Task 2. Project Work Plan

Investigation of the Effects of Abundant Faunal Species on P Cycling in the Everglades Storm Water Treatment Areas (STAs)

I. BACKGROUND AND CONCEPTUAL MODEL

The Science Plan for the Everglades Stormwater Treatment Areas was established to investigate the critical factors that collectively influence STA performance and to fulfill the requirements of the Consent Orders between the Florida Department of Environmental Protection (FDEP) and the South Florida Water Management District (District) associated with the National Pollution Discharge Permits (NPDES) and Everglades Forever Act (EFA) Permits for the Everglades STAs (SFWMD, 2013). An important project associated with the scientific investigations addressed in the Science Plan is the evaluation of phosphorus sources, forms, flux and transformation processes in the STAs. This project addresses the following key and sub-questions:

Can internal loading of phosphorus to the water column be reduced or controlled, especially in the lower reaches of the treatment trains?

What are the sources (internal/external, plants microbial, wildlife), forms, and transformation mechanisms controlling the residual P pools within the different STAs and are they comparable with what is observed in the natural system?

Can the biogeochemical or physical mechanisms be managed to further reduce soluble reactive, particulate and dissolved organic P concentrations at the outflow of the STAs?

The detailed study plan (DSP) indicates that this project is comprised of multiple independent but interconnected study components. Collectively, they are designed to generate quantitative information pertinent to STA function and performance optimization at low phosphorus concentrations. One component of the project focuses on the potential role of aquatic fauna on STA performance.

The DSP also outlines key and sub-questions that specifically relate to the influence of wildlife on STA performance. These include:

What is the influence of aquatic animals, especially fish, on the reduction of phosphorus in the STAs?

- Do fish and macroinvertebrates affect P import, export, or nutrient cycling within the system enough to alter outflow TP concentrations?
- What rates of TP cycling can be expected in the STAs from fish and macroinvertebrates? How significant is faunal recycling to ambient P turnover?
- What is the form and availability of excreted TP for the dominant faunal components?
- How does the grazing of submerged aquatic vegetation (SAV; e.g., hydrilla) affect STA functionality? What are the effects of herbivory in terms of SAV growth, health, biomass, and TP uptake?

