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 Restoration Strategies Science Plan (RSSP) 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 P cycling and STA performance. This aspect is the subject of this SOW.

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 wildlife and fisheries on the reduction of phosphorus in the STAs?

- *Do fish and wildlife affect P import, export, or nutrient cycling within the system enough to alter outflow TP concentrations?*
- *What are the primary diets of waterfowl and submerged aquatic wildlife in the STAs?*

- *What rates of TP cycling can be expected in the STAs from wildlife, fish and macro-crustaceans? How significant is faunal recycling to ambient P turnover?*
- *What is the form and availability of excreted TP for the dominant faunal components?*

The particular component of the DSP being addressed in this Work Order focuses on the role of aquatic fauna on the performance of the STAs.

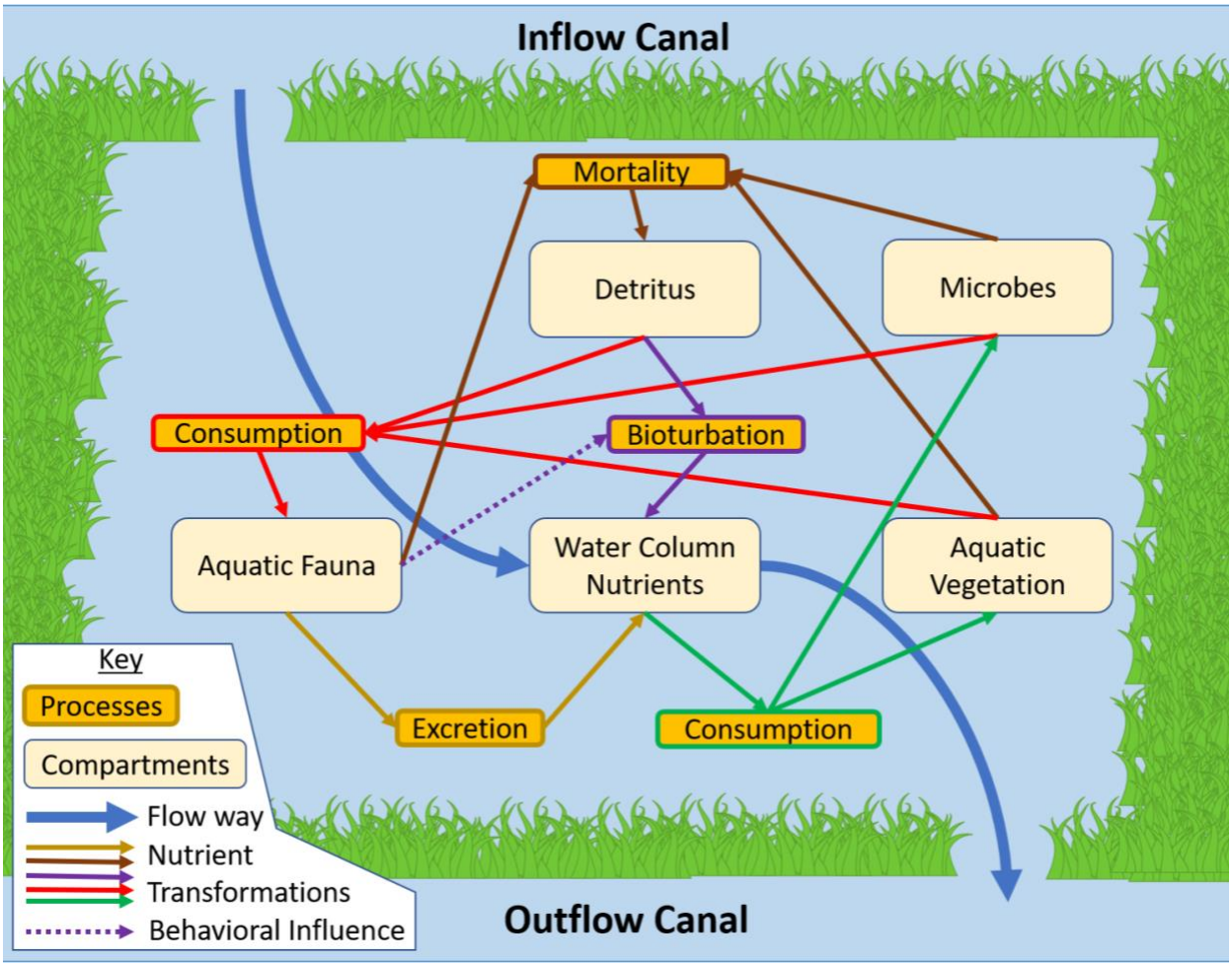

Figure 1. A conceptual model of the potential roles of aquatic fauna in nutrient cycling in the STAs. The large squares represent the five main nutrient compartments in the STAs: Detritus, Microbes, Aquatic Fauna, Water Column Nutrients, and Aquatic Vegetation. Arrows represent processes of nutrient transformation between nutrient compartments, and are color coded for each type of process: consumption (red), bioturbation (purple), decomposition (brown), excretion (yellow), photosynthesis (green).

Fauna are increasingly recognized as important in nutrient cycling in aquatic systems (Vanni 2002). The abundance and species composition 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 moving stored phosphorus from the sediments and transporting it, via excretion, back into the water column. This pathway through benthic feeding is particularly important in the STAs where the goal is to get TP accreted into the floc and sediments as a treatment process. Second, aquatic fauna can have important indirect effects through modifications of the environment (e.g. bioturbation; Vanni 2006). Finally, aquatic animals can act as important P sinks and vectors of P-transport, especially by large, mobile animals such as fish and crocodilians. The information being collected through the field experiments and monitoring in this project will provide a means of quantifying P sinks and doing a first estimation of the possible role of P vectoring via animals.

Given the potential pivotal role of aquatic fauna in STA nutrient cycling, a pilot study was initiated in 2015 (Work Order: 4600003032-WO01), as part of the RSSP, 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-loading through excretion and bioturbation experiments.

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

Data from the pilot study also revealed that fish and invertebrate distributions are highly heterogeneous in space and time compared to those of other wetlands in south Florida. While spatial and seasonal variation were accounted for in the pilot study through appropriate sample sizes based on power analyses, there remains the potential for large inter-annual fluctuations in population sizes and community composition due to annual changes in hydrology, nutrient loading, habitat structure, disturbance events, and related ecological dynamics (e.g., Dorn & Cook 2015). Additional sampling is needed to quantify annual variation and to improve the precision of the faunal biomass estimates for parameterization of the P-budgets.

