probability [p] < 0.05) lower than inflow TP concentrations at all water level treatments except the control, representing a TP removal of 76% in the deeper treatments. Outflow total nitrogen (TN) concentrations were also lower than inflow concentrations during all sampling events (23% removal); however, differences were not significant. Mean adult cattail densities during the 10-month inundation period showed a 23 and 26% decline in the high and extreme water level treatments, respectively, compared to the control; however, the differences were not significant. In contrast, the mean juvenile populations were significantly ($p < 0.05$) reduced at all water depths greater or equal to 84 cm. Lower juvenile populations in the deeper treatments resulted in lower adult densities measured in the same deeper water level treatments, since there was a decrease in the pool of individuals that could potentially develop into adult cattails.

Leaf elongation rates (LER) were significantly ($p < 0.05$) higher in cattail plants from the deeper water level treatments, with the higher rates measured during the more active growing period (July–October). LER responses to deep water conditions may explain in part why cattail plants from deepest treatments were not stressed to the point of irreversible damage causing a total collapse of the cattail population. In their effort to survive under deep water conditions cattail can grow taller facilitating the gas exchange between the above- and belowground parts of the plant (Grace 1989, Bailey-Serret and Voesenek 2008). Photosynthetic rates during the entire monitoring period were significant ($p < 0.05$) only between the control and the deepest treatment (124 cm). Transpiration and water use efficiency across all treatments during the entire study were not significantly different. However, when comparing across events, all gas exchange rate parameters tended to increase toward the end of the growing season (October–November), which may indicate that slightly cooler temperatures provided less stressful conditions for growth.

In this study, adaptations to water depths by cattail occurred mostly through changes in shoots and ramets (the base of the cattail plant at the soil surface) and less through biomass allocation. As water depths increased, cattail height and weight significantly ($p < 0.05$) increased, i.e., fewer but significantly larger ramets were measured in the deeper water treatments. Allocation between aboveground (64%) and belowground (36%) biomass was not significantly different among water depth treatments. In part, the lack of response of biomass allocation is due to the trade-off between fewer but larger ramets measured in the deeper treatments and higher density of smaller cattail plants measured in the shallower treatments. Similarly, tissue nutrient concentrations were not significantly different with increases in water depth. However, high nutrient concentrations in tissues associated with resource storage (i.e., shoot bases), indicate that cattail stored nutrients and allocated resources for shoot growth even under deep water depths, a strategy that improve its chances to survive deep water conditions. These results show that cattail communities in the STA may tolerate water levels up to 84 cm for 10 months. However, more research is needed to study the duration of inundation threshold at water depths greater than and equal to 84 cm (2.75 feet) where normal operations of water depths (38 cm; 1.25 feet) in the STAs do not result in reduced treatment due to cattail loses. Final results of this study are reported in Appendix 5C-1 of this volume.

USE OF SOIL AMENDMENTS/MANAGEMENT TO CONTROL PHOSPHORUS FLUX (SOIL MANAGEMENT STUDY)

Michael Chimney

The purpose of the Soil Management Study is to investigate methods that may reduce flux of soluble reactive P (SRP) from the soil to the water column in the Everglades STAs. Reduction of internal P flux, in turn, may result in lower TP concentrations at STA outflows. These methods include soil amendments (e.g., materials typically rich in metal cations, i.e., aluminum, calcium, iron or magnesium, that readily bond with SRP) and soil management techniques (e.g., soil inversion to bury surface soils that have a high P content). Phase I of the study proposed two options for conducting large-scale field trials within the STAs: (1) leverage a remediation project that used soil inversion (deep tilling) to bury copper-enriched agricultural soils in the STA-1W Expansion Area #1 and (2) build large-scale sub-cells in four STAs to test various amendment technologies (Chimney 2015; **Table 5C-4**). Considering the uncertainties in treatment efficacy, potential adverse effects on STA operations, the estimated cost of large-scale field amendment trials, and the practicality of implementing these technologies at full-scale in the STAs, option one was chosen to determine the effect of inverting soils on P flux after flooding (Chimney 2015, 2017). The inversion of the soil column through deep tilling buried the oxidized, P-rich higher copper content soils bringing lower-P subsoil to the surface. Consideration of additional studies to test soil amendments and other management techniques outlined in Chimney (2015) has been discontinued due to the uncertainties described above.

Table 5C-4. Progress to date of the Soil Management Study.

Task	Status and Key Findings	Publications and Reports
Phase I: Literature review	Completed in October 2015. A literature review of technologies, including a synthesis of relevant SFWMD supported projects and an assessment of feasibility of implementing at full-scale in the STAs.	Chimney 2015
Phase II: Small-scale experiments	A decision was made not to proceed with Phase II, which would screen a variety of soil amendments/management techniques identified in Phase I	Chimney 2015 Chimney 2017
Phase III: Large-scale field trials	Ongoing. A field-scale treatment of soil inversion for copper remediation in the STA-1 W Expansion Area #1 was initiated in 2017. Water and soil samples have been analyzed along with SAV surveys as part of this study to determine if soil inversion improves initial and long-term STA performance in reducing discharge P concentration.	Josan et al. 2019 DBE 2019b DBE 2020a,b,c,d DBE 2021a

Construction of the STA-1W Expansion Area #1 added three new treatment cells to STA-1W: Cells 6, 7, and 8. Soils were tilled to a depth of at least 60 cm in all of Cell 7 and a portion of Cell 6 to remediate for high copper levels in the surface soils (AECOM Technical Services, Inc. 2016). Cell 8 surface soil does not have high copper levels and was not tilled. A preliminary study was conducted to characterize these tilled and untilled soils (Josan et al. 2019). The soil brought to the surface through tilling included peat (inverted-peat) and marl (inverted-marl), which is an amalgam of calcium carbonate ($CaCO_3$) solids that include organic material as well as phosphorus. The median TP content of the inverted-peat and inverted-marl soils (527 and 160 milligrams per kilogram [mg/kg], respectively) was significantly lower than the median TP content of the untilled soils (830 mg/kg).

P flux was measured in soil cores collected from these inverted and untilled areas (Josan et al. 2019); these cores were flooded with low-P STA-1W outflow water and incubated under dark, aerobic conditions. After 42 days of incubation, the average water column TP concentrations for untilled, inverted-peat and inverted-marl soils were 156, 68, and 13 µg/L, respectively. The corresponding mean SRP flux rates in these cores were 3.3, 1.6, and 0.1 milligrams P per square meter per day (mg $P/m^2/d$), respectively. The soil core incubations suggested that deep tilling may be beneficial in reducing the flux of P from flooded soil to the overlying water column.

The STA-1W Expansion Area #1 was flooded in 2019 after all soil tilling was completed. The FDEP permit start-up criteria required to initiate flow-through operation were met; however, normal operation of this facility was initially delayed due to ongoing construction activities (i.e., STA-1W Expansion #2 and the STA-1W Refurbishment projects). In the interim, grids of geo-referenced sample sites were established in Cells 7 (the study's tilled-soil treatment; 25 sites) and 8 (the study's untilled-soil control; 34 sites). Baseline water and soil samples were collected in April 2019 from a subset of sites in both cells (DBE 2019b, 2020d). Average surface soil TP content was similar at the tilled and untilled sites: 963 ± 154 mg/kg and 821 ± 31 mg/kg, respectively. Surface water TP concentrations varied considerably within both cells, with TP concentrations ranging from 40 to 636 µg/L in Cell 7 and 43 to 155 µg/L in Cell 8. Field surveys were conducted at all sites in Cells 7 and 8 during April 2019, August 2019, May 2020, and August 2020 to document the grow-in and species composition of the SAV community (DBE 2019b, 2020c,d, 2021a). Four SAV species have been observed to date: chara (*Chara* sp.), southern water nymph (*Najas quadalupensis*), spiny water nymph (*Najas marina*), and floating bladderwort (*Utricularia gibba*). SAV was more abundant in Cell 8 compared to Cell 7. Now that normal flow-through operation of the STA-1W Expansion Area #1 has begun, the treatment efficacy of Cell 7 (tilled soil) can be compared to Cell 8 (untilled soil) using weekly measurements of inflow and outflow TP concentrations in both cells.

EVALUATION OF FACTORS CONTRIBUTING TO THE FORMATION OF FLOATING TUSSOCKS IN THE STAS (TUSSOCK STUDY)

Mark Clark[1], Katie Glodzik[1], and Orlando Diaz

The purpose of the Tussock Study is to determine key factors that cause the formation of floating wetland communities (mats, islands, and complexes) in the STAs. This will be accomplished through an evaluation of physical, chemical, and biological conditions that occur in areas of healthy cattail coverage and how these conditions differ from areas where floating wetland communities occur as mats, islands, or complexes in the STAs. The goal of this study is to provide management and operational guidelines to prevent formation of floating wetland communities and reduce their effect on STA performance. These effects include increased turbidity, shading of SAV, scouring of surrounding vegetation, creation of short circuits of water flow, and clogging flow structures. Phase I included a literature review of floating wetland research, a nomenclature scheme that described various types of floating vegetation and floating mats in the STAs (**Figure 5C-3**), and an assessment of current areas that contain floating cattail communities/tussocks in STA EAV cells (**Table 5C-5**).