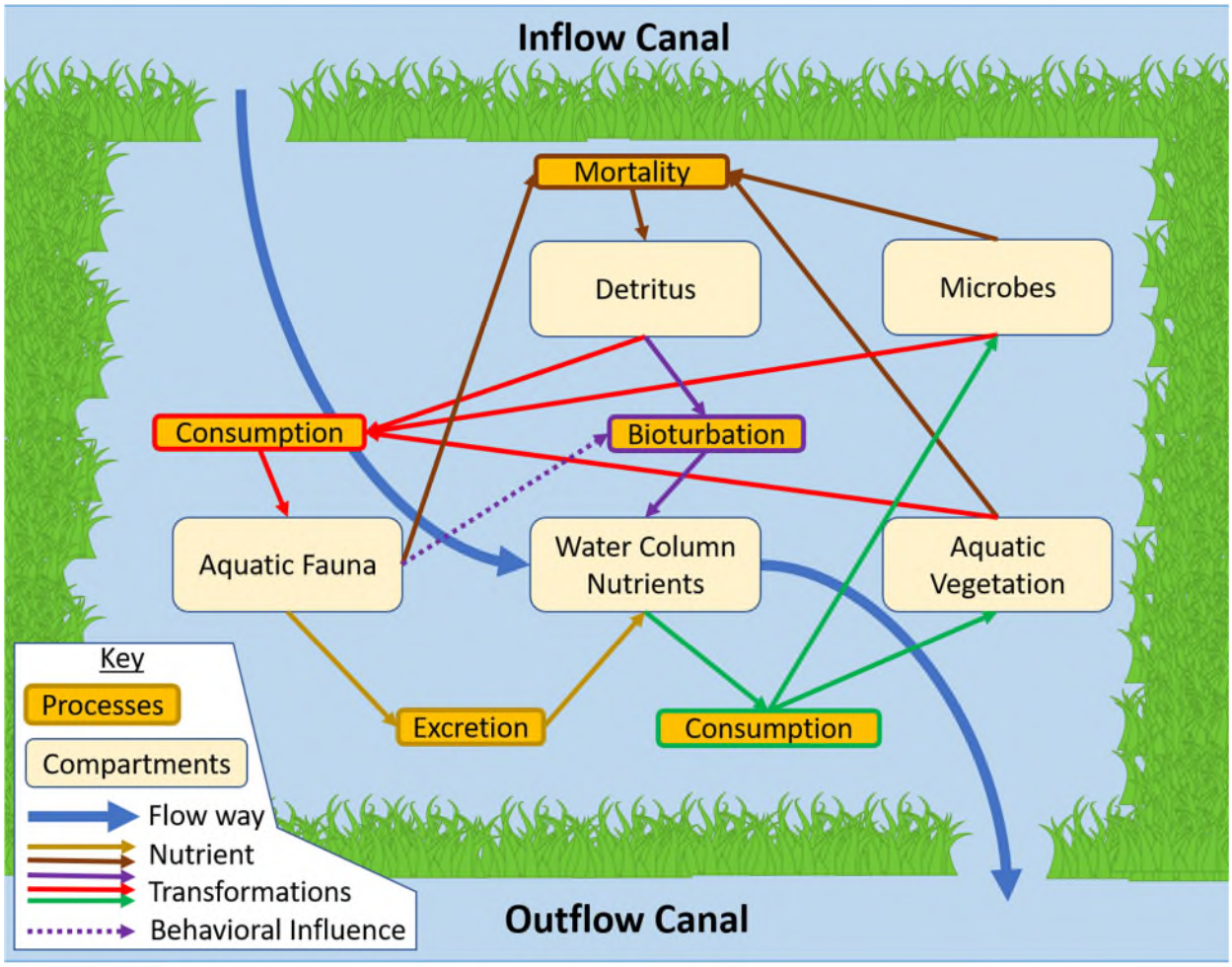


Figure 1. A conceptual model of the potential roles of aquatic fauna in nutrient cycling in the STAs. The large squares represent the five main nutrient compartments in the STAs: Detritus, Microbes, Aquatic Fauna, Water Column Nutrients, and Aquatic Vegetation. Arrows represent

processes of nutrient transformation between nutrient compartments, and are color coded for each type of process: consumption (red), bioturbation (purple), decomposition (brown), excretion (yellow), photosynthesis (green).

Aquatic animals are increasingly recognized as important in nutrient cycling in aquatic systems (Vanni 2002). The abundance and mix of aquatic animals can affect water column nutrient concentrations via multiple different pathways. First, they can do so directly by mobilizing nutrients stored in animal or plant material through consumption and excretion. This is important because it fundamentally alters the nature of biological materials and rates of nutrient cycling by converting organic P from body tissues into a soluble, more labile form that is excreted into the water column. This process may be particularly important to water-column nutrient levels when the phosphorus originates from benthic or detrital sources because this can serve as a 'new' source of phosphorus (Vanni et al. 2006). In this respect, the large populations of benthivorous fishes within the STAs such as Tilapia and Catfish could be important for transport stored phosphorus from the sediments into the water column via excretion. Second, aquatic fauna can have important indirect effects through modifications of the environment (e.g. bioturbation; Vanni 2006) and by top-down effects involving predator limitation of prey/food resources and cascading effects (Dorn 2013, Kellog and Dorn 2012). Top-down effects may impact nutrient cycles if they impact the abundance of key grazers or bioturbators, altering the efficiency of animal mediated nutrient cycles. A potentially important top-down effect in the STAs is faunal consumption and removal of SAV. For example, a strong effect was recently evident in an outflow cell of STA-1E when the rapid population growth of a non-native apple snail considerably reduced SAV biomass and STA performance. Finally, aquatic animals can act as important P- and N- sinks and vectors of P- and N-transport, especially by large, mobile animals such as fish and crocodilians.

Given the potential pivotal role of aquatic fauna in STA nutrient cycling, a pilot study (2015) and the first two years of sampling (2017-18) have already been completed (Work Order: 4600003032-WO01, WO01R1, and WO03), as part of the Restoration Strategies Science Plan, to explore the role of aquatic animals on nutrient cycling and P transformations and to determine whether their effects are relevant to STA functioning. Specifically, this involved quantifying aquatic animal assemblages in STA outflow cells, and estimating the contribution of select species to P- and N-loading through excretion and bioturbation experiments.

Results from this study have shown that many species (e.g. small fish species, grass shrimp, and benthic feeding large-bodied fishes) are exceptionally abundant in the STAs, and far exceed those in neighboring Everglades marshes. These faunal populations have the potential to affect P- and N-cycling by changing P and N concentrations in the water column, by transforming P and N to more labile/recalcitrant forms, by transporting P and N to areas where it has significant impacts on STA functionality, and by consuming significant quantities of SAV. Understanding these affects will require quantifying 1) the contributions of additional key species to P- and N-loading through excretion and bioturbation, 2) the effects of fish herbivory

on SAV growth and biomass, and 3) the redistribution of P within the outflow cells through faunal movements.

Data from the past work also revealed that fish and invertebrate distributions are highly patchy in space and time compared to those of other wetlands in south Florida. Furthermore, SAV communities also show great spatiotemporal variation. Given the high degree of variation of faunal and infaunal communities, sampling efforts need to be continued to improve and maintain relevance of areal estimations for parameterizations of P-budgets.

It was also determined that certain non-native fish species (e.g., Tilapia) were considerably less abundant in the electrofishing data than expected given their observed abundances in the STAs. Some undersampling was expected, but the observed high abundance of Tilapia in the STAs and their potential importance in nutrient cycling suggests that further understanding of this sampling bias is required.

II. OBJECTIVES

The primary goals of this study are to quantify the areal biomass and community compositions of fishes and other aquatic fauna and their effects on water quality in the outflow cells of the STAs. Specifically, the following data shall be collected: 1) biomass (kg ha^{-1}) and community composition of small-bodied fish (<8 cm standard length) and macroinvertebrates (e.g. crayfish, gastropods) in STA outflow cells for two years in the spring, summer and autumn, 2) biomass (kg ha^{-1}) and community composition of large-bodied fish (>8 cm standard length) in STA outflow cells for two years in spring, summer and autumn, 3) estimates of electrofishing sampling bias of non-native fishes using controlled densities of fishes, 4) estimates of mass-specific P excretion rates of abundant species, 5) experimental estimates of enhanced water column TP caused by faunal bioturbation, 6) experimental estimates of SAV herbivory by aquatic herbivores, and 7) (dependent upon STOP/GO conditions) movement and distribution data of most abundant large fish species using radio tracking to understand faunal mediated P-redistribution. Faunal biomass, stoichiometry and excretion results will be combined to estimate areal (per ha) P consumption and excretion by the entire faunal community. Faunal community uptake and release of P will be compared to relevant external sources and uptake of P such as inflow load, load from soil to water column, and P sequestration in the macrophyte biomass. Bioturbation and herbivory estimates will be used to evaluate the potential of aquatic animals to alter the efficiency of benthic sequestration of TP that may be included in future P budgets. Management recommendations shall be provided based on the results of the above studies.

