SECTION 9: EVALUATE THE ROLE THAT ROOTED FLOATING AQUATIC VEGETATION (FAV) HAVE IN LOWERING STA TP DISCHARGE CONCENTRATIONS

9.1 OVERALL STUDY PLAN SUMMARY

The purpose of this study is to evaluate if plant communities or habitat patches (hereafter referred to as patches) in STAs with rooted floating aquatic vegetation (hereafter referred to as rooted FAV patches) can provide additional reduction in STA discharge phosphorus (P) concentrations. If warranted, this study will assess the P uptake and storage of selected rooted FAV patches in STAs as compared to selected SAV patches (hereafter referred to as SAV patches).

In general, STAs are composed of emergent aquatic vegetation (EAV) cells that provide an initial reduction in P concentrations followed by SAV cells that provide additional reduction in P concentrations. Patches of rooted FAV occur in the STAs, largely in SAV cells, that are dominated by *Nymphaea odorata* (white water lily), *Nelumbo lutea* (American lotus), and *Nuphar lutea* (spatterdock). Previous District research, field observations, and a recent mesocosm study (Chapter 5B, SFER 2015) suggest that white water lily (and possibly other species of rooted FAV) may have the potential to provide additional reduction in water column P concentrations in downstream STA cells at least under some circumstances.

A reconnaissance will be performed to identify specific rooted FAV patches and SAV patches in STAs for further study. The objective of this study is to determine if there is a benefit to fostering vegetation communities with rooted FAV because this type of vegetation may provide P reduction and storage mechanisms that SAV alone lack. If rooted FAV patches examined show advantages in P reduction relative to SAV patches, then management actions may be recommended to promote establishment of rooted FAV patches in the downstream end of STAs.

9.2 BASIS FOR THE PROJECT

Key Science Plan Question Study Addresses

- Key question 3: What measures can be taken to enhance vegetation-based treatment in the STAs and FEBs?

9.3 BACKGROUND/LITERATURE REVIEW

Numerous studies indicate that physical, chemical, and biological functions provided by rooted FAV in wetlands affect P storage and cycling and hence the P concentration in the water column (Caraco et al. 2006, Dacey 1980, Kok et al. 1990, Mawson et al. 1983). One important consideration is the role of rooted FAV in altering P transport into the water column across the soil-water interface. Rooted FAV may reduce P diffusion from the soil to the overlying water via various mechanisms. These plants in general possess efficient inner air transport system to bring atmospheric oxygen down to the roots and thereby create a local aerobic zone in the rhizosphere (Caraco et al. 2006, Dacey 1980). The oxygenated rhizosphere near the soil surface normally inhibits the diffusion of P into the overlying water column (Mawson et al. 1983). In addition, the P concentration gradient between the interstitial or porewater of the soil and the overlying water column affects the P flux or rate of release (Reddy and DeLaune 2008). The uptake of P from the porewater by FAV roots may reduce the P concentration gradient between the soil-water interface and hence reduce P flux from the soil to the water column. Further, the dense belowground biomass of rooted FAV may also physically prevent P diffusion from soil and provide a more long term storage mechanism.

Rooted FAV may directly increase P uptake (and particulate settling) via large leaf blades and long petioles as well as through decomposition of organic matter, thus improving P removal capacity (Seo, et al. 2005, 2010). The canopy structure of rooted FAV may increase resistance to flow and reduce damage to SAV by large storms with high flow rates. Moreover, the ecophysiology of rooted FAV tend to promote P retention. Rooted FAV have extensive belowground rhizomes, low above- to belowground biomass allocation, high tissue nutrient concentration, high nutrient translocation from shoots to roots, and slow turnover rates that result in greater belowground nutrient storage and retention than in vegetation without these characteristics (Chapin 1980, Chapin et al. 1982, Miao and Zou 2012). Thus, the presence of rooted FAV may have other benefits for improved water quality in the STAs. Furthermore, the presence of rooted FAV may have an impact on SAV sustainability by buffering strong winds and flows.