[1] Soil and Water Science Department, University of Florida, Gainesville, Florida.

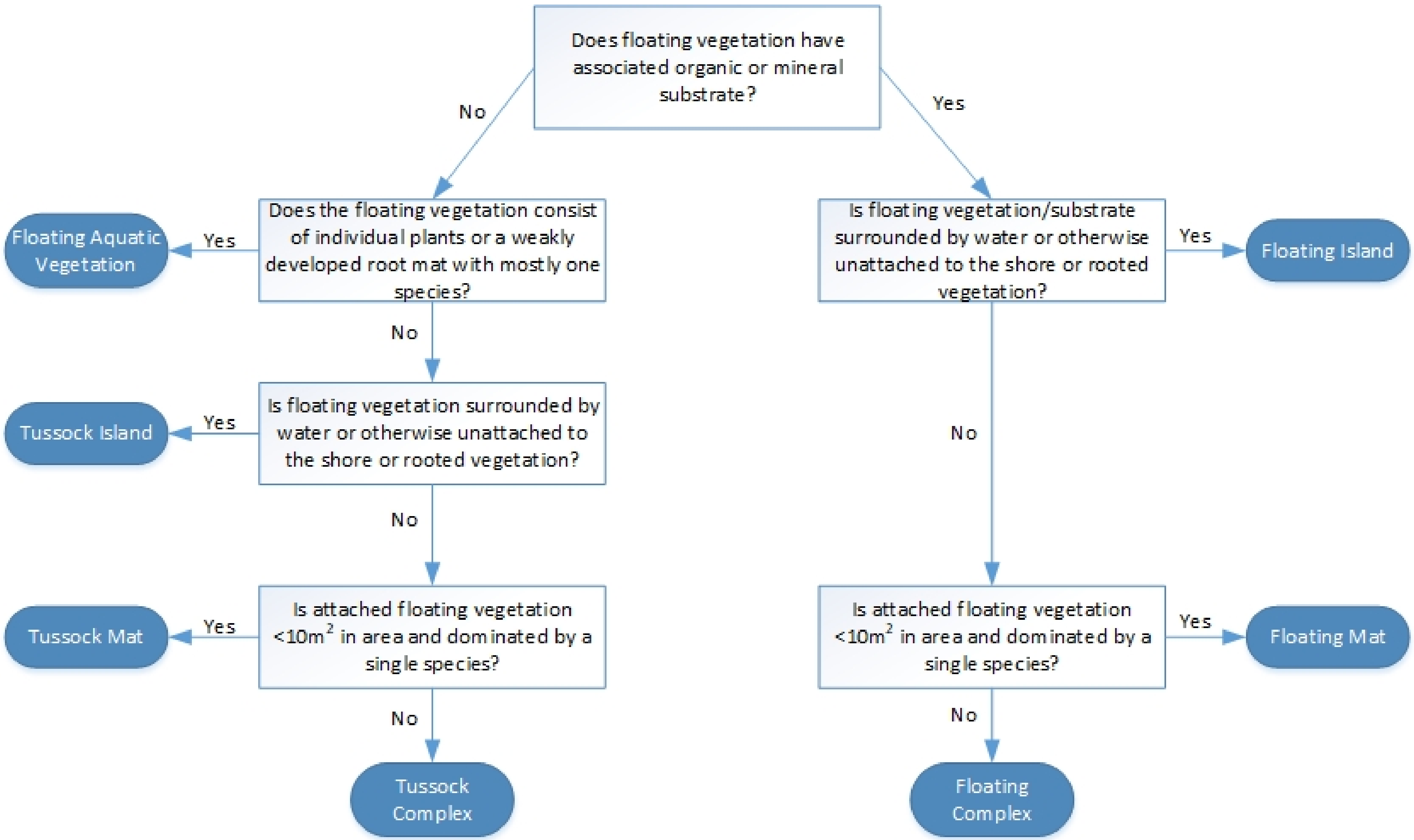

Figure 5C-3. Dichotomous key to the floating wetland nomenclature. (Note m^2 – square meter.)

Table 5C-5. Status of tasks for the Tussock Study.

Task	Status and Key Findings	Reports
Phase I		
Literature review	Task is complete.	Clark 2019b
Floating wetlands nomenclature	Task is complete. A hierarchical nomenclature scheme was developed to describe floating wetlands in STAs (**Figure 5C-3**).	Clark 2019c
Assessment of floating tussocks coverage in STA EAV cells	Task is complete. A multispectral camera on a UAS was used to determine if tussocks could be observed in areas that otherwise would not be apparent in satellite imagery.	Clark and Glodzik 2019
Thermography assessment	Task is complete. A thermal camera on a UAS was used to determine if thermal reflectance of vegetation can differentiate between emergent and floating *Typha.*	Clark 2019a
Phase II		
Data mining	Task is complete. Historical data related to formation of floating wetland communities in STAs was evaluated to determine if significant statistical relationships with water levels, soil P, and past agricultural history could be found.	Clark and Glodzik 2020
Refine UAS methodology and workflow	Task is complete. Flight planning, flight implementation, image post processing and GIS classification protocols were summarized. Post Processing Kinematic GPS correction significantly improved accuracy. A point sampling approach is recommended over ortho-mosaic stitching, due to less sensitivity to weather and sun angle conditions.	Clark and Glodzik 2021
UAS assessment of STA EAV cells	Task delayed due to COVID-19 restrictions. This task will map tussocks in 21 STA Cells where EAV is dominant to find floating wetland communities.	
Typha wetland buoyancy model	Task delayed due to COVID-19 restrictions. This task will estimate the potential for formation of floating wetland communities based on buoyancy measurements and water conditions.	
Evaluation of findings and final report	Task delayed due to COVID-19 restrictions. Final report and recommendations to reduce potential of floating wetland communities.	

Global and local terminologies were found in the literature review that described many of the floating wetland communities in the STAs. The review did not find a universal terminology or classification scheme for these floating complexes, despite such floating communities being found often in wetlands around the world from the tropics to the tundra. However, floating substrate (presence/absence), horizontal connectivity, and dominant vegetation were often components of terms used to describe floating wetlands. These descriptive terms were used to develop a floating wetland nomenclature that describes the variety of emergent plant communities in the STAs (**Figure 5C-3**; Clark 2019c).

The floating wetland nomenclature uses a hierarchical terminology. "Tussock" and "floating aquatic vegetation (FAV)" are floating vegetation with no associated organic substrate (**Figure 5C-3**). "Floating island" or "floating mat/complex" describes floating vegetation with associated substrate or unvegetated floating substrate. An "island" is free floating substrate not connected along any side, and a "mat" or "complex" is floating substrate connected along at least one side. The differentiation between "mat" and "complex" is based on size and species richness. For areas less than 10 square meters (m^2) and or composed of a single species the term "mat" is used. For contiguous areas greater than 10 m^2 or composed of multiple species, the area is termed a "complex".

The nomenclature for these floating wetlands can be defined further based on dominant vegetation type. For example, floating substrate dominated by *Typha* that is not attached to any adjacent vegetation and composed of organic matter would be labeled "*Typha* floating island". If that same *Typha*-dominated floating substrate were attached to other vegetation, and the area was greater than 10 m^2, the area would be labeled "*Typha* floating complex". A floating substrate area with mixed herbaceous vegetation that is contiguous and attached to non-floating vegetation would be labeled "marsh floating complex". STA-1W Cell 2A contains a variety of these floating wetlands (**Figure 5C-4**).

Floating wetland communities were identified and measured in two EAV cells of the STAs: STA-1W Cell 2A and STA-2 Cell 7 using UAS (Clark and Glodzik 2019). Post-processing and classification schemes were developed to identify floating wetland communities in these EAV cells. Multispectral drone imagery data discovered *Typha* floating complexes that otherwise would not be apparent in satellite imagery.

Thermal imagery was evaluated as a method to find floating wetland communities with a thermal-infrared camera attached to the UAS. It was expected that healthy cattail would have distinctly different thermal signatures from floating wetland communities. However, thermal signatures were not unique among the various floating and non-floating wetland communities assessed. Differences detected were strongly influenced by time of day reducing the ability to detect floating wetlands with thermal infrared imagery (Clark 2019a). Under very specific conditions (at solar noon when shadows are minimal) thermal-infrared imagery, when added to red, green, blue, near infrared, and red-edge spectral images, may support the differentiation between emergent and floating *Typha*. The influence of localized hot and cold spots can be reduced by aggregating pixels, which would reduce variability, allowing the underlying integrated energy balance to be expressed.