III. SCOPE OF SERVICES

This Work Order requires considerable expertise and experience in: 1) Everglades aquatic food web and community ecology, 2) population dynamics of Everglades fish and

macroinvertebrates, 3) a general understanding of how aquatic fauna mediate nutrient cycling in aquatic environments, 4) predator-prey interactions and cascading effects among assemblages of snails, crayfish (snail predators), large-bodied fish, 5) aquatic faunal sampling design and implementation, 6) experimental design, and 7) advanced statistical analysis, interpretation and synthesis of data. The work will be performed by Florida International University (Hereafter referred to as UNIVERSITY)

To accomplish the project goals the UNIVERSITY shall:

a) Quantify 1) large-bodied fish and 2) small-bodied fish/ macroinvertebrate density and biomass, in STA outflow cells following methods developed in Work Orders 4600003032-WO01, WO01R1, and WO03.

b) Develop and conduct a field study to calibrate the electrofishing technique for abundant non-native fishes.

c) Measure rates of P release from excretion and bioturbation for dominant taxa using methods developed in Work Order 4600003032-WO03.

d) Develop and conduct an experimental study to quantify faunal herbivory.

e) Calculate areal P excretion estimates of faunal communities.

These objectives shall be accomplished by a combination of field sampling, field experimentation, laboratory processing, and analyses. Sampling sites will be the same as those used in Work Order 4600003032-WO01, WO01R1, and WO03 plus the addition of sampling outflow cells in STA-3/4 for quantifying large-bodied fish. Specific requirements associated with each objective are described in detail in section IV below. Deliverables will be stored by the District on the RSSI server and data will be uploaded and stored within ERDP.

IV. WORK BREAKDOWN STRUCTURE

Task 1. Project Kick-off Meeting

The UNIVERSITY Principle Investigator and other key scientists involved with the project shall attend a project kickoff meeting with the District project team within three weeks after issuance of this Work Order. During the kickoff meeting, specific details regarding the study, timelines, project deliverables, and expectations will be discussed. Contact information for key personnel and their roles and responsibilities from both the UNIVERSITY and the District project teams shall be provided during the kick-off meeting.

Deliverables

A draft memorandum summarizing minutes of the kick-off meeting and a list of action items for both the District and the UNIVERSITY.

Final memorandum summarizing minutes of the kick-off meeting and a list of action items for both the District and the UNIVERSITY.

Task 2. Project Work Plan

The UNIVERSITY shall develop a Draft Project Work Plan in accordance with the project objectives and discussions at the kickoff meeting. District staff shall review and provide comments within two weeks following receipt of this Draft Project Work Plan. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Project Work Plan within two weeks of receiving such comments. The Draft Project Work Plan must include, at a minimum, the following:

- Project description and objectives
- Detailed description of tasks including sampling approaches/methodologies and deliverables, including any relevant literature supporting the proposed methods
- Detailed experimental design of the herbivory experiment, SOPs- including sampling approaches/methodologies and deliverables, including any relevant literature supporting the proposed methods
- A comprehensive breakdown of the services required by the District laboratory for each sub-study in the project (study parameters, number of samples, type of analyses for each sample, and expected schedule of delivery). A contingency plan and cost estimate for the laboratory work shall also be provided in the event that the District laboratory is unable to fully support the project's analytical needs.
- Conceptual model of the potential roles of aquatic fauna in STA nutrient cycling
- SOPs, analytical methods and data quality objectives
- Project Management information detailing the staffing arrangements, roles, and responsibilities
- Schedule of activities
- UNIVERSITY's contingency plan in case of staff turnover
- UNIVERSITY's QA/QC procedure to ensure that the quality of staff work is acceptable and all project deliverables are correct and accurate
- A copy of all necessary permits for collecting and handling animals

The District approved Final Project Work Plan shall become the binding document for this Work Order. Any changes to the Statement of Work will be incorporated in a work order revision executed by the parties.

Deliverables

Draft Project Work Plan

Final Project Work Plan

Task 3. Quantify faunal biomass and community composition in STA outflow cells

Quantification of the biomass and composition of: 1) large-bodied fish and 2) small-bodied fishes and macroinvertebrates shall follow the same sampling approaches developed and employed in Work Orders 4600003032-WO01, WO01R1, and WO03. Sampling will be focused on the same outflow (SAV) cells in STAs -1W, -1E, -3/4, and -2 from the previous Work Order. Throw-trap sampling will be continued in STA 2, while electrofishing will be conducted in all four STAs. Sampling will occur during the spring, summer and fall over two calendar years.