In contrast, SAV have very limited or no belowground roots and therefore no root storage of P. They also lack an oxidized rhizosphere to minimize soil P release to the water column. SAV have faster decomposition and nutrient turnover rates that may lead to greater P release to the water column. Overall, the ecological functions associated with P storage and cycling that SAV species lack may be complimented by rooted FAV and therefore rooted FAV patches may provide a greater reduction in P concentrations in downstream STA cells than SAV patches. However, the net effect of these rooted FAV patches on low-level P concentrations in the STAs needs to be demonstrated through field investigation.

White water lily historically and currently is a dominant species within sloughs in Everglades oligotrophic P environments where the most readily available form of P (soluble reactive phosphorus, SRP) is low and organic and particulate forms of phosphorus are relatively high (Hagerthey et al. 2008, McCormick et al. 2009, Saunders et al. 2014). Miao and Zou (2012) and Miao (2015) found that white water lily was the most efficient in accumulation of nutrients (both P and N) in its tissues, particularly belowground rhizomes among Everglades macrophytes.

Information on vegetation communities in the STAs indicates that rooted FAV thrive in certain areas. Thriving and naturally-occurring patches of white water lily occur in various SAV cells including; STA-1W Cell 5B and Cell 4, STA-1E Cell 4S, STA -2 Cell 3, STA-3/4 Cell 1B. American lotus patches are found in SAV cells; STA-1W Cell 5B, STA-1E Cell 4N and Cell 4S, STA-3/4 Cell 1B, and STA-5/6 Cell 1B, Cell 2B and Cell 3B and have been expanding quickly Spatterdock patches occur in STA-5/6 Cell 3B. In STA-1E Cell 4S SAV experienced catastrophic damage by invasive exotic apple snails but the American lotus persisted and maintained their ability to grow, reproduce, and expand. Thus, rooted FAV may be able to thrive at nutrient levels under existing ecological conditions typical of the downstream STA cells.

9.4 DETAILED STUDY PLAN AND EXPERIMENTAL DESIGN

The overall goal of this study is to evaluate the role that rooted FAV patches may play in the lower reaches of the STA treatment trains (i.e., in low-P SAV cells) in reducing outflow P concentrations to meet STA TP discharge limits. An in-situ field study will be performed in two phases (if results from the first phase warrant a second phase). The objective of Phase 1 of the in-situ field study is to compare differences between rooted FAV patches (treatment) and SAV patches (control) in terms of the concentration of phosphorus in the water column. The objective of Phase 2 of the in-situ field study, if warranted (STOP/GO) will be to continue to compare differences between rooted FAV patches (treatment) and SAV patches (control) in terms of phosphorus in the water column, as well as to compare differences in P storage in rooted FAV, SAV, floc and soil.

9.4.1 Study Plan Description

Phase 1 in-situ study

Phase 1 of the in-situ field study consists of evaluating phosphorus in the water column in rooted FAV and SAV patches to determine if there are differences in these patches. If differences exist in the phosphorus concentrations in the water column between rooted FAV patches and SAV patches then Phase 2 of the in-situ study will be implemented.

Phase 2 in situ-study

Phase 2 of the in-situ field study would consist of including an evaluation of phosphorus storage in rooted FAV, SAV, floc and soil in rooted FAV patches and SAV patches. Phase 2 is designed to determine if rooted FAV patches show advantages in internal storage of phosphorus that provide additional reduction in phosphorus in the water column.

The study aims to potentially compare several specific types of rooted FAV patches with SAV patches. This could be implemented with a typical factorial study with one factor (patch type) and several types of vegetation (several types of rooted FAV and SAV). However, all rooted FAV patches do not occur in a single SAV cell. Instead, they are distributed either in different SAV cells or at different locations in the same cell. Thus, it is not practical to apply classical factorial designs, including block design, split-plot design, and nested design for the study.

Miao and Carsten (2006) and Miao et al. (2009) addressed the design and data analysis of large-scale real-world field studies that are not appropriate for classical statistical designs due to either one or multiple issues associated with the study unit, such as replication, independence, and large spatial variation, etc. Various non-traditional study designs have been suggested and applied, including the Treatment/Control design (e.g., Miao et al. 2009). This design, usually consists of a treatment and a control, and has been proposed as early as in 1950s (Hasler et al. 1951) and continuously developed over approximately six decades (Box and Tiao 1965, 1975, Osenberg and Schmitt 1996, Pickett 1989, Stewart-Oaten 1996, Underwood 1993 and 1994).

