Date: 2/8/2018

Detailed Study Plan
Restoration Strategies Science Plan
Improving Resilience of Submerged Aquatic Vegetation in the STAs

Water Quality Treatment Technology
Applied Science Bureau
South Florida Water Management District

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Overall Study Plan Summary

Submerged aquatic vegetation (SAV) plays a significant role in removing nutrients, especially phosphorus (P), from the water column in downstream treatment cells of the Everglades Stormwater Treatment Areas (STAs). SAV species, especially *Chara sp.* (e.g., Muskgrass) can grow rapidly, provided the right water chemistry (e.g. inorganic carbon, low P) and adequate water levels are maintained. Sudden declines of SAV, (e.g. *Chara sp.*) have been observed in different parts of the STAs resulting in a shift from clear water to turbid water and a reduction of P removal.

The causes of such die offs have not been examined and to date no data analyses has been conducted to explain the sudden loss of SAV or the gradual transition to different species in the STAs. Understanding these sudden shifts in vegetation could provide information to improve management of the STAs leading to increased resilience of the target SAV species.

The purpose of the study is to investigate the effects of operational (e.g., water depth, flow velocity, nutrient concentration, and mineral concentration) and natural environmental conditions (e.g., seasonality, light penetration, disease, clonal stresses, competition with epiphytic periphyton) on the SAV health in the STAs. The study also will investigate allelopathic effects from *Chara sp.* that may inhibit growth of other species. SAV loss due to herbivory or extreme weather events is being addressed in another study.

The study will be conducted in two phases, with a STOP/GO decision at the end of Phase 1. The knowledge and data gaps will be addressed in Phase 1 along with the list of questions through a literature review and an analysis of the period of record STA research. This information should result in a set of significant factors to be investigated in Phase 2.

Basis for the Project

The project will address the following key SFWMD Restoration Science Strategies Plan (RSSP) Question:

- What measures can be taken to enhance vegetation-based treatment in the STAs?

And sub-questions:

- What factors improve system resilience and sustainability in SAV cells: e.g. diversity, nutrient load management, water level?
- How do water depths and soil characteristics affect sustainability of dominant vegetation?
- What is the effect of SAV compartment size on SAV sustainability?
- Will rotation of SAV and emergent vegetation cover enhance the sustainability of SAV cells?
- What are the most important drivers and stressors of Chara sustainability and resilience?

An additional question to be addressed is:

- What is the optimal growth condition for dominant SAV species in the STAs, in terms of hydrologic and hydraulic conditions, nutrient levels, and mineral content in the water column?

Background/Literature Review

The construction and operation of sizeable freshwater treatment wetlands, known as the Everglades Stormwater Treatment Areas (STAs), are mandated by the Everglades Forever Act (EFA) (Section 373.4592, Florida Statutes) and are an integral part of State and Federal efforts to preserve the remaining Everglades ecosystem (Chapter 5B, SFER, 2017). STAs are composed of open water, emergent plants, floating and submerged vegetation and have retained a significant amount of P that otherwise could have been released into the Everglades Protected Areas (Reddy et. al., 2002; SFWMD, 2017). More than half of the area in the STAs are occupied by submerged aquatic (SAV) vegetation (Pietro et al., 2006; Chimney, 2017), whereas the rest of the STA areas are dominated by emergent aquatic vegetation (EAV) interspersed with floating aquatic vegetation (FAV). SAV plays a very significant role in removing nutrients, especially P, from the water column from the downstream treatment cells of the STAs. Therefore, the long-term sustainability of SAV is a critical factor in determining the success of P removal from the impacted water coming from agricultural and urban runoff. Recent field surveys of the SAV cells documented the loss of SAV resulting in easily suspended solids in the water column, and potentially reducing the treatment efficacy of a STA cell. To date, there has been no data analyses that can explain the sudden loss of submerged vegetation or the gradual transition to different species in the STAs. Understanding these sudden shifts in vegetation could provide information to improve management of the STAs leading to increased resilience of the target SAV species.