Deliverables

The UNIVERSITY shall prepare Draft Task Reports that summarize the aquatic faunal community and biomass results for each sampling year. The Draft Task Reports will include Introduction, Methods, Results, and Discussion sections, and all data, statistical output, tables and figures from the task. Data relevant to this task should be provided in a tabular format within Microsoft Excel. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses. District staff shall review and provide comments within one week following receipt of each Draft Task 3 Report. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Task 3 Report within two weeks of receiving such comments.

Draft Task 3 Report for year 1

Final Task 3 Report for year 1

Draft Task 3 Report for year 2

Final Task 3 Report for year 2

Task 4. Measuring animal impacts on P turnover via bioturbation

Impacts of bioturbation on water column TP and TN were assessed during fall and spring in the past work orders. However, our original fall work (2018) was preliminary as methods were improved. Our spring 2019 experiments provided our first dataset for all species targeted by

this effort. We will repeat the fall experiment in 2019 using methods developed in 2018 to yield data from the wet and dry seasons. Blue Tilapia (Oreochromis aureus), Largemouth Bass (Micropterus salmoides), and Sailfin Catfish (Pterygoplichthyes spp.) are used in this work; Blue Tilapia and Largemouth Bass were chosen because of their high abundance in STA fish surveys, and Sailfin Catfish were chosen for their known potential for bioturbation. We stock field enclosures (approx. 2.25 m^2) with three densities of each target species (0, average density for the area, max recorded density for the area).

Power analyses using within treatment means and standard deviations (SD) from previous trials of the bioturbation experiment suggest that the study design suffered from low statistical power because of low replication. We used a power analysis to estimate the sample size needed to yield the observed mean differences between treatments to significant at the P=0.05 level using the lowest and highest within-treatment standard deviations (SD) from our experimental trials. When the highest within-treatments SD was used, the power analyses suggested that the total sample size needed was substantially higher than the previously employed 9 (3 per treatment). When the lowest within treatments SD was used, the power analysis suggested a total sample size close to or lower than the proposed 27 per species (3 treatments with 9 replicates). For example, the activity of Largemouth Bass had the smallest impact on water column P concentrations of the three species, and yet their per capita influence in the 2.25 m^2 enclosures was to increase water column P by 0.0033 mg/L (15% of the control P = 0.0220 mg/L). When the highest within treatment SD (0.0066 mg/L) was used, the power analysis suggested a total sample size of 87 (29 per treatment). However, when the same analysis was done using the lowest SD (0.0033 mg/L), the suggested total sample size is 27 (9 per treatment). It is likely that increasing the per treatment sample size from 3 to 9 will decrease the within-treatment variance to a sufficient level to yield statistical significance for a treatment effect equivalent to 15% increase of the mean. Largemouth Bass had the lowest per capita effect size (15%) compared to Blue Tilapia (29%) and Sailfin Catfish (54%), and power analyses for each suggested sample sizes smaller than those illustrated above for Largemouth Bass. In response to these results we propose a modified experimental design to maximize replication.

This new experimental design will use 27 enclosures for each species (9 per treatment). In order to accomplish this with similar funds as the previously proposed methods, we propose shortening each experiment to two weeks. Previous results showed no significant difference between weeks nor a trend in concentrations of nutrients beyond the second week. Furthermore, in order to minimize contamination and artificial bioturbation (by us rather than the fish) we will only sample floc, water, and periphyton from plastic patches attached to walls inside the enclosures prior to the start of the experiment (day 0) and at completion (day 14). Periphyton from periphytometers (Chick et al 2009) will be collected at completion of the experiment (Day 14).

Comparison of District Laboratory Services for proposed and accepted methods

Proposed: 3 species * 27 enclosures * (3 water samples + 2 floc samples + 2 periphyton samples + 1 biofilm sample = 8 samples per enclosure) * 2 analytes (TP and TN) + 27 equipment blanks = **1,296 analyses + 54 EB analyses**

Previously Accepted: 3 species * 9 enclosures * (5 water samples + 5 floc samples + 3 periphyton samples + 2 biofilm samples = 15 samples per enclosure) * 2 analytes (TP and TN) + 12 equipment blanks = **810 analyses + 24 EB analyses**

Deliverables

The UNIVERSITY shall prepare a Draft Task Report that summarizes the impact of bioturbation in recycling P from the STA benthos. The Draft Task Report will include Introduction, Methods, Results, and Discussion sections, and all data, statistical output, tables and figures from the task. Data relevant to this task should be provided in a tabular format within Microsoft Excel. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses. District staff shall review and provide comments within one week following receipt of the Draft Task 4 Report. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Task 4 Report within two weeks of receiving such comments.

Draft Task 4 Report

Final Task 4 Report.