Historical data from the Everglades STAs were analyzed to identify statistically significant predictors of floating wetland communities across EAV cells (Clark and Glodzik 2020). Ratings of floating wetland community coverage for each treatment cell were obtained by interviewing SFWMD scientists responsible for on-the-ground management of STA vegetation. The results suggested a higher likelihood of floating vegetation occurrence in treatment cells with greater high water levels (characterized as Q90 or 90^{th} percentile of water level recorded in the cell), cells that had lower TP soil storage at the start of cell operations, and cells built on agricultural land that was farmed in recent years prior to cell construction. These results are considered preliminary until a more quantitative estimate of floating wetland community coverage can be determined using UAS imagery.

The processes and procedures using UAS classification to find floating wetlands in the STAs were optimized and improved in Phase II (Clark and Glodzik 2021). Findings provide a detailed summary of flight planning, flight implementation, image post processing, and GIS classification protocols. Use of post processing kinematic GPS correction significantly improved accuracy during photo capture allowing faster post processing of imagery and creation of high quality orthomosaic imagery for GIS community classification. Weather conditions for optimal stitching of mosaic images and consistent spectral reflectance are rare in STAs and therefore, large-scale mapping of STAs is not practical. Instead, a UAS point sampling approach that is less sensitive to weather conditions will be used as an alternative means to assess distribution of floating wetlands within the STAs as part of this investigation.

Direct measures of floating wetland buoyancy components and a model to indicate the potential for tussock formation are currently being developed as part of Phase II. Field sampling to assess various components of buoyancy, including above and below water vegetation, soils, and decomposition gases as well as root and rhizome connectivity with sediment were collected in spring 2021. This information will be integrated into a buoyancy model that can predict the effect of changes in water level and soil cohesiveness on buoyancy and the likelihood of floating wetland formation.

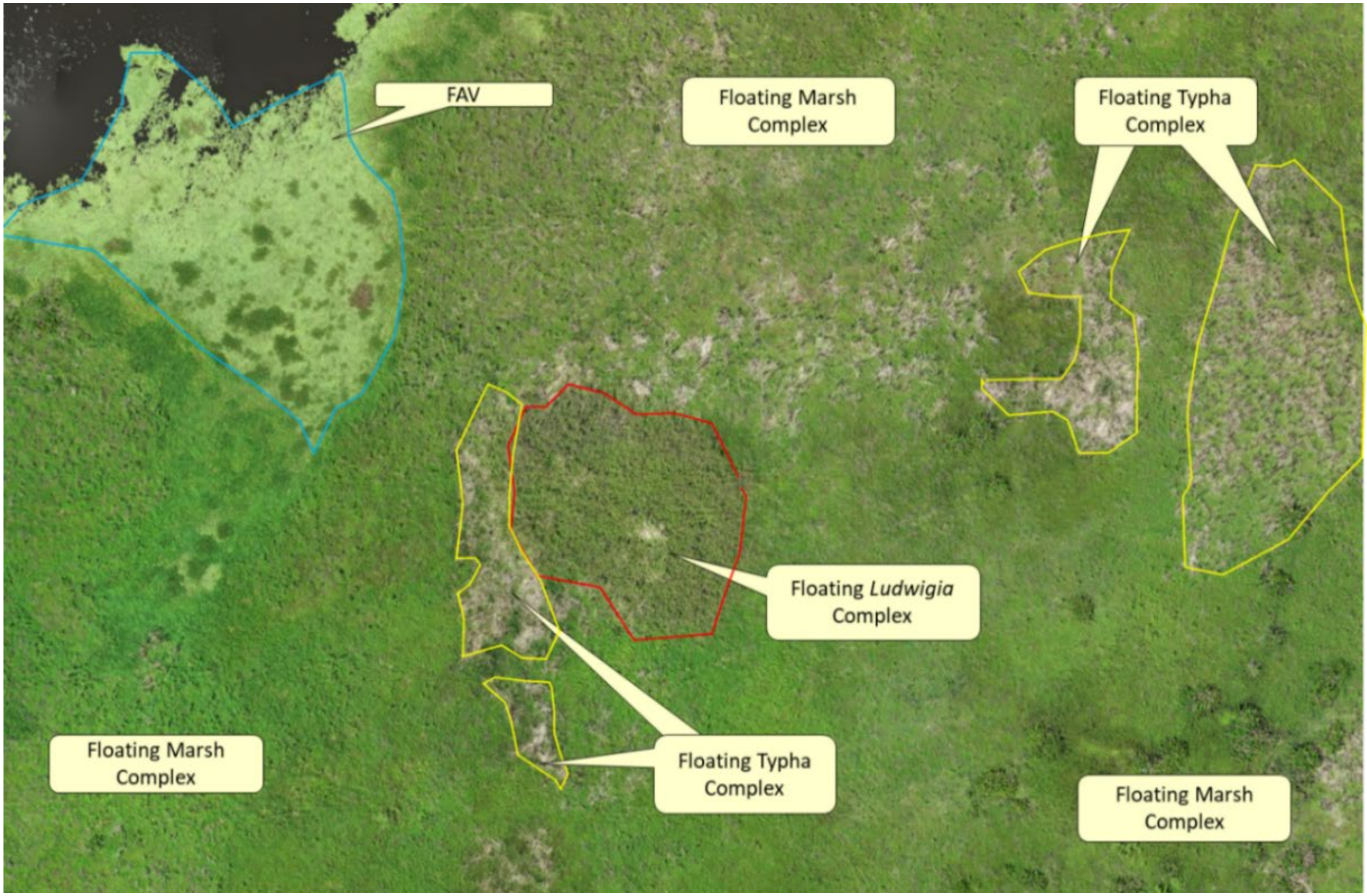

Figure 5C-4. Examples of floating wetland nomenclature applied to an aerial image of STA-1W Cell 2A.

INVESTIGATION OF THE EFFECTS OF ABUNDANT FAUNAL SPECIES ON P CYCLING IN THE STAS (FAUNAL STUDY)

Mark Barton[2] , Joel Trexler[1], Mark Cook, and Sue Newman

The purpose of the Faunal Study is to evaluate the influence of large populations of fish and aquatic invertebrates on P reduction in STAs. This study (1) estimates standing stock biomass of small fish (< 8 cm standard length) and aquatic macroinvertebrates in STA-2, and large fish (> 8 cm standard length) in STAs 2, 1E, 1W, and 3/4; (2) estimates mass-specific P excretion rates of the most abundant species; and (3) evaluates the potential of benthic aquatic species to enhance water column nutrient concentrations through bioturbation. Biomass and excretion estimates are combined and scaled up to estimate areal (per ha) P excretion by the aquatic animals in STA-2 (i.e., rates of P released to the water column via excretion, micrograms P per hectare per hour [µg P/ha/h]). Excreted loads of P were compared to external loads of P and other important nutrient-cycling pathways in the STAs. Bioturbation estimates will be used to evaluate the potential of aquatic animals to reduce the effectiveness of STA P removal. These estimates will be included in nutrient budgets supporting management actions aimed at improving STA performance.

The influence of animals on forms, fluxes, and transformations in the Everglades STAs is an important uncertainty for their efficient management for P removal. Animals, including waterbirds, fish, and macroinvertebrates, are important for nutrient cycling within aquatic ecosystems (Vanni 2002, Doughty et al. 2016). The abundance and diversity of aquatic animals can affect water column nutrient concentration through several pathways. Animals function as a source of internal nutrient loading by directly mobilizing benthic or particulate nutrients through their feeding and excretion (Vanni et al. 2006). They can also have important indirect effects through modifications of the environment (e.g., bioturbation; Vanni et al. 2006) and by consumptive effects through predation that result in interactive effects felt through the food web (Kellogg and Dorn 2012, Dorn 2013). Bioturbation, including all animal activities leading to mixing of the substrate, particle ingestion, and defecation, elevates water column nutrients through the resuspension of benthic nutrients and seston. Top-down effects may modify nutrient cycles by altering the abundance of species and impacting the efficiency of animal-mediated nutrient cycles.

Previous results from this work, reported in Appendix 5C-3, 5C-4, 5C-1 of the 2018, 2019, and 2020 SFERs, suggest a pivotal role of animals in STA nutrient cycling, but further work is needed on their direct and indirect roles in nutrient cycles and their impact on P transformations in the STAs (**Table 5C-6**). This year, despite several challenges with weather and COVID-19, work continued to estimate aquatic animal contributions to water column nutrient concentrations by fish excretion and bioturbation. To improve the robustness of efforts to scale up rates of P transformation from these processes, another year of estimates of biomass of fish, amphibians, and aquatic macroinvertebrates from STA-2 and aquatic animal community composition in STAs 3/4, 1E, and 1W was conducted. This is ongoing work with two more years of study planned, but interesting new results came to light this year that will be highlighted here.