The Treatment/Control design approach will be applied to the field study. Paired patches consisting of a rooted FAV patch (i.e., treatment) and a SAV patch nearby, without rooted FAV, (i.e., control) will be selected potentially for each type of rooted FAV patch. Each paired patch will have two replicates and each paired patch should be similar in terms of spatial coverage area (size), shape, flow direction, dominant SAV species, etc., to the extent possible.

9.4.2 Experimental Design and Study Plan Components

The study consists of the following tasks including reconnaissance and patch selection, Phase 1 in-situ field study, and if warranted, development of sampling methodology and the Phase 2 in-situ field study. Data management and analysis and reporting will be provided for each in-situ study phase.

Patch selection will be performed by the project team that will include an external contractor. The Phase 1 in-situ field study (including patch setup and field sampling) will be performed by an external contractor with input from internal project team members. If warranted (STOP/GO) sampling methodology development and the Phase 2 in-situ field study (including setup and field sampling) will also be performed by an external contractor with input from internal project team members.

Task 1 Reconnaissance and Patch Selection

Three rooted FAV plant species will be evaluated including, white water lily (*Nymphaea odorata*), American lotus (*Nelumbo lutea*), and Spatterdock (*Nuphar lutea)*. These species occur in SAV cells interspersed with various species of SAV (**Figure 1**). These patches vary widely in spatial extent, ranging from 20 m^2 to 100 m^2.


a. STA 2 Cell 3
b. STA-1W Cell 5
c. STA-1W Cell 5
d. STA-1W Cell 5
e. STA-1W Cell 5
f. STA-1ECell 4
g. STA-5/6 Cell 3B

Figure 1 Patches of white water lily (a-d) American lotus (e-f) in SAV cells, and spatterdock (g).

White water lily, American lotus, and spatterdock are found in multiple SAV cells (**Figure 2**). White water lily patches are found in western half of Cell 5B and Cell 4 of STA-1W, in south end of Cell 3 of STA-2; and in the south end of Cell 1B of STA-3/4. American lotus patches are found in Cells 4N and 4S of STA-1E, the southwest portion of Cell 5B of STA-1W, Cell 2B of STA-3/4 and in Cells 1B and 2B of STA-5. Spatterdock patches are found in Cell 3B of STA-5/6.

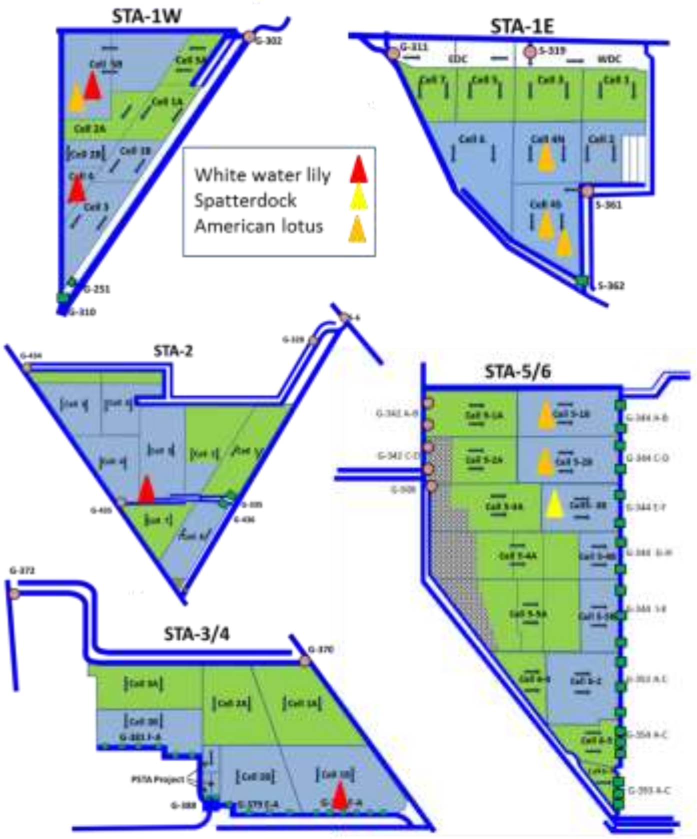

Figure 2

Figure 2 Distribution of three types of rooted FAV patches in SAV cells in STAs, including white water lily (▲) American lotus (▲), and spatterdock (▲).