Mechanisms of P Removal by SAV

Aquatic vegetation can reduce P concentrations of water in STAs, through particulate settling, and P uptake directly by SAV and associated periphyton. Generally, most STA flow-ways are configured so that the upstream cell is primarily EAV while the downstream cell is primarily SAV (Figure 1). An exception is STA-2 where three of the original flow-ways are configured as single cell areas with either EAV (Cell 1) or SAV-dominated (Cell 3), while Cell 2 was recently transformed into EAV-SAV community.

Generally, SAV and the associated periphyton community sequester P rapidly and store it within SAV biomass. Research indicates that SAV wetlands are more effective than EAV at removing P from the water column (Brenner et al., 2006; Knight et al., 2003; Gumbricht, 1993a; Dierberg et al., 2002; Knight et al., 2003). Kadlec and Knight (2009) indicate that SAV is more effective than EAV for phosphorus removal at low concentrations (about 100 µg/L) in a subtropical climate. However, SAV is less effective at higher phosphorus concentrations (about 1000 µg/L). An exception to these findings is the long-term performance of STA-2 Cell 1, a predominantly cattail cell with a sparse sawgrass community that has achieved period of record outflow TP flow-weighted mean (FWM) concentrations of 14 ppb compared to the adjacent SAV cell (Cell 3) that achieved 18 ppb.

A healthy SAV community within the water column allows it to remove nutrients from water effectively and minimizes the effects of hydraulic short-circuiting. When P loading into the STAs was controlled such that overloading was prevented, SAV cells with associated periphyton, demonstrated that outflow P concentrations could be reduced to below 20 ppb (Dierberg et al., 2002; Pietro et al., 2006a).

Factors Influencing SAV Growth

The literature demonstrates a tremendous variation in response of various *Chara* species to high and low P concentrations. For example, growth of *Chara globulairs* was low at TP concentrations of 15-30 µg/l (Forsberg, 1965), and growth of Chara *vulgairs* was low at TP concentrations of 10 µg/l (Hough & Putt, 1988). At high inorganic P concentrations up to 1000 mg P/m^3, Blindow (1988) found no effect on the growth of *Chara tomentosa* and *Chara hispida*. Kufel and Ozimek (1994) observed effective growth of *Chara aspera* at soluble reactive P concentrations up to 770 mg P/m^3. Laboratory experiments demonstrated that the addition of as much as 10 mg/l of NH_4^+ or NO_3^- stimulated the growth of *Chara* spp., although additions of concentrations higher than 10 mg/l of NH_4^+ reduced shoot length (Simons and Nat, 1996). Therefore, an accurate assessment and identification of the various Chara species and varieties is paramount to understanding their potential to grow under various P and N regimes.

Ceratophyllum demersum (Coontail) thrives under high alkalinity (Spence, 1967), and is a nitrophilous plant which required higher organic nitrogen levels in the surrounding water at least for a part of the year (Goulder and Boatman, 1971). Development and morphology of Coontail was not influenced by the nitrates in water column up to 105 mg/l, whereas higher concentrations of ammonia affected growth and morphology (Best, 1980). Potamogeton and Chara species are associated with high alkalinity (0.7 – 3.14 mEq of HCO_3/l) and pH (7.7 -9.6), however *Potamogeton pecttinatus*, and *Myriophyllum spicatum*, can grow in non-calcareous water of high pH. Through the STAs' operational history, large-scale losses of SAV have occurred as well as transitions to different species over time.

Water clarity is an important factor that governs the photosynthetic activity of SAV. Phillips et al. (1978) found that in exceptionally shallow lakes like the STAs, phytoplankton development may not decrease light adequately to slow the development of SAV and recommended that shading by epiphytic growth was the essential factor causing SAV decay. Other authors later confirmed their findings (Sand-Jensen and Borum, 1991, Jones et al., 1999; Roberts et al., 2003). Recent field surveys also documented the production of epiphytic growth on SAV species, however, the exact cause/timing of proliferation of various epiphytic communities in STA waters is still a mystery!