Task 5. Measuring animal impacts on P turnover via excretion

Excretion rates will be measured on aquatic fauna using short-term incubations based on the methods developed in Work Order 4600003032-WO03 (i.e., methods comparable to Schaus et al. 1997, Torres and Vanni 2007, Capps and Flecker 2013). During that work order, we completed winter (January) excretion incubations for the most abundant species (Blue Tilapia, Largemouth Bass, Sailfin Catfish, Eastern Mosquitofish (Gambusia holbrooki), Bluefin Killifish (Lucania goodei), and Sailfin Molly (Poecilia latipinna)). Because nutrient regeneration can also differ by temperature (Higgins et al. 2006), incubations shall be conducted for the same species in summer (July/August). In addition, incubations for 4 additional abundant species will be conducted to improve community-wide estimates of nutrient regeneration through fish excretion: Mayan Cichlids (cichlasoma europthalmus), Florida Gar (Lepisosteus platyrhincus), Bowfin (Amia calva), and grass shrimp (Palaemonetes paludosus). Water samples will be collected from the field, filtered, and used to estimate dissolved P and N before and after 1 h incubations in bags (0.26 and 7 L containers) following Schaus et al. (1997) and Capps and Flecker (2013) and modified for work in South Florida. No-animal control bags will be conducted on the same days and with the same protocol as fish incubation bags. Water samples will be stabilized and delivered to the water quality lab of the District for quantification of P and N concentration (TP, TDP, TN, and NH_4-N). Dissolved oxygen (DO) will be measured at the start and end of each experimental trial. A second set of trials will be conducted in oligohaline regions of WCA 2A to provide a baseline for interpreting fish contributions to STA ecological P cycling.

Deliverables

The UNIVERSITY shall prepare a Draft Task Report that summarizes impacts of excretion in recycling P into the water column. The Draft Task Report will also discuss the role of aquatic faunal excretion rates for individuals of key species in shaping the P budget and efficiency of P retention in the STAs. The Draft Task Report will include Introduction, Methods, Results and Discussion sections, and all data, statistical output, tables and figures from the task. Data relevant to this task should be provided in a tabular format within Microsoft Excel. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses. District staff shall review and provide comments within one week following receipt of the Draft Task 5 Report. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Task 5 Report two weeks of receiving such comments.

Draft Task 5 Report

Final Task 5 Report

Task 6. Correcting for electrofishing sampling bias

The CPUE electrofishing method of Task 3 requires appropriate calibration to estimate non-native fish biomass per unit area (kg ha^{-1}). Ideally, we will use replicated ponds that can be stocked with a known density of key species; Blue Tilapia and Mayan Cichlids to represent non-native species known to be under-represented in electrofishing samples, and Florida Gar, Largemouth Bass, and a sunfish species to represent native species already documented to be well-sampled (Chick et al. 1999). This design will permit regression of catch versus 'true' density as illustrated in Dorn et al. (2005). If ponds are not available, two alternative approaches will be evaluated for completion of this task. If block nets are available as used in Chick et al. (1999; enclosed 1 Ha of marsh), we will use them to create enclosures that can be stocked as planned for ponds. If ponds and block nets are not available, we will locate an isolated canal or marsh habitat that can be sampled thoroughly by electrofishing. Over a 10 day period, we will repeatedly collect, mark, and release all targeted taxa that are captured. At the end of this period, a large seine net or comparable sampling method will be used to exhaustively sample and determine the relative abundance of species present. We will use Closed Capture statistical models available in programs such as MARK (White 2007; see also https://sites.warnercnr.colostate.edu/gwhite/analysis-marked-animal-encounter-data/) to estimate population size from the capture-recapture data. These estimates will be used to create relative abundance estimates of the species that can be compared to estimates from the end-of-project exhaustive sample; deviations in these relative abundances are indicative to electrofishing bias and permit estimates of relative capture efficiency. The finalized methodological design for correcting for electrofishing sampling bias will be described in detail in the project Work Plan (Task 2) based on availability of STA experimental ponds, block nets, or other study areas.

Deliverables

The UNIVERSITY shall prepare a Draft Task Report that summarizes the electrofishing calibration results. The Draft Task Report will include Introduction, Methods, Results, and Discussion sections, and all data, statistical output, tables and figures from the task. Data relevant to this task should be provided in a tabular format within Microsoft Excel. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses. District staff shall review and provide comments within one week following receipt of the Draft Task 6 Report. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Task 6 Report within two weeks of receiving such comments.

Draft Task 6 Report

Final Task 6 Report.

Task 7. Herbivory experiment

Several of the aquatic faunal species common in the STAs are herbivorous and have the potential to affect nutrient dynamics by reducing standing crops of submerged aquatic vegetation. The most abundant large fishes (>8cm maximum length) in the STAs that include plant matter (live vascular plants) in their diets are the non-native Nile Tilapia (e.g., Khallaf and Alne-na-ei 1987) and Blue Tilapia (e.g., McDonald, 1987; Zale & Gregory, 1990). Two small fish species (< 8 cm maximum length) are substantially or obligately herbivores or detritivores. Sailfin Mollies are the third most abundant small fish in the STAs and Flagfish are the fifth most abundant and have been shown to be herbivorous or detritivorous, or both by gut content analysis (Loftus 2000) and molecular diet markers (Belicka et al. 2012; Sanchez and Trexler 2018). The most abundant macroinvertebrate, the Ramshorn Snail, is an herbivorous scraper that is also abundant in the Everglades ecosystem (Williams and Trexler 2006; Ruehl and Trexler 2011, 2015). Grass Shrimp are abundant in the STAs and may include some algae in their diets, but they are primarily carnivores based on molecular analysis (stable isotopes and fatty acids: Williams and Trexler 2006; Belicka et al. 2012) and feeding studies. Grass Shrimp have been shown to contribute to nutrient regeneration, possibly because of their mode of feeding on inhabitants of periphyton mats (Geddes and Trexler 2003).