Three major factors should be considered in selecting vegetation patches for the field study: size, age, and the micro-hydraulics in the area where the paired patches will be located. Patch size is important. Unfortunately, there is, currently, insufficient background information to estimate an appropriate patch size for the study. The strategy is to select patches that are as large as possible. In addition, the size of the control patch should be similar to the treatment patch.

The age of the vegetation in patches is important. This is because certain ecosystem processes (such as sediment P retention) operate, in general, at a longer temporal scale (years). Well established mature vegetation patches are more suitable for the study. There is currently information about the age of a specific rooted FAV patches in the STAs. In general, it is assumed that larger patches are older than smaller patches.

Finally, the micro-hydraulics in the study area should be considered, including any short circuiting, flow direction, and/or any apparent known differences in micro-topography. For the Treatment/Control design, the control patch should be located in the nearby area where the treatment patch is located, but distant enough to not be affected by the treatment patch. The control patch should be close enough to have a similar positioning to inflow.

Potentially, three paired patches will be selected by the project team. Airboat trips will be taken to inspect known patches. At each patch, observations will include, but not limited to 1) the size of the rooted FAV patches 2) whether there exists a nearby compatible SAV patch (control) of a similar size 3) the distance of the control patch away from the treatment patch, 4) whether the paired patches receive water flow from a similar direction (i.e. no short circuits), and 5) GPS coordinates. After the rooted FAV patches (treatment) and SAV patches (control) are selected the patches will be marked for identification.

Task 2 Phase 1 In-situ Field Study

The objective of the Phase 1 in-situ field study is to compare differences in water quality between rooted FAV patches (treatment) and SAV patches (control) in terms of phosphorus in the water column. The experimental design and Sampling Plan for Phase 1 will be developed to address measurement in the water column and will include:

- The number of samples
- Sampling regime (transect, grid, or random in a grid)
- Type of measurement (grab and/or auto-sampler)
- Sampling frequency
- Seasonality (wet and dry seasons)
- Water depth
- Measurement in the water column for P species and other water quality parameters

Tentatively, field sampling will be conducted for two seasons: a summer wet season (June, July, and August) and a winter dry season (December, January, and February).

Task 3 Sampling Methodology Development and Phase 2 In-situ Field Study

If differences exist that indicate that the concentration of phosphorus species in the water column in rooted FAV patches is lower than in SAV patches then Phase 2 of the in-situ study will be implemented if warranted. The objective of the Phase 2 in-situ field study is to compare differences between rooted FAV patches (treatment) and SAV patches (control) in terms of P storage in rooted FAV, SAV, floc and soil.

The results from Task 1 (Reconnaissance and Patch Selection) and Task 2 (Phase 1 In-situ Field Study) will be used to develop the experimental design and Sampling Plan for the Phase 2 in-situ study. This could potentially include the use of an enclosure to compartmentalize the patches. The experimental design and Sampling Plan for Phase 2 will be developed to address the following:

- The number of samples
- Sampling regime (transect, grid, or random in a grid)

- Type of measurement (grab and/or auto-sampler)
- Sampling frequency
- Seasonality (wet and dry seasons)
- Water depth measurement
- Measurement in the water column for P species and other water quality parameters.
- Measurement of rooted FAV, SAV, soils and floc:
  - Biomass
  - Nutrient content
  - Enzyme activity
  - Redox
  - Other parameters
- In addition porewater chemistry and phosphorus flux may also be measured

9.4.3 Data Management and Reporting

All data and field notes will be maintained for the project by the project study leader. All data products and associated metadata shall undergo a quality assurance/ quality control screening. All sampling procedures laboratory analyses, and documentation must comply with Chapter 62-160, FAC and the associated field sampling SOPs. Accuracy and precision in laboratory analysis will be validated and reflected in the data sets and reports. Quarterly progress reports and a final report will be prepared by the project team. A final report for each in-situ study phase will be provided with findings and recommendations.

9.4.5 Study Schedule

Phase I:	Initiate work – FY2016
	Complete work – FY2017
Phase II:	Initiate work – FY2017
	Complete work – FY2018

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