II. OBJECTIVES

The primary goals of this study are to quantify the areal biomass and community composition of fishes and other aquatic fauna and their effects on water quality in the outflow cells of the STAs. It is not feasible to quantify all animal species in the STAs so we are focusing on those community components that are most abundant based on the pilot study. Specifically,

the following data shall be collected over the next year: 1) biomass (kg ha^{-1}) and community composition of small-bodied fish (<8 cm standard length) and macroinvertebrates (e.g., crustaceans, gastropods) in STA outflow cells 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 in spring, summer and autumn; 3) estimates of mass-specific P excretion rates of abundant species; and 4) experimental estimates of enhanced water column TP caused by faunal bioturbation. 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 using data from other projects in the PFLUX program. Bioturbation 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.

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 and birds (crayfish predators); 5) aquatic faunal sampling design and implementation; 6) experimental design, quality assurance processes in research; 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 Order: 4600003032-WO01.
c) Measure rates of P release from excretion and bioturbation for the dominant taxa using methods developed in Work Order: 4600003032-WO01.
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 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 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, quality assurance 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
- A comprehensive breakdown of the services required by the District laboratory for each sub-study in the project. This monitoring plan should include study parameters, number of samples, type of analyses for each sample, and expected schedule of delivery. The Project Manager shall ensure that the monitoring plan is provided to the District's Analytical Services section for review and revision as needed. 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 combined modern literature concepts with those gained from the pilot study.
- A quality assurance plan providing analytical methods and data quality objectives and linking to other sections of the workplan providing methodological detail.
- Project Management information detailing the staffing arrangements, roles, and responsibilities
- Schedule of activities
- UNIVERSITY's contingency plan for employing and training new staff 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. This material shall be detailed in a quality assurance plan.
- 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 Order: 4600003032-WO01. Sampling will be focused on the same outflow (SAV) cells in STAs -1W, -1E, and -2 from the previous Work Order, as well as additional sampling for large-bodied fishes in outflow cells of STA-3/4.