Prior regional estimates indicated that fish excretion has a substantial effect on water column TP (Barton et al. 2020b). These estimates used excretion rates determined during the winter dry months of the year and focused on the six most abundant of the more than 30 species collected in the STAs. To improve estimates of TP recycling by excretion, the experiment was repeated with the original six species to obtain summer excretion estimates for eastern mosquitofish (*Gambusia holbrooki*), bluefin killifish (*Lucania goodei*), sailfin molly (*Poecilia latipinna*), largemouth bass (*Micropterus salmoides*), blue tilapia (*Oreochromis aureus*), and sailfin catfish (*Pterygoplichthys multiradiatus*). Furthermore, excretion rates for three new species—Mayan cichlid (*Mayaheros urophthalmus*), Florida gar (*Lepisosteus platyrhincus*),

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and grass shrimp (*Palaemonetes* spp.)—were investigated during summer and winter conditions. Future regional estimates should be more accurate using summer and winter excretion rates for the three most abundant small fish, the most abundant aquatic invertebrate, and the five most abundant large fish.

Table 5C-6. Status of tasks for the Faunal Study.

Task	Status and Key Findings	Publications and Reports
Community analysis	Field work was completed in 2019 and 2020 aside from interruptions due to COVID-19. Preliminary analysis indicate that small fish and macroinvertebrates biomass may have decreased over this time period in the STAs, and further analyses will aim to understand why this decrease was observed. Electrofishing field work was also completed in 2019 and 2020, aside from March and June 2020 due to COVID-19 and equipment failure. Further electrofishing is ongoing. Analyses will investigate whether large fish communities follow similar decreasing trends as small fish and invertebrate communities.	Barton and Trexler 2020
Bioturbation by large fish	This task was met with several challenges associated with COVID-19 and weather. Despite extensive efforts to keep the project on schedule, the field work for this task could not be completed as scheduled. The experiment has been rescheduled a third time and is currently underway.	Barton et al. 2020d
Fish excretion rate estimation	Field work for this task was completed in 2019 and 2020. Data from this work will expand our knowledge of excretion rates for the three most abundant small fish and 3 most abundant large fish to include seasonal rates. This work has also added summer and winter excretion data for three additional species. This expanded data set will be used to improve regional estimates of excreted P and N in the STAs.	Barton et al. 2020c

While the three most abundant large fish species (largemouth bass, blue tilapia, and sailfin catfish) excreted substantially less per unit mass than the three small fish (eastern mosquitofish, bluefin killifish, and sailfin molly), their contribution of TP to the water column was dominated by TP released from the substrate through bioturbation (Barton et al. 2020b). Bioturbation rates of these species were determined in spring but may differ throughout the year with changing activity levels caused by temperature, rainfall, and spawning behavior. A final bioturbation experiment was attempted on two separate occasions to establish seasonal bioturbation rates; however, these efforts were suspended in April 2020 due to Florida International University COVID-19 protocols, and again in November 2020 due to heavy rains associated with Tropical Storm Eta that flooded the experimental enclosures (**Figure 5C-5**). The experiment has been rescheduled a third time and is underway as of summer 2021.

Throw-trap surveys were completed successfully in June and September 2019, and March and September 2020, allowing for updated biomass estimations for small fish and macroinvertebrate communities. A throw-trap survey was also attempted in June 2020, but high waters in STA-2 prevented successful completion. Small fish average monthly biomass was 181 and 224 kilograms per hectare (kg/ha) in STA-2 Cells 3, 4, 5, and 6 in June and September 2019, respectively. These values were consistent with previously reported values for the same cells in September 2018 and March 2019 (199 and 198 kg/ha, respectively). However, the biomass of small fish in STA-2 decreased substantially in March and September 2020 (80 and 87 kg/ha, respectively). This decrease was attributed to a decrease in fish biomass in Cells 4 and 5, from 85 and 76 kg/ha, respectively, in September 2019 to 15 and 12 kg/ha, respectively, in March 2020. In September 2020, water levels in Cell 5 were too high for throw-trap sampling and the biomass data cannot be compared to previous sampling periods, but Cell 4 biomass did increase from 15 kg/ha in March 2020 to 38 kg/ha in September 2020.

Figure 5C-5 The bioturbation enclosures before (top) and after (bottom) Hurricane Eta's heavy rains.

The decrease in estimated areal biomass may result from a decrease in the number of fish caught in throw traps or a change in dominant vegetation/habitat type. In Cell 4, the change coincides with a decrease in vegetative cover; bare habitats (areas where more than 50% of the top down coverage is bare substrate) made up 17% of Cell 4 in July 2019 and increased to 42% in April 2020. Furthermore, the average biomass per throw-trap sample across all habitat types decreased from 8.9 ± 0.5 grams per square meter (g/m^2) in 2019 to 4.4 ± 0.7 g/m^2 in 2020. In Cell 5, the areal coverage of dominant vegetation did not change. This cell is largely dominated by bare habitat (76% of total areal cover), and the dominant vegetation is *Najas guadalupensis* (southern water nymph; 10%). The average biomass of fish collected from samples collected in *N. guadalupensis* remained consistent from June 2019 to March 2020 (9.4 ± 0.6 g/m^2), but the average biomass of fish per throw-trap sample in bare habitat decreased from 5.87 g/m^2 in June 2019 to 0.74 g/m^2 in September 2019 and 0.23 g/m^2 in March 2020.

A similar decrease in biomass is seen with macroinvertebrates collected in throw-trap samples in STA-2 Cells 3, 4, 5, and 6 but the change occurs earlier, between June 2019 (160 kg/ha) and September 2019 (68 kg/ha). While invertebrate biomass was consistently low in Cell 5 in June 2019, September 2019, and March 2020 (6.2 ± 2.1 kg/ha), the invertebrate biomass in Cell 4 decreased throughout the same months (96, 52, and 11 kg/ha, respectively). Previous analyses showed that invertebrate density was strongly correlated with vegetative cover (Barton et al. 2020a), and the observed decrease in invertebrate biomass coincides with the decrease in vegetative habitat in Cell 4. Furthermore, the consistently low invertebrate biomass in Cell 5, where bare habitat is dominant, further supports that invertebrates prefer vegetated habitats.

Electrofishing surveys were continued in July and October 2019 and October 2020 allowing for updated biomass estimates for large fish communities. March and June 2020 electrofishing surveys were attempted. The March survey could not be completed when fieldwork was stopped by COVID-19 protocols at Florida International University. The June survey was hindered by generator malfunction. The generator was replaced and electrofishing surveys have resumed. The electrofishing data have not yet been analyzed and it remains to be seen if a similar decrease in large fish biomass will be observed, as was seen in small fish and macroinvertebrates. It is unclear what caused these decreases in fish and invertebrate biomass, but further analyses will investigate changes in water level and other environmental factors. These changes in biomass will affect our estimates of the impact that fish and invertebrate communities have on water column TP and TN. Fluctuation in aquatic animal abundance will offer insight into their role in nutrient cycling within the STAs and whether their management can be used to improve the efficiency of nutrient removal from surface water passing through the STAs.

IMPROVING RESILIENCE OF SAV IN THE STAS (SAV RESILIENCE STUDY)

Jacob Dombrowski and Kevin Grace[3]

The purpose of the SAV Resilience Study is to assess physical, chemical, and biological processes that reduce the sustainability of SAV communities. SAV is an important and abundant component of the STAs. Substantial declines in SAV coverage and density have occurred at times in several STA cells, which could impair nutrient removal performance of these systems (Dombrowski et al. 2020). Factors that may contribute to SAV species distribution, persistence, colonization, and recovery in the STAs include SAV biology, water chemistry, nutrient loading, soil/sediment chemistry including deposition of fine marl sediments, physical characteristics, herbivory, and interactions among these factors (DBE 2018). Temporal changes of soil characteristics and extreme weather events (e.g., hurricanes and drought) also have resulted in stress on SAV in the STAs. The SAV Resilience Study is comprised of four sub-studies: (1) effect of marl soil on SAV growth (complete), (2) effect of nutrient loading rates on *Chara* sustainability (ongoing), (3) effect of dry out on SAV growth (complete), and (4) impediments to SAV growth in STA-2 FW 3 (complete; **Table 5C-7**).

The first sub-study to evaluate marl soil accumulation on SAV growth sought to address the question: Does growth of selected SAV species become impaired by the accumulation of new marl sediments, as compared to growth on the substrate (previously-farmed muck soil) present at the time of STA construction? Leaf tissue nutrient accumulation, biomass growth, root development, and water column P reduction by two common rooted SAV species, *Najas guadalupensis* (southern water nymph) and *Potamogeton illinoensis* (Illinois pondweed), and a non-rooted macroalgae, *Chara* spp., were monitored. Each SAV species was grown within outdoor mesocosms on three different substrate types: farm muck (recently farmed soil, typical of STA start-up), aged muck (soil from SAV-dominated STAs that has been operational for an extended period), and marl (substrate from within SAV beds that represents the current condition). Following establishment, plant growth continued for 20 weeks while water was exchanged with fresh, low-nutrient water every 2 weeks. Plant tissues were harvested at 10 and 20 weeks, and analyzed for total dry weight, ash-free dry weight, macronutrients (P, N, carbon, and calcium), and micronutrients (iron, manganese, copper, boron, zinc, nickel, and molybdenum). Soil samples were taken at the same time steps as plant tissues and analyzed for the same micro- and macronutrients as well as bulk density. Water was sampled inside each replicate during and 14 days following water exchange. This sub-study was completed in WY2020 (Dombrowski and Grace 2020).