The impact of herbivory will be examined experimentally by establishing the same 2.25-m^2 enclosures used for the bioturbation study (Task 5) in areas with emergent vascular plants. Blue Tilapia will be stocked at ambient density in nine enclosures and nine will be left without Blue Tilapia addition to serve as controls. For a third set of nine, the plastic sheets used to enclose fish will be replaced with a 2.54-cm wire mesh that excludes large fish but permits small fish and invertebrates to enter the enclosure (Dorn et al. 2006; Chick et al. 2008). A single run of this experiment will use 27 enclosures (3 treatments x 9 replicates). The species composition, density, and biomass of emergent vegetation at the start and end of each

experiment, duration 3 weeks, will be recorded to determine effects of fish on vascular plant standing crop. Plastic-strip periphytometers will be employed in the enclosures, as used in the bioturbation study, to document biofilm grazing, which may be elevated in the treatments excluding large fish, but permitting small ones to recruit. Tilapia enclosures will be electrofished to remove the fish at the end of the experiment. The Tilapia will be euthanized to permit analysis of their gut contents. Following capture of the Tilapia, two 1-m^2 throw trap samples will be collected in each enclosure to document the small fish and macroinvertebrates present. This experiment will be replicated twice in May-June, once in 2020 and again in 2021, in areas with high density of vascular plants typical of STA 2; once in an area with dense Chara sp and another time in an area with Potamogeton illinoensis. The location and vegetation type will be determined at the project kick-off meeting.

The resulting data will include biomass and nutrient status of vascular plants and biofilms, including periphyton, for treatments with large Blue Tilapia present, large Blue Tilapia absent but small fish and macroinvertebrates present, and large Blue Tilapia absent and elevated small-fish and macroinvertebrate density. By comparing the plant and biofilm standing crops among these treatments, the per capita impact of large Blue Tilapia and the community of small fish and macroinvertebrates on autotrophic biomass can be estimated. Vascular plant, periphytometer biofilm, and benthic floc stoichiometry (C, N, and P) will be measured at the start and end of these experimental trials, both from in the enclosures and from nearby reference sites.

Deliverables

The UNIVERSITY shall prepare a Draft Task Report that summarizes impacts of herbivory in on vascular plant and algae standing crops. The Draft Task Report will include Introduction, Methods, Results and Discussion sections, and all data, statistical output, tables and figures from the task. Data relevant to this task should be provided in a tabular format within Microsoft Excel. All statistical output and code shall be provided with sufficient notation to allow for replication of analyses. District staff shall review and provide comments within one week following receipt of the Draft Task 7 Report. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Task 6 Report two weeks of receiving such comments.

Draft Task 7 Report for Year 1

Final Task 7 Report for Year 2

Task 8. Scaling up the P budget parameters, Final Report and Presentation

The UNIVERSITY shall submit for District review and approval, a Draft Final Project Report summarizing all work performed and all technical data collected from all study components. The UNIVERSITY shall analyze and integrate data from Tasks 3, 4, 5, 6, and 7 from the current contract, as well as from previous Work Orders 4600003032-WO01, WO01R1, and WO03, and provide meaningful interpretation of the data with emphasis on the implications of communities for short-term and long-term performance of the STA.

This shall include integrating species-specific areal biomass and excretion rate data to generate estimates of total community excretion estimates. Areal estimates of P resuspension based on field density and mesocosm results will also be obtained to scale up mesocosm findings. These estimates, in $\mu g\ P \bullet ha^{-1} \bullet$, shall be compared with concentrations in the surface water as well as changes across the STA cells (input and output values) to consider the magnitude of the source of P internal recycling compared with external sources and change in the P concentration as the water moves through the cells. The calculated P excretion rates and resuspension rates will be used to parameterize P-budgets for the STAs.

The UNIVERSITY shall discuss the relevance of these results in terms of STA management. The Final Project Report shall include at a minimum the following: Introduction, Methods, Results and Statistical Analyses, Discussion, and Conclusions sections with documentation of any associated problems or constraints.

The District's project team will review the Draft Project Report and provide comments and edits to the UNIVERSITY within two weeks of receipt of the Draft Project Report. The UNIVERSITY shall also present final project results to District staff. The UNIVERSITY will revise the Draft Project Report based on written comments from the District and submit the Final Project Report prior to the end date of this contract.

The UNIVERSITY is not required to prepare manuscripts for publication as part of this contract. However, should the UNIVERSITY decide later to use the collected data for publication, any manuscripts shall be jointly prepared and published with relevant District staff.

Deliverables

Draft Final Project Report

Final Project Report

Presentation of the results of the Final Project Report to the District

VI. QUALITY ASSURANCE PLAN

University Staffing Contingency and Training: FIU has a large staff of technicians with training in throw-trap sampling and electrofishing in the Trexler lab that can be called upon to complete tasks in this project if the original staff are unable to continue the work. The lab currently has six full-time technicians who routinely conduct these tasks for other Everglades projects. The lab has two additional post-doctoral research associates, one with experience in electrofishing and the other with experience collecting and processing samples for stoichiometric analysis. This staff includes personnel who have recently completed experimental studies of a similar nature to those in the project for other Everglades work, notably the DECOMP Physical Model project. The laboratory maintains SOP documents for the specific projects in this contract that can be accessed by these staff and implemented efficiently. FIU has several Principal Investigators with extensive experience in managing similar project to this one, should PI Trexler become unavailable. These personnel include Drs. Gaiser, Rehage, and Fourqurean, who are experienced in working with SFWMD projects.