The role of dissolved organic carbon in nutrient cycling in STAs is less understood. The steady drainage loads from agricultural runoff usually results in browning of the surface water. The browning is typically the result of organic macromolecules, or humic constituents commonly referred to as dissolved organic carbon or simply DOC. Additionally, the degradation of EAV and SAV communities along the STA flow paths also produce DOC. The presence of DOC in the water column decreases the harmful effects of ultraviolet radiations (UVR), thus seen as a sunscreen that shields submerged flora and fauna from the harming impacts of UVR. The direct exposure to UVR provides both beneficial as well as cellular damaging effects to biota (Williamson et al., 2001). However, during daytime, the ultraviolet component of sunlight interacts with DOC in water resulting in the formation of chemically reactive and biologically toxic compounds including hydroxyl and alkyl-peroxyl radicals (Mill et al., 1980), superoxides, and hydrogen peroxide (H_2O_2) (Cooper and Zika, 1983). In a very recent laboratory experiment (Wolf et al., 2017), the combination of DOC and UVR produced substantial amounts of reactive oxygen species (ROS), including H_2O_2 that caused DNA damage in Daphnia. The authors believed that photo activated DOC lead the production of ROS. Most of the freshwater oxidative stress studies are performed in lakes and rivers with DOC content ranging from 3 mg/L to 20 mg/L (Häkkinen et al., 2004).

To understand the behavior of DOC at inflow and outflow structures, an analysis of period-of-record of DOC data from the STAs showed no statistically significant difference between average DOC concentrations recorded at inflow (28.9 mg/l) and outflow (25.5 mg/l) structures. The presence of high amounts of carbon in the STA waters may trigger the production of oxidative radicals peaking during the daytime. These oxidative radicals, especially hydrogen peroxide (H_2O_2), may enhance the hydrolysis and mineralization of organic matter which can release P and other nutrients to the water column.

The literature gathered from various freshwater ecosystems suggests that the STAs provide favorable conditions for the generation of oxidants and radicals, and H_2O_2 has a longer half-life and can be measured by simple methods (Copper et al., 1989b). As far as is known no quantitative data exists on the H_2O_2 production and its associated impacts on flora and fauna in the constructed wetlands of south Florida. Therefore, studying the impact and quantification of major oxidative radicals along with their half-life periods can provide further insights about the mechanism of P release and uptake at the terminal treatment cells. This information could be used to improve the SAV resiliency and optimizing STA treatment capacity to reach Water Quality Based Effluent Limits.

Conceptual STA Model

A Stormwater Water Treatment Area is composed of several P removal pathways mediated by various plant communities and their placement along the water flow (Figure 1).

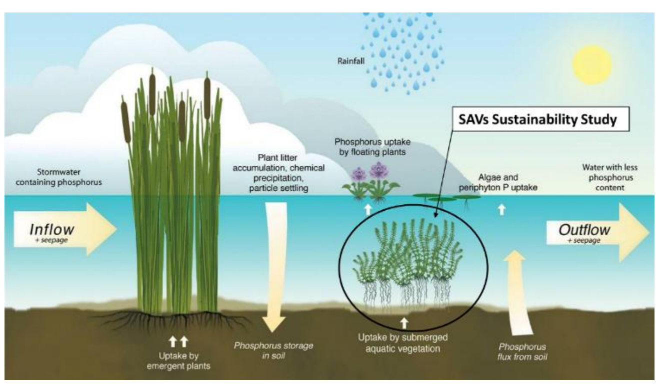

Figure 1. A conceptual model showing the role of different plant communities and their interaction with soil on Phosphorus cycling in STAs.