[3]DB Environmental Laboratories, Inc., Rockledge, Florida.

Table 5C-7. Status of the tasks for the SAV Resilience Study.

Task	Status and Key Findings	Reports
SAV growth as affected by accumulation of new marl sediments	Task was completed in September 2019. Growth and nutrient content of rooted macrophytes *Najas guadalupensis*, *Potamogeton illinoensis*, and the non-rooted macroalgae *Chara* spp. were evaluated in mesocosms of newly-accrued STA marl soil, recently-farmed muck soil, and aged muck soils.	DBE 2020a
Chara sustainability at different nutrient loading regimes	Task is ongoing. *Chara* spp. are evaluated in mesocosms of different PLRs. Interactions between water column P and vegetation density, dissolved oxygen, pH, temperature, and light conditions are examined. Various manipulations have been added to determine the conditions that result in vegetation collapse.	DBE 2020b,h
STA dryout	Task completed June 2020. Soil cores from STA-1E Cell 6 and STA-2 Cell 3 were dried or kept hydrated to examine the influence of drawdown on SAV germination and growth. Dried cores had faster and higher germination rates, though no difference was observed between wet and dry cores for SAV growth.	DBE 2020b
Impediments to SAV growth in STA-2 FW 3	Task was completed in July 2020. SAV growth was evaluated in corrals placed on bare soil in STA-2 FW 3. Corrals were inoculated with *Chara* spp., *Najas marina*, *Potamogeton illinoensis*, or *Najas guadalupensis*. *Chara* and *Najas marina* grew well in monoculture, while little to no growth was detected for *Najas guadalupensis or Potamogeton illinoensis*. In polyculture, *Najas guadalupensis* exhibited higher growth while *Najas marina* had little to no growth.	DBE 2020b

The second sub-study, Effect of Nutrient Loading Rates on *Chara* Sustainability, examines the potential for the common macroalgae *Chara* spp. to suddenly and rapidly decline or collapse. Specifically, the sub-study seeks to answer the following questions: Does *Chara* have an upper limit to its tolerance to nutrient loading? and Are there early signs of declining health in STA *Chara* beds? Mesocosms inoculated with *Chara* are subjected to either low (~0.3 g P/m^2/yr), moderate (~1 g P/m^2/yr), or high (~2 g P/m^2/yr) PLRs. The initial mesocosms included low TP soil to focus on the effects of external loading. Additional mesocosms were established in WY2021 that contain higher TP soil to focus on the effects of internal loading. Nutrient loading regimes are intended to mimic the conditions at the inflow, middle, and outflow of an STA FW. The relative density and nutrient removal performance of *Chara* are assessed as indicators of SAV 'health'. *Chara* growth is monitored over time alongside routine water quality sampling. Water physical chemistry parameters (dissolved oxygen, temperature, and pH) are recorded at continuous 15-minute intervals. Additional measurements of soil redox potential, photosynthetically active radiation (PAR), and vegetation relative density are measured monthly. During WY2020, the mesocosms were subjected to a drawdown during the dry season to replicate seasonally low water levels, followed by a pulse phase during the transition from dry to wet season.

Results to date show *Chara* density responds to external nutrient loading across the range of PLRs applied in this study, with the increased densities under high and moderate PLR treatments (**Figure 5C-6**). P removal efficiency follows the nutrient loading gradient as well; however, the moderate and low loading treatments produce the lowest outflow P concentration (12 and 13 µg/L, respectively). Although P removal efficiency remains highest within the high loading treatment, anoxic conditions have developed near the soil surface in these treatments. There is high light limitation within these treatments with < 5% PAR remaining at the soil surface as compared to 5 to 20% and 40% PAR remaining in the moderate and low loading treatments, respectively. Despite the development of light-limiting conditions, a total collapse of biomass has not occurred in any treatment. The high loading treatment also had the greatest response to the drawdown and pulse phases, where outflow TP increased during the drawdown/pulse and remained higher during the return to normal operations. This may indicate a greater degree of stress by external P loading, as the moderate and low loading treatments returned to (or improved) their outflow TP concentrations following the drawdown/pulse phases. This experiment will continue into WY2022 and will focus on the comparison of internal and external P loading on *Chara* sustainability.

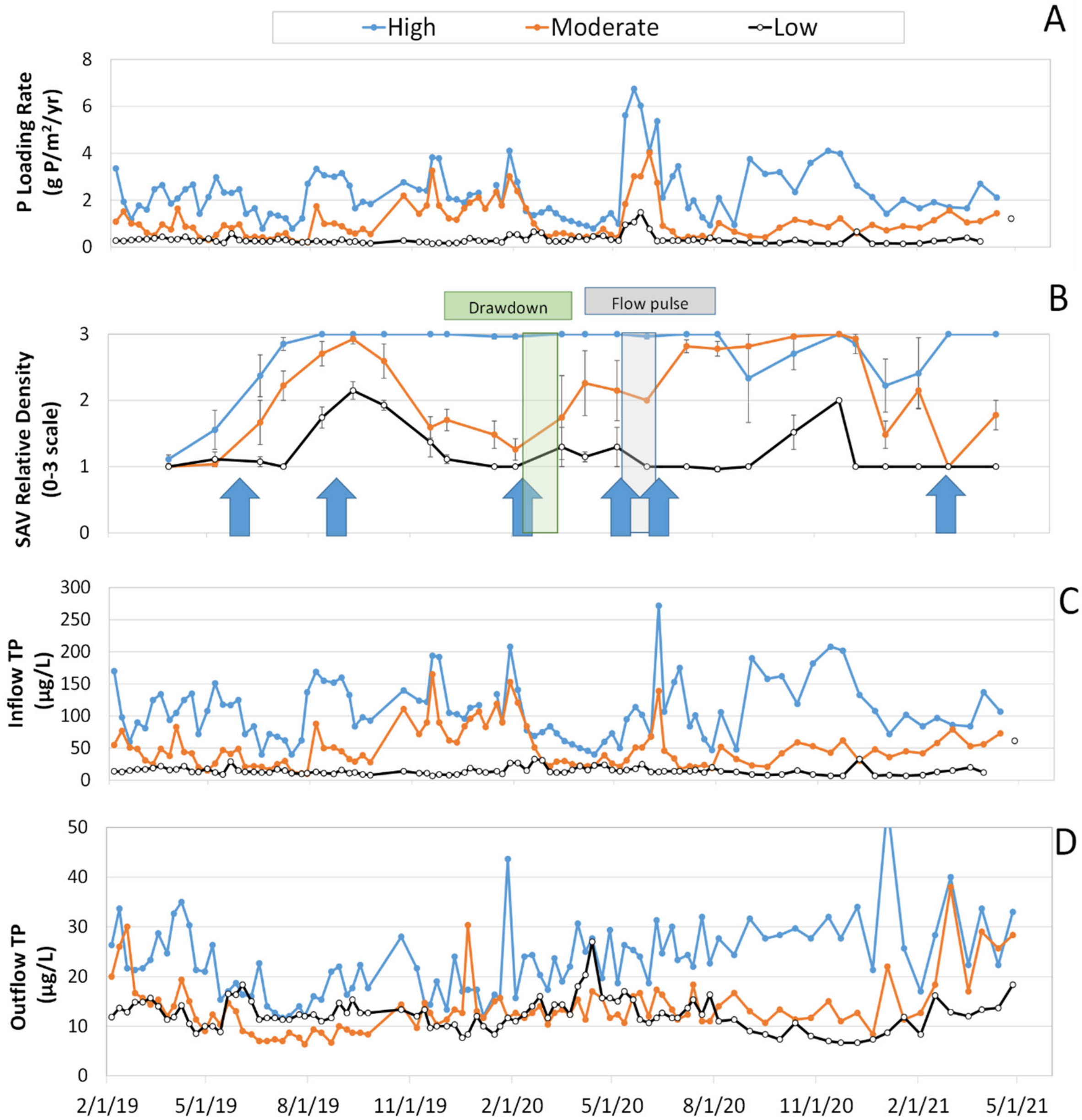

Figure 5C-6 (A) Phosphorus loading rate during the entire study period. (B) Relative density of *Chara* spp. in mesocosms during the period between March 27, 2019, and May 6, 2020. Values represent the average (± standard error) of monthly values in triplicate mesocosms under each of three loading treatments. A value of 3 indicates higher density (> 67% coverage). The arrows indicate plant tissue sampling events during the study. (C and D) Time series of weekly inflow and outflow TP concentrations. Error bars denote the standard error around the mean of outflow TP concentrations from triplicate mesocosms under each nutrient treatment.