QA/QC Plans: See accompanying document.

VII. MANAGEMENT

Principal Investigator: Dr. Nathan Dorn (formerly Dr. Joel Trexler)

Roles and Responsibilities:

- Financial management
- Overseeing experimental design, data analysis, and productions of deliverables.

Co-PI: Dr. Janelle Goeke (formerly Dr. Mark Barton)

Roles and Responsibilities:

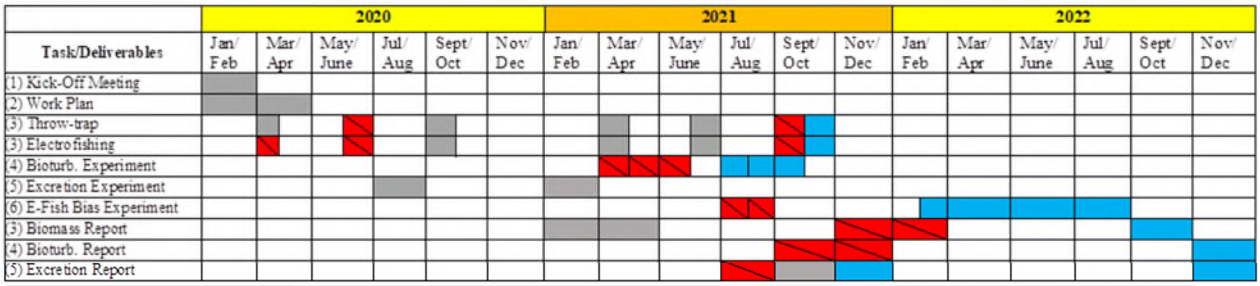
- Experimental design and implementation
- Leading/Overseeing field work and sample processing
- Data analysis
- Production of deliverables

Lead Technician: Ariana Jonas (formerly Matthew Lodato)

Roles and Responsibilities:

- Overseeing/leading field work and samples processing if Co-PI is unavailable.

VIII. SCHEDULE OF ACTIVITIES



Task/Deliverables	2020						2021						2022					
	Jan/ Feb	Mar/ Apr	May/ June	Jul/ Aug	Sept/ Oct	Nov/ Dec	Jan/ Feb	Mar/ Apr	May/ June	Jul/ Aug	Sept/ Oct	Nov/ Dec	Jan/ Feb	Mar/ Apr	May/ June	Jul/ Aug	Sept/ Oct	Nov/ Dec
(1) Kick-Off Meeting																		
(2) Work Plan																		
(3) Throw-trap																		
(3) Electrofishing																		
(4) Bioturb. Experiment																		
(5) Excretion Experiment																		
(6) E-Fish Bias Experiment																		
(3) Biomass Report																		
(4) Bioturb. Report																		
(5) Excretion Report																		

Task/Deliverables	2023						2024				
	Jan/ Feb	Mar/ Apr	May/ June	Jul/ Aug	Sept/ Oct	Nov/ Dec	Jan/ Feb	Mar/ Apr	May/ June	Jul/ Aug	Sept/ Oct
(6) E-Fish Bias Report											
(7) Herbivory Experiment											
(8) Final Report											
(9) SFER/Manuscript											

Red cells are sampling events that have been removed from the previous version. Red cells that are crossed out were directly impacted by the covid-19 pandemic, whereas the remaining red cells with a single diagonal slash were postponed to accommodate changes earlier changes in the timeline. Blue cells are those that were added, and gray cells were not changed. Yellow cells are STOP/GO tasks.

IX. PERMITS

Florida Fish and Wildlife Conservation Commission

Commissioners
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Michael W. Sole
Vice Chairman
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Joshua Kellam
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Gary Lester
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Executive Director
Eric Sutton
Executive Director

Thomas H. Eason, Ph.D.
Assistant Executive Director

Jennifer Fitzwater
Chief of Staff

850-487-3796
850-921-5786 FAX

Managing fish and wildlife resources for their long-term well-being and the benefit of people.

620 South Meridian Street
Tallahassee, Florida
32399-1600
Voice: 850-488-4676

Hearing/speech-impaired:
800-955-8771 (T)
800 955-8770 (V)

MyFWC.com

January 3, 2019

Joel Trexler
Department of Biological Science
Florida International University
Marine Science Building, Room 350
3000 NE 151st
North Miami, FL 33181

Dear Dr., Trexler:

Attached is Scientific Collector's Permit S-19-05 permitting Joel C. Trexler (including your Ichthyology class when accompanied by you), Nathan Evans, Alex Mercado-Molina, Sofia Burgos, John Gatto, Tom Granger, Iris Krehahn, Peter Flood, Erin McCarthy, Matthew Ladato, Lauren Ramos, Nichole Strickland, Somers Smott, Scott McCleay, Alain Duran, Domenique Olesen, Mark Barton, Martha Zapata, Erika Somoza, Jeff Kline, and Mark Cook of Florida International University to take and possess freshwater fish in the State of Florida for scientific purposes only. Please carefully review the provisions stated on this Scientific Collector's Permit.

Please mark your boat and/or equipment with the name of the institution or agency you represent so that the public will be informed as to your purpose.