Study Hypotheses

The operating hypotheses for this study are:

1. Growth and sustainability of SAV are effected by operational as well as natural environmental conditions.
2. *Chara sp.* has allelopathic effects that inhibits growth and establishment of epiphytic communities.
3. Presence of residual herbicides ≥ than the lethal plant dose in STA water affects SAV growth.

Study Plan Description

The purpose of this study is to investigate the effects of operational (e.g. water depth, flow velocity, nutrient concentration, and mineral concentration) and natural environmental conditions (e.g. seasonality, light penetration disease, clonal stresses, competition with epiphytic periphyton) on the SAV health in the STAs. The study will be conducted in multiple phases, with a STOP/GO decision for continuing after each phase.

The objectives of Phase 1 will include:

- Review of literature – expand and finalize existing literature review on SAV biology and factors that influence SAV growth. Gather information on SAV community management.
- Historical SAV data analysis – analyze existing data and information, gathered through years of SAV surveys and water quality data in selected STA cells. Both vegetation maps and vegetation field surveys will be used to identify SAV affected areas and associated time periods. Evaluation of operational ranges, including hydrological conditions, water quality data, before, during and after the observed SAV decline in selected affected cells will be performed. Specifically, stage, flow, soil and water nutrient status, residual herbicide concentrations, and species coverage will be examined to determine the role of selected variables (both controllable and uncontrollable) towards the growth cycles of SAV. The overarching goal of this task is to gain understanding of SAV growth behaviors under different hydrological regimes and nutrient inputs and narrow down factors which can be used to develop research methods.
- Develop methods –propose experimental and analytical methods to answer study questions.
- Study plan development – if continuation to Phase 2 is warranted based on Phase 1 findings, develop Study Plan for Phase 2 implementation.

The Phase 2 Work Plan will be developed as a deliverable from Phase 1, and may involve experimental treatments and field surveys, measurements, and sampling. It is anticipated that the study will be conducted in selected SAV cells and/or *in-situ* mesocosms. Initially the study will focus on Chara and southern Naiad and will be planned for two and a half years. We anticipate that 2.5 yrs. would isolate some of the response variables that control SAV resiliency, however, it may take longer time to evaluate their effectiveness. The target response would be SAV growth (density, biomass), health (relative index), species turnover, and tissue and soil chemistry. During the field study we anticipate following test samples could be submitted to the laboratory for analysis:

Water samples: ammonium, nitrate, TN, TP, DOC, TDP, SRP, TDS, turbidity, LTSS, Ca, Mg, Na, inorganic N, inorganic carbon, micro-nutrients, dissolved oxygen.

SAV samples: TN, TC, TP, TCa, TMg, TNa, Ash-free Dry Weight (Loss on ignition)

Floc/marl samples: TN, TC, TP, TCa, TMg, DOC, inorganic carbon, Ash-free Dry Weight (Loss on ignition)

In-situ field measurements: weather, light penetration, water velocity, water temperature, pH.

Data Management

All water quality data produced by the District's laboratory will be loaded into DBHYDRO. The District's laboratory staff performs the necessary data verification prior to loading to DBHYDRO. Ecological data, other than water quality, will be loaded into ERDP database. The study Project Lead or designated scientist will perform data validation prior to loading into the database. Any data, metadata, figures, maps, or other related information that is not compatible with either ERDP or DBHYDRO databases will be loaded into the MORPHO database.

Data generated by the contractor will be reviewed by the District Study Lead, then loaded into either the ERDP or MORPHO. A detailed description of the data, including method of collection and experimental design, will be filed in to MORPHO.

The Applied Sciences Bureau data steward will oversee controlling the data loading and distribution.

Data Analysis

R and JMP Statistical Packages will be used for statistical analyses to determine significance of trends, relationships, and differences in effects of different variables . Spatial analyses of results, where applicable, will be done using ARC GIS software.

Reporting

The Project Lead will meet with the project team and contractors on a routine basis to ensure that the project stays on time and on budget. The Project Lead will summarize the findings and status on a quarterly basis and provide quarterly progress reports to management. Study presentations also be delivered when requested, e.g., for the District's Science Plan team, Restoration Strategies workshops, forums and Long-Term Plan meetings.