The third sub-study, STA Dryout, examined temporary drawdowns as a potential management tool to consolidate and compact soil, which could improve the germination and growth of SAV communities. Consolidation could expedite SAV recovery in FWs following large-scale losses of SAV, such as occurred in STA-2 FW 3 in 2017. This FW previously contained dense beds of *Chara*, *N. guadalupensis*, and *P. illinoensis*, thus a viable seedbank may exist within the soil. To examine the effects of drawdown on soil consolidation and SAV germination, a phased approach was taken where soil cores were first taken from STA-2 FW 3 and STA-1E Cell 6. The latter cell currently supports species similar to those previously found in STA-2 FW 3. Cores were subject to controlled drying, including thorough dryout (dry) or partial dryout, or left in a field-moist (wet) condition. Subsamples of each treatment core were taken to assess bulk density and soil P after a drying period. Each treatment was then monitored for SAV germination over a 6-week period following rehydration. Following germination, the sub-study entered a growth phase where vegetation was removed from each soil and fresh *N. guadalupensis* was inoculated into growth cylinders placed onto the dry and wet soil treatments. SAV biomass was then harvested after 12 weeks and used to calculate the overall growth rate. The growth phase of this sub-study was completed in WY2021 (Dombrowski and Grace 2020). Growth rates of *N. guadalupensis* were not significantly different when compared between the dry and wet treatments. Development of *N. guadalupensis* roots was significantly lower in the higher bulk density soil of the dry treatments. Other volunteer SAV species (notably *Chara*) germinated and grew in many of the growth cylinders, especially in the dry treatments, which may have limited *N. guadalupensis* growth both above- and belowground. Net growth with all SAV species included was positive for all but one of the growth cylinders, but was not significantly different between treatments. In summary, drying the STA soil improved *Chara* germination, but did not significantly affect SAV growth and instead, appeared to limit root development by *N. guadalupensis*.

The fourth sub-study, Impediments to SAV Growth in STA-2 FW 3, examines if growth of SAV taxa is impeded by current soil conditions. A complete SAV collapse occurred in 2017 in STA-2 FW 3 following a gradual decline in SAV coverage. To date, recolonization by common SAV taxa has been limited, presumably due to unfavorable conditions that have developed within STA-2 FW 3. To assess SAV growth rate, a series of netted corrals (1.2 meters (m) x 1.2 m) inoculated with common SAV taxa were used. Following initial reconnaissance surveys for appropriate site conditions (deep marl sediment and a persistent lack of vegetation), net corrals (sample size [n] =20) were established in WY2020 within the FW where SAV has not yet reestablished. Two corrals were established as unplanted controls, while the remainder were inoculated either in mono- or polyculture with taxa historically found in STA-2 FW 3. SAV taxa included were *Chara*, *Najas marina*, *P. illinoensis*, and *N. guadalupensis*, with both *Najas* species included within the polyculture corrals. Grazing by large-bodied fish (i.e., *Oreochromis aureus*) may also be a contributing factor to SAV decline within STA-2 FW 3, thus select polyculture treatments had 2 fish added in addition to SAV. Following inoculation, SAV were allowed a 9-week growth period during June–July 2020. All SAV biomass was then collected, sorted by species and above- versus belowground biomass, and weighed to determine a final growth rate. Within the monoculture corrals, *Chara* and *N. marina* increased in biomass during the growth period while *P. illinoensis* and *N. guadalupensis* had little to no growth. This is consistent with observations from recent surveys in STA-2 FW 3, where species composition has shifted to almost exclusively *Chara* and *N. marina*. Within the polyculture treatments, *N. guadalupensis* exhibited higher growth rates than *N. marina,* which had little to no growth. However, reduced *N. guadalupensis* biomass was found in the polyculture treatments that contained fish. This suggests that *O. aureus* may prefer to eat *N. guadalupensis* over *N. marina*.

QUANTIFYING LIFE CYCLE AND P UPTAKE AND RELEASE FROM PERIPHYTON AND PHYTOPLANKTON COMMUNITIES (PERIPHYTON STUDY)

Kathleen Pietro

The purpose of the Periphyton Study is to evaluate nutrient dynamics of periphyton and phytoplankton in downstream areas of STA treatment FWs where TP concentrations are very low (≤ 20 µg/L). Periphyton is defined in this study as the biological matrix of algae, fungi, bacteria, and detritus attached to submerged surfaces in the water column of STAs. This study focuses on measurements of nutrient uptake and release, growth, respiration, senescence, and death. These processes may be significant factors for P removal in the outflow regions of the STAs.

Phase I of this study reviewed periphyton literature regarding nutrient uptake, growth, respiration, and death in tropical and subtropical oligotrophic (low nutrient) or mesotrophic (moderate nutrient) freshwater wetlands subjected to relatively low P conditions (≤ 20 µg/L; Laughinghouse et al. 2019; **Table 5C-8**). A list of methods to measure biomass, metabolism, rates, pigments (chlorophylls and phycocyanin), taxonomic composition, cell biovolume, amplicon sequencing, metagenomics, metatranscriptomics, stable isotopes (^{31}P, ^{32}P, ^{15}N, ^{14}C), and enzyme activity was compiled.

Phase II will begin in July/August 2021 with a study designed to measure the bioavailability of DON and DOP to periphyton in laboratory incubations. The study will compare periphyton communities found in EAV and SAV communities and their effect on water quality within the outflow region of STA-3/4 Cell 2B. Additional periphyton studies from Everglades STAs, Everglades Water Conservation Areas (WCAs), and the Everglades National Park will be compiled and evaluated in a component of the Data Integration and Analysis Study (See the *Data Integration and Analysis (Data Integration Study)* section later in this chapter). Phase III will provide a snapshot of biogeochemical and genetic responses of periphyton in low TP water column concentrations in SAV and EAV areas of an STA outflow cell.

Table 5C-8. Status of tasks for the Periphyton Study.

Task	Status and Key Findings	Publication or Report
Phase I: Literature review of periphyton nutrient cycling in tropical and subtropical oligotrophic systems	This task was completed in 2019.	Laughinghouse et al. 2019
Phase II: Laboratory incubations	The tasks include measuring bioavailability of DOP and DON by periphyton collected from EAV and SAV dominated areas located in the outflow region of STA-3/4 Cell 2B.	
Compilation of microbial-related research from STAs, WCAs, and Lake Okeechobee	This task is included as a part of the Data Integration Study.	DBE 2021d
Phase III: Design mesocosm and field-scale study to measure periphyton life cycle and nutrient uptake rates within EAV and SAV communities.	This task is in the planning stages. Specifics are still to be determined.	

L-8 FEB AND STA OPERATIONAL GUIDANCE (L8-FEBOG STUDY)

Matt Powers

The purpose of the L8-FEBOG study is to support operational guidance through an evaluation of water quality, stage, flow, and groundwater. The primary objective of this FEB is to attenuate peak flows and temporarily storing stormwater runoff to assist in the operation of STA-1E and STA-1W and help improve treatment performance (SFWMD 2015).

In WY2018, FEB outflow TP concentrations were higher than inflow TP concentrations (Xue 2019). Elevated TP outflow concentrations occurred from May to June 2017 and during April 2018 when little flow entered the FEB and water levels were low. This is undesirable as elevated TP concentrations from the FEB will increase P loading to the downstream STAs, making it more difficult for the STAs to meet the WQBEL. Although the L-8 FEB was not designed to remove P, an increase in TP from inflow to outflow was not anticipated. Three sources of TP that could cause this increase have been postulated and will be evaluated in three phases. Phase I will look at groundwater. Phase II will look at sediment resuspension and/or soil erosion. Phase III will look at biological sources such as mussels, fish, and plankton (**Table 5C-9**). For Phase I, monthly water quality samples were collected in the interior compartments of the FEB and quarterly water quality samples were collected in the surrounding groundwater wells from January to August 2019 (**Figure 5C-7**). Phase II began in September 2019, focusing on the resuspension of sediments into the water contributing to elevated TP in the FEB, and is ongoing.

Table 5C-9. Status of tasks of the L8-FEBOG study.

Task	Status and Key Findings	Reports
	Phase I	
Detailed study plan	This task is complete. The plan describes Phase I water sampling and potential phases to evaluate sediments, runoff, and biota as sources of P.	SFWMD 2019
Phase I: Water quality sampling and summary final report	This task is complete. Surface water and groundwater from January to August 2019 were characterized. Groundwater TP concentrations were much less than average TP concentrations in the FEB indicating that groundwater was not a source of TP. A budget of the L8-FEB shows that groundwater contribution of TP is minimal.	DBE 2020e
	Phase II	
Surface water monitoring	This task is complete. Higher TP and resuspended material were found in samples taken during high flow events and after the events ended.	DBE 2020f
Sediment characterization	In laboratory tests, sediment samples from the interior of the FEB and canals were resuspended into beakers of water significantly increasing water column TP. Soils from the surrounding levees did not increase TP as much.	
Event based surface water quality sampling	Analyses of autosamples taken from the inflow cell to L8-FEB showed increasing TP during and after a major flow event.	
	Phase III (TBD)	

Figure 5C-7. Sampling sites of the L8-FEBOG study.