All Scientific Collector's Permits expire December 31, 2019. If you wish to have your permit renewed, please write to us 30 days before the expiration date or 30 days before you will need the permit. At that time, consideration will be given to renewal. A report is required by the terms of the permit. The report must include location of collection, sizes, and number collected. In addition, please provide either a final/completion report or a progress/status report that discusses progress relating to objectives and conclusions. The report must provide the Permit number which authorized the work performed in the report.

Please submit the report when you request renewal. Failure to do so is just cause for refusal to reissue your permit.

Sincerely,

Barron Moody
Biological Administrator II

Attachment

cc: L/E Commander

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SCIENTIFIC COLLECTOR'S PERMIT

Number: S-19-05
Date: 01/03/19

A - Under authority of Title 68A-9002 Florida Administrative Code, permission to collect freshwater fish is hereby granted to: Joel C. Trexler (including your Ichthyology class when accompanied by you), Nathan Evans, Alex Mercado-Molina, Sophia Burgos, John Gatto, Tom Granger, Iris Krehahn, Peter Flood, Erin McCarthy, Matthew Ladato, Lauren Ramos, Nichole Strickland, Somers Smott, Scott McCleay, Alain Duran, Domenique Olesen, Mark Barton, Martha Zapata, Erika Somoza, Jeff Kline, and Mark Cook of Florida International University to take and possess freshwater fish in the State of Florida for scientific purposes only.

B - Permit Conditions:

1. Permit Number, collection date, locations, species, number of fish to be collected, names of personnel, and description of collecting equipment shall be provided in writing prior to collection to: Regional Fisheries Administrator, Florida Fish and Wildlife Conservation Commission, Regional Office, 8535 Northlake Blvd., West Palm Beach, FL 33412, Fax No. 561-625-5129 and Law Enforcement Dispatcher, Florida Fish and Wildlife Conservation Commission, Regional Office, 8535 Northlake Blvd., West Palm Beach, FL 33412, Fax No. 561-357-4203. Also contact Kelly Gestring at 561-391-6409 prior to scheduling sampling to prevent conflicts with ongoing Florida Fish and Wildlife Conservation Commission sampling in the same areas (Geographic locations B, C, and E only).

2. Collection methods: Dipnets, seines, backpack electrofisher, minnow traps, throw traps, pop nets, and boat electrofisher. Fyke-nets may be used in Everglades National Park and Water Conservation Area 3A and 3B. Fyke-nets shall be 8.5 meters in length with a 6-8 meter lead, two 3 meter wings and a 1 X 0.6 meter opening. Fyke-nets shall have 1.25 cm mesh with a throat opening of 9 X 9 cm. Net openings to be fitted with PVC bars in a manner to prevent capture of medium and large alligators

3. Use of fish toxicants such as rotenone is prohibited.

4. Geographic location:
   A. Water Conservation Areas 2A, 2B, 3A, 3B (Everglades Wildlife Management Area; including canals, e.g. L-67A, L-29).
   B. Tamiami Canal, C-111 Canal and C-111E Canal.
   C. Marsh east of L-30 Canal.
   D. Everglades National Park (with Federal approval).
   E. L-31N and L-31W.
   F. Stormwater Treatment Areas (with South Florida Water Management District approval).
   G. Region wide (seine only).
   H. WCA 1 (Loxahatchee NWR; with Federal approval).

5. Except as provided herein, all fish must be released alive, unless it is a previously unrecorded exotic species which should be retained, and Florida Fish and Wildlife Conservation Commission be alerted promptly.

6. Species to be taken: Most freshwater fish species to be live-released on site. The following total yearly numbers may be sacrificed. Largemouth bass not to exceed three pounds (25 annually), Sunfish (Lepomis spp.; 500 annually), Bullheads (50 annually), Pickerel (20 annually), Cichlids (except butterfly peacocks; (500 annually), Florida gar (50 annually), Lake Chubsucker (50 annually), Bowfin (50 annually). Small marsh fish (e.g. cyprinids) may be retained.

7. No species classified as endangered, threatened or species of special concern may be taken. Freshwater aquatic life classified as prohibited or conditional may not be transported alive.

8. This permit shall not apply to State and Federal Wildlife Refuges, management areas or parks unless specifically provided herein. Permission is granted to collect fish in Everglades and Francis Taylor Wildlife Management Areas, and the C-111 portion of East Everglades WMA. Upon approval by the appropriate state or federal agency, this office has no objections to fish collections in Stormwater Treatment Areas, Everglades National Park, Big Cypress National Preserve, and WCA 1 (Loxahatchee Wildlife Refuge).

9. Permittee must be in possession of this permit while collecting.

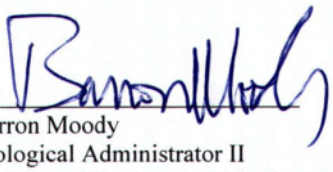
10. A report is required 90 days after completion of collection activities or upon request for permit renewal. The report must include location of collection, sizes, and number collected. In addition, please provide either a final/completion report or a progress/status report that discusses progress relating to objectives and conclusions. The report must reference the Permit Number authorizing the completed work.

11. The purpose of this permit is to authorize collection of fish for education and scientific research and monitoring.

12. This permit may be revoked for failure of the permittee to abide by permit conditions and rules of the Commission.

13. This permit is not transferable and expires December 31, 2019.

Eric Sutton
Executive Director

By 
Barron Moody
Biological Administrator II
Division of Freshwater Fisheries Management

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