An update on the study progress and findings will be prepared for the annual South Florida Environmental Report (SFER). At the project conclusion, a final report will be prepared describing all the study components and summarizing the findings, conclusions, and recommendations.

Contractual laboratory

A majority of the analyses will be conducted at DB Environmental Laboratories as part of the current EAA-EPD contract.

Equipment

TBD

Facilities

Laboratory analyses will be done at the District lab and DB Environmental lab. *In-situ* mesocosm studies will be conducted at chosen STA cell by the DB Environmental scientists. District lab analyses will need to be approved through the EMRT process during the Phase 2 development.

Study Schedule

	7/17 – 9/17	**10/17 – 12/17**	**1/18 – 3/18**	**4/18 – 6/18**
Literature Review				
Data Analyses and Report				
Develop Methods				
Study Plan Development				

Project Deliverables

1. Extend the present literature review conducted by District scientist. District Project Lead/Scientist will provide an EndNote Literature library to the DBE staff.
2. Collect time series data from inflow and out flow structures of each STA, and from the selected STA Cells that have been historically surveyed by District and DBE.
3. Draw information from the STA vegetation surveys and provide time series maps.
4. Develop methods which will be used in Phase 2.
5. Develop study plan to be implanted in Phase 2, if warranted, after considering STOP/GO decision.

Budget and Anticipated Resource Requirements

List Functions	Skill of Functional Employees	Identify Employees	Total FTEs Required for Complete Project
Scientific Project Lead	Field monitoring/Communication Logistics	Manohardeep Josan	0.25
Technical Project Manager	Wetland biogeochemistry	Delia Ivanoff	0.1
Lead Scientist	Data analysis	M. Chimney	0.1
Principle Scientist	Technical review	T. James	0.1
Business lead	Project systems support	Kim O'Dell	0.1
Total Resource Requirements			**0.65**

Literature Cited

Best, E.P.H. 1980. Effects of nitrogen on the growth and nitrogenous compounds of Ceratophyllum demersum. Aquat. Bot. 8: 197-206.

Blindow, I. 1988. Phosphorus toxicity in Chara. Aquat. Bot. 32: 393–395.

Brenner, M., D. Hodell, J. Curtis, W. Kenney, B. Leyden, B. Gu, J. Newman. 2006. Mechanisms for organic matter and phosphorus burial in sediments of a shallow, subtropical, macrophyte-dominated lake. J. Paleolimnol. 35:129-148.

Cooper, W.J. and R.G. Zika. 1983. Photochemical Formation of Hydrogen Peroxide in Surface and Ground Waters Exposed to Sunlight. Science. 220(4598): 711-712.

Dierberg, F. E., T. A. DeBusk, S. D. Jackson, M. J. Chimney and K. C. Pietro. 2002. Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: response to hydraulic and nutrient loading. Water Res. 36: 1409-1422.

Forsberg, C. 1965. Environmental conditions of Swedish charophytes. Symp. Bot. Upps. 18: 1–67.

Goulder, R. and D.J. Boatman. 1971. Evidence that Nitrogen Supply Influences the Distribution of a Freshwater Macrophyte, Ceratophyllum Demersum. J. Ecol. 59(3): 783-791.

Gumbricht, T. 1993. Nutrient removal processes in freshwater submersed macrophyte systems. Ecol. Eng. 2(1): 1-30.

Häkkinen, P.J., A.M. Anesio, and W. Granéli. 2004. Hydrogen peroxide distribution, production, and decay in boreal lakes. Can. J. Fish. Auqat. Sci. 61(8): 1520-1527.

Hansson, L.A. 1988. Effects of competitive interactions on the biomass development of planktonic and periphytic algae in lakes. Limnol. Oceanogr. 33: 121–128.