Phase I results indicate stratification of P by depth with the highest P concentrations, primarily in particulate form, at the bottom of the FEB. Groundwater is not a source of PP or TP as samples from groundwater wells surrounding the FEB had lower PP and TP concentrations than FEB surface water (**Table 5C-10**). P loading from groundwater seepage was relatively small compared to surficial inflow and contributed an estimated 6 to 14% of the total P load to the FEB (DBE 2020e). Groundwater has been excluded as the major contributor of P in discharges from the FEB.

Collection and analysis of Phase II data are ongoing. Preliminary analysis of sediments collected from inside the FEB indicate these sediments contain P-enriched organic matter that can be resuspended and remain in suspension for over a week (**Table 5C-10**). These resuspended sediments result in elevated turbidity and elevated PP and TP concentrations in the water column. The effect of inflow and outflow structure operations on TP concentrations is ongoing.

Table 5C-10. Groundwater and surface water quality values from the L8-FEBOG Study.

Parameter (Units [a])	Groundwater		Surface Water	
	Mean	Range	Mean	Range
Dissolved Oxygen (mg/L)	0.6	0.0–2.7	6.1	0.0–12.8
pH	7.2	6.9–7.6	8.3	7.3–10.0
Conductivity (µS/cm)	1,739	516–9,229	1,623	1,226–2,624
Temperature (°C)	25.6	24.3–27.9	23.2	16.9–29.0
Total Phosphorus (mg/L)	0.028	0.008–0.075	0.112	0.025–0.675
Particulate Phosphorus (mg/L)	0.013	0.002–0.042	0.095	0.021–0.511
Total Dissolved Phosphorus (mg/L)	0.015	0.006–0.033	0.017	0.004–0.164
Soluble Reactive Phosphorus (mg/L)	0.012	0.004–0.028	0.012	0.001–0.153
Dissolved Organic Carbon (mg/L)	14.2	6.5–18.8	18.8	16.8–21.2
Total Nitrogen (mg/L)	1.9	0.4–6.0	2.2	1.2–4.3
Ammonia (mg/L)	1.1	0.1–5.0	0.2	0.0–2.5
Nitrate + Nitrite (mg/L)	0.006	0.005–0.015	0.475	0.005–2.330
Hardness	470	163–1,029	352	308–487
Alkalinity (mg/L)	277	148–610	221	186–284
Chloride (mg/L)	265	22–2,320	268	192–670
Sulfates (mg/L)	184	18–712	156	116–266
Dissolved Calcium (mg/L)	132	49–353	99	88–121
Dissolved Potassium (mg/L)	7.1	3.7–18.3	8.9	8.4–13.1
Dissolved Magnesium (mg/L)	24	6–102	24	22–43
Dissolved Sodium (mg/L)	190	24–1,588	184	128–461

a. Key to units: °C – degrees Celsius; µS/cm – microsiemens per centimeter; and mg/L – milligrams per liter.

QUANTIFYING THE RECALCITRANCE AND LABILITY OF P WITHIN STAS (BIOMARKER STUDY)

Elise Morrison[4], Sue Newman, Jill King, and R. Thomas James,

The purpose of the Biomarker Study is to evaluate relationships between organic matter (OM) and P to capture the sources and potential turnover of P within the STAs (**Table 5C-11**). This work builds on a pilot project that was previously conducted in STA-3/4 (Morrison et al. 2020). Water samples were collected from the major inflow structures to the STAs and a major outflow structure from Lake Okeechobee, which can deliver lake water to the STAs, to characterize the OM and P of STA source water. Water samples were analyzed for nutrients, P speciation, microbial bioassays, and dissolved OM composition. Data analysis is ongoing. A workplan was developed that includes the evaluation of potential faunal biomarkers as tracers of fish inputs of OM and P. Samples have been collected in collaboration with the Faunal Study, biomarkers have been analyzed, and data analysis is ongoing. In addition, in situ litterbag studies will evaluate the changing OM in STA litter and floc, and the surrounding water. The overall goal of the study will be to evaluate how much of the total DOP and DON in the surface water at the outflow region in a well-performing STA is bioavailable. This information should indicate the contribution to water column P from the internal and external sources, which may suggest further management actions to reduce these contributions, thus improving STA performance.

Table 5C-11. Status of Biomarker Study tasks.

Task	Status	Reports
Detailed work plan	Complete	University of Florida 2021b
Source water characterization	Samples retrieved; analysis is ongoing.	
Faunal excretion and bioturbation analyses	Samples received, analyses ongoing.	
In situ experiments	Experimental design approved (work plan). Prototype litter bags constructed and deployed in July 2021.	

[4] Department of Environmental Engineering Sciences, University of Florida, Gainesville, Florida.

DATA INTEGRATION AND ANALYSIS (DATA INTEGRATION STUDY)

R. Thomas James

The purpose of the Data Integration Study is to integrate STA and Science Plan data and documents to support management decision making. A number of tasks will be undertaken to achieve this purpose: (1) review and summarize results from Science Plan reports and publications as well as other relevant STA reports, (2) compile, review, and analyze STA and Science Plan study nutrient, biological, and ancillary data, (3) continue development of STA P models and/or development of new models (empirical and deterministic) to support understanding of P dynamics in the outflow regions of the STAs, (4) determine missing relevant information and methods through a data gap analysis, and (5) develop a guidance document to provide potential STA design, operation, and management strategies to attain lower TP discharges to meet the WQBEL at the STA outflows.

Four sub-studies have been implemented thus far to support the first three tasks (**Table 5C-12**). These include (1) Water, Vegetation, and Soil Relationships in the Everglades STAs, (2) Integration of DB Environmental Microbiological Studies in Wetlands and STAs of South Florida, (3) Development of Simulation Models for Everglades STAs, and (4) Comparison of STAs and WCA-2A Transect Water Quality Data. Of these four sub-studies: Water, Vegetation, and Soil Relationships in the Everglades STAs is complete. The other sub-studies are ongoing.

The Water, Vegetation and Soil Relationships in the Everglades STA sub-study explored and characterized relationships among water, soil, and vegetation variables in STA outflow regions. This study compiled available databases of water quality, soils, and vegetation coverage, and tissue nutrient information and then focused specifically (and solely) on assessing internal conditions and data relationships in STA outflow regions, using data from full-scale and mesocosm-scale studies, for further insights on STA performance and apparent limits.

Several observations were made from the field and mesocosm studies. Consistently low P concentrations were found in outflow regions with dense SAV, but high density did not guarantee reaching the target concentrations (≤ 13 ug/L; DBE 2020g). The macroalgae *Chara* was often the densest SAV community and outflow regions where it was abundant produced the lowest TP concentrations. Rooted vegetation, including vascular SAV species (*N. guadalupensis* and *P. illinoensis*) and cattail, likely add P to the water column through extraction from the soils into the aboveground plant tissue. This extraction resulted in higher TP concentrations than found in areas of dense *Chara*. In a batch microcosm study, vascular SAV species (*N. guadalupensis* and *P. illinoensis*) accumulated slightly higher tissue P from the soil resulting in higher water P concentration than for microcosms of *Chara*. Outflow TP concentrations from STA FWs were not related to TP in floc or surficial soil from this region most likely due to the sparseness of contemporaneous soil and water column TP data in the same location, and the variation in water column TP caused by other proximate factors. However, controlled mesocosm-scale studies found that outflow P was indeed related to soil TP concentration.

The Integration of DB Environmental Microbiological Studies in Wetlands and STAs of South Florida sub-study compiled a substantial amount of microbial research that has been carried out over the past 23 years into a summary document (DBE 2021c). The information is wide ranging from enzyme activity, periphyton biomass, and growth rates and factors affecting the periphyton communities within the STAs. One of the major conclusions is that there is a P threshold below which periphyton flourishes, removing P from the water column and maintaining low P conditions. When P concentrations are above this threshold, the periphyton community declines. Thus, P loads into these regions where substantial periphyton communities exist must be kept low. To maintain low P concentrations in these regions, options to reduce internal loading from soils by removal, capping, or burial may be considered.

Table 5C-12. Status of the tasks for the Data Integration Study.