Hough, R. A. and D. A. Putt. 1988. Factors influencing photosynthetic productivity of Chara vulgaris L. in a moderately productive hardwater lake. J. Freshwat. Ecoi. 4: 411–418.

Jones, J.I., J.O. Young, G.M. Haynes, B. Moss, J.W. Eaton, and K.J. Hardwick. 1999. Do submerged aquatic plants influence their periphyton to enhance the growth and reproduction of invertebrate mutualists? Oecologia. 120: 463–474.

Kadlec, R.H. and S.D. Wallace. 2009. Treatment Wetlands, Second Edition. Taylor and Francis Group, Boca Raton, FL.

Knight, R. L., B. Gu, R. A. Clarke and J. M. Newman. 2003: Long-term phosphorus removal in Florida aquatic systems dominated by submerged aquatic vegetation. Ecol. Eng. 20: 45-63.

Kufel, L. and T. Ozimek. 1994. Nutrient Dynamics and Biological Structure in Shallow Freshwater and Brackish Lakes. Mortensen, E., Jeppesen, E., Søndergaard, M. and Nielsen, L.K. (eds), pp. 277-283, Springer Netherlands, Dordrecht.

Kufel L and I. Kufel. 2002. Chara beds acting as nutrient sinks in shallow lakes—a review. Aquat. Bot. 72(3-4): 249-260.

Pietro, K. C., M. J. Chimney and A. D. Steinman. 2006. Phosphorus removal by the Ceratophyllum periphyton complex in a south Florida (USA) freshwater marsh. Ecol. Eng. 27: 290-300.

Pietro, K., R. Bearzotti, M. Chimney, G. Germain, N. Iricanin, T. Piccone, and K. Samfillippo. 2006a. Performance and optimization of the Everglades stormwater treatment areas. In: 2006 South Florida Environmental Report. South Florida Water Management District, West Palm Beach. p. 5-1 to 5-85.

Phillips, G.L., D. Eminson, and B. Moss. 1978. A mechanism to account for macrophytedecline in progressively eutrophicated freshwaters. Aquat. Bot. 4: 103–126.

Reddy, K. R., J. R. White, Y. Wang and J. Newman. 2002. Stability and Bioavailability of Recently Accreted Phosphorus in Stormwater Treatment Areas. pp. 100. University of Florida, Gainesville, FL.

Roberts, E., J. Kroker,S. Körner, and A. Nicklisch. 2003. The role of periphyton during the re-colonization of a shallow lake with submerged macrophytes. Hydrobiologia. 506: 525–530.

Sand-Jensen, K. and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. 41: 137–175.

SFWMD. 2013. Restoration Strategies Regional Water Quality Plan: Science Plan for the Everglades Stormwater Treatment Areas. South Florida Water Management District, West Palm Beach, FL. June 2013. Available online at www.sfwmd.gov/rs_scienceplan.

Simons, J. and E. Nat. 1996. Past and present distribution of stoneworts (Characeae) in The Netherlands. Hydrobiologia. 340(1): 127-135.

Spence, D.H.N. 1967. Factors controlling the distribution of freshwater macrophytes with particular reference to the Lochs of Scotland. J. Ecol. 55(1): 147-170.

Valley R.D., W. Crowell, C.H. Welling, and N. Proulx. 2006. Effects of a Low-dose Fluridone Treatment on Submersed Aquatic Vegetation in a Eutrophic Minnesota Lake Dominated by Eurasian Watermilfoil and Coontail. J. Aquat. Plant Manage. 44: 19-25

Williamson, C.E., P.J. Neale, G. Grad, H.J. De Lange, and B.R. Hargreaves. 2001. Beneficial and detrimental effects of UV on aquatic organisms: implications of spectral variation. Ecol. Appl. 11(6): 1843-1857.

Wolf, R. T. Andersen, D.O. Hessenand, and K. Hylland. 2017. The influence of dissolved organic carbon and ultraviolet radiation on the genomic integrity of Daphnia magna. Funct. Ecol. 31(4): 848-855.