Sub-study	Status and Key findings	Tasks Supported [a] and Reports
Water, Vegetation, and Soil Relationships in the Everglades STAs	This sub-study is complete. It included the (1) compilation of data sets of water quality, sediment quality, and aquatic plants and (2) analysis of vegetation effects on water quality, soil effects on water quality, and joint soil-vegetation interactive effects on water quality. The four main conclusions from the report are: (1) annual average outflow TP concentrations were lower from FWs densely vegetated with SAV in the outflow region, (2) outflow TP concentrations from STA FWs were not related to floc or surficial soil TP in the outflow regions, (3) water column TP and soil TP were related in mesocosm studies, and (4) SAV tissue P is closely related to soil P, based on targeted field samples, a controlled microcosm study using several soils and plant species, and field monitoring data from the STA-3/4 PSTA Cell.	Task 1 DBE 2020g
Integration of DB Environmental Microbiological Studies in Wetlands and STAs of South Florida	This sub-study is ongoing. Work done so far includes (1) compilation of a bibliography of microbial related research from WCAs, STAs and the Everglades, (2) review of the sections related to microbial studies in the *Evaluation of Soil Biogeochemical Properties Influencing Phosphorus Flux in the Everglades Stormwater Treatment Areas (STAs) Final Report* (University of Florida 2019), (3) compilation of data from microbial studies conducted over the past 20 years, and (4) development of a report discussing the microbial studies carried out over the past 20 to 30 years (DBE 2021d).	Tasks 1 and 2 DBE 2021c, d
Development of Simulation Models for Everglades STAs	This sub-study is ongoing. Work done so far includes (1) compilation of information relevant to STA component interactions, (2) development of a framework for a biogeochemical model, and (3) compilation of the information needed to update the Comprehensive Aquatic Ecosystem Model (CASM) to analyze food web components in the STAs.	Tasks 1 and 3 ESA, Cardno, and Coastal Ecosystems 2021
Comparison of STAs and WCA-2A Transect Water Quality Data	This sub-study is ongoing. Tasks completed include (1) compilation of literature and reports dealing with water quality in wetlands and statistical analyses, (2) compilation of monthly average inflow and outflow water quality information and internal average wetland measurements for the period of record for all STAs, (3) compiled available transect information for STA FWs and WCA-2, and (4) development of piecewise structural equation models to determine potential water quality components affecting P outflow.	Tasks 1,2, and 3

a. The tasks supported are as follows:

1. Review and summarize results from Science Plan reports and publications as well as other relevant STA reports.
2. Review and analyze STA and Science Plan study data related to P processing and removal.
3. Development of STA P models.

The Development of Simulation Models for Everglades STAs sub-study has compiled STA research that describes key interactions between STA wetland components including inflows and outflows, transport, plant communities, soils, and fauna. The sub-study reviewed essential publications and reports and identified missing data. Matrices of these direct interactions demonstrate that a substantial number of direct interactions among the components of STA wetlands have been studied and or measured. Some key interactions not identified are being studied currently in ongoing Science Plan studies such as the

Biomarker, Periphyton, and Faunal studies. The wealth of data available to develop paired component interactions is very robust and should lead to useful models.

The Comparison of STAs and WCA-2A Transect Water Quality Data sub-study has developed an extensive literature survey of wetland water quality research. In addition, it compiled transect data and extensive monthly averaged data for the period of record of each STA for inflow and outflow water quality and internal STA conditions. These data are being examined with piecewise structural equation models and other statistical techniques to understand what factors contribute to variation in TP outflow concentrations, support TP removal at low TP concentrations, and inhibit TP removal.

P REDUCTION DYNAMICS IN STA-1E, STA-2, STA-3/4, AND STA-5/6 (P DYNAMICS STUDY)

Jill King and Odi Villapando

The purpose of the P Dynamics Study is to examine the mechanisms and factors influencing outflow TP concentrations within under-performing STA FWs, which are not currently meeting the WQBEL. Using lessons learned from a previous Science Plan study (P Flux Study) completed in 2019, similar research is being conducted in selected under-performing FWs. In addition, effects of low and high flow will be evaluated. To determine the causes of underperformance, similar approaches to those used in the P Flux Study will be used to identify major P forms/storages and chemical and biological processes related to P uptake and regeneration. Historical data analysis also will be used to evaluate and compare the various characteristics of well- and under-performing STA FWs to determine causes of under-performance.

This study will address specific questions related to P removal and return as follows:

1. How does the spatial distribution of water column P differ between FWs?
2. How is the internal P profile of these FWs influenced by hydrologic conditions (no flow versus high flow)?
3. Are the differences in FW performance related to internal P loading? If so, what are the sources and mechanisms of internal P loading? Does internal P load vary with season (wet: high PLR versus dry: low PLR)?
4. How do soil and vegetation conditions influence P removal and internal loading in these FWs?

There are three major efforts in this study (**Table 5C-13**). The first effort, conducted by DB Environmental Laboratories, Inc., involves opportunistic, spatially intense water quality sampling under no flow and high flow conditions together with an assessment of vegetation and soil conditions in the outflow region of the study FWs. These measurements will occur in multiple under-performing FWs to capture the variable conditions experienced across the STAs. This effort will address several outstanding questions about STA performance:

1. Does under performance arise from ineffective removal of inflow P or from excessive internal P return (internal loading)?
2. Are there specific problematic internal zones impairing under-performing FWs?

3. Are equilibrium P concentrations higher in under-performing systems than in well-performing FWs?
4. Are equilibrium P concentrations related to soil or plant nutrient concentrations?

The second effort, conducted by the University of Florida, evaluates biotic and abiotic factors and mechanisms regulating P dynamics in the study FWs. Specific objectives of the study are to determine the following:

Spatial (inflow to outflow) patterns in basic soil physico-chemical properties

Reactive and non-reactive pools of P in floc and soils

P adsorption and desorption characteristics of soils

Potential rates of key microbial and biogeochemical processes in floc and surface soils regulating P flux from soil to the water column

Physical and chemical characteristics of particulates in the water column and floc of selected STA sites

Comparing data from under-performing systems with data from well-performing systems will improve our understanding of the external and internal drivers that regulate the performance of these STAs, especially in the lower reaches of the treatment trains.

The third effort, conducted by SFWMD, is collecting water quality data under normal STA operations during the wet and dry seasons to complement the other study efforts. Sites established from inflow to outflow will be equipped with automated instrumentation during the established sampling period to allow for continuous monitoring. Water quality parameters along with measurements of field conditions such as water depth, light, turbidity, water temperature, and dissolved oxygen, will be evaluated.

The study will be conducted over three years (2021 through the end of 2023) with SFWMD sampling beginning in February 2021. The DB Environmental Laboratories, Inc. project component began in January 2021 with historical data analysis. The University of Florida project component began in March 2021.

Table 5C-13. Status of tasks for project efforts under the P Dynamics Study.

Task	Status and Key Findings	Reports
Dynamics in the STAs (DB Environmental Laboratories, Inc.)		
Kick-off Meeting	This task is complete.	
Project Work Plan	This task is complete.	DBE 2021b
Data Analysis of Historical Performance Transition Events	This task will evaluate existing data sets to evaluate the causes of under performance and identify any unique characteristics of well- and under-performing STA cells through the review of historical performance transition events. Findings to date indicate that most under-performance events are associated with disturbance, and that SRP is an important fraction of outflow during under performance.	
Spatial Water Quality Monitoring and Vegetation Surveys.	This task is ongoing and will collect surface water samples under targeted flow conditions across a spatially distributed set of stations throughout the selected FWs. In addition, semi-quantitative vegetation surveys will document vegetation health and coverage. To date, “no flow” sampling events have been performed in STA-2 FWs 3 and 4 and STA-5/6 FW 1. “High flow” sampling events have been conducted in STA-1E Central and Eastern FWs. A high flow and no flow sampling event are planned for each study FW during the first two years of the study.	

Table 5C-13. Continued.

Task	Status and Key Findings	Reports
Soil and Vegetation Sampling and Analysis	This task is ongoing and will collect soil and vegetation in each of the study FWs once in association with a no flow event. To date, soil and vegetation sampling has been completed in STA-2 FW 4 and STA-5/6 FW 1.	
Final Data Analysis and Synthesis Report and Manuscript	This task will be completed in the third year of the study.	
Evaluation of Biogeochemical Factors and Processes (University of Florida)		
Kick-off Meeting and Project Orientation	This task is complete.	
Project Work Plan	This task is complete.	University of Florida 2021a
Soil Sample Collection	This task is ongoing and involves soil sampling from transect and benchmark sites from inflow to outflow sites in selected FWs. To date, 39 sediment cores have been taken from two transects: STA-2 FW 2 and STA-2 FW 4.	University of Florida 2021c
Soil P Fractionation	This task will assess relative proportion of reactive and stable P pools in the different soil layers and explore correlative relationships of these P pools with soil physical and chemical characteristics, and sorption and desorption characteristics.	
P Sorption or Desorption Characteristics of STA Soils	This task will determine P sorption and release properties of soils in all the study sites.	
Seasonal Assessment of Biogeochemical Indicators.	This task will document seasonal patterns of biogeochemical properties and processes at the different regions of selected FWs from samples collected in the previous task. Water-extractable nutrients, enzyme activities (phospho-monoesterase, phospho-diesterase, ß-glucosidase, and leucine aminopeptidase), microbial biomass, anaerobic respiration, and organic N and P mineralization will be evaluated.	
Characterization of Ultra-fine Particulate P in the STAs	This task will evaluate significance and composition of <0.45 micron (µm) particulate P at the inflows and outflows of the STAs and how vegetation may affect this particulate P at the outflow.	
Annual Reports and Publications	These are to be completed annually and at the completion of the study.	

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