We will survey small-bodied fish and macroinvertebrate composition and biomass in STA 2 outflow cells 3, 4, 5, and 6. Small-bodied fish (< 8 cm standard length [SL]) and macroinvertebrates will be quantified using 1-m^2 throw traps (Jordan et al. 1997, Dorn et al. 2005) in open water and submerged aquatic vegetation (SAV) habitats to estimate density and species composition. All captured individuals will be euthanized with MS222, preserved with formalin in the field, and processed in the lab in the weeks following the sampling. Large-bodied fish (≥ 8 cm SL) abundance will be quantified as mean catch-per-unit-effort (CPUE) based on replicated 5-min electrofishing transects (Chick et al. 1999) in STA-1E, STA-1W, and STA-2). All large-bodied fishes will be identified to species, measured for length (cm) and mass (g), and released within 200 m of their capture location.

To incorporate spatial variation in animal density and associated habitat types within the STA outflow cells, a random sampling design stratified by vegetation type will be used. Sampling effort (128 throw-trap samples and 64 electrofishing transects) will continue at stations sampled during Work Order: 460003032-WO01, which were proportionally stratified based on the areal coverage of the four dominant habitats (*Ceratophyllum demersum*, *Najas* spp., *Chara* spp., open water) found in the submerged aquatic vegetation (SAV) outflow cells. To capture seasonal variation, throw-trap samples and electrofishing samples will be collected in September 2018, March 2019, and June 2019. Four electrofishing transects will be completed in each submerged aquatic vegetation (SAV) outflow cell in STA-1E (cells 2, 4N, 4S, and 6), STA-1W (cells 3, 4, 1B, 2B, 5B), STA-2 (cells 3, 4, 5, 6), and STA-3/4 (cells 1b, 2b, 3b). In each outflow cell, three electrofishing transects will be conducted in the shallow, vegetated, marsh habitat and one transect conducted in the deeper, canal habitat located on the periphery of each cell. This sampling scheme accounts for the greater availability of the marsh habitat relative to the canal habitat in each cell. The same general electrofishing transect locations will be sampled during each sampling event.

Deliverables

The UNIVERSITY shall prepare Draft Task Reports that summarize the aquatic faunal community and biomass results for the sampling year. The Draft Task Reports shall include Introduction, Methods, Results, and Discussion sections, and all data, statistical output, tables and figures from the task. Data relevant to this task shall be provided in a tabular format using 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
Final Task 3 Report

District Laboratory Services
None.

Task 4. Measuring animal impacts on P turnover

Impacts of bioturbation on water column TP will be assessed through a field mesocosm experiment based on the methods employed in Work Order: 4600003032-WO01. This study will be completed in December 2018 and April 2019 to capture seasonal variation in aquatic productivity and activity.

We will assess the effects of bioturbation on TP and TN concentration in the water column via *in situ* mesocosm experiments containing Largemouth Bass (*Micropterus salmoides*), Blue Tilapia (*Oreochromis aureus*), and Orinoco Sailfin Catfish (*Pterygoplichthys multiradiatus*). Mesocosm enclosures (1.5 m X 1.5 m; 2.25 m^2) will be stocked with three densities of each target species including high-density (2 individuals), low-density (1 individual), and fishless-control treatments (3x 3 treatment levels, 3 species = 27 enclosures). The high-density treatment is approximately equal to the estimated density of the focal species observed during prior electrofishing surveys in the STAs. The low-density treatment is approximately equal to the average density of the focal species observed during prior electrofishing surveys in the STAs. The fishless control treatments will contain no individuals. To ensure the fish do not escape, the bottoms of the enclosures will be buried 20-30cm into the substrate.

Enclosures will be placed in the field at least five days prior to stocking with fish. Samples of floc and water will be collected from each enclosure every 7 days after the start of the experiment for 4 consecutive weeks. Water samples (125 mL) will be collected via mid-water-column grab sampling. Water samples will be acidified with sulfuric acid to a sample pH of between 1.3 and 2 then transported to the SFWMD Nutrient Analysis Laboratory, on ice, within 72 hrs of collection for analysis. Floc samples will be collected using a 5.08-cm diameter coring device. Floc cores will be sectioned and measured in the field and the top "pourable" floc layer retained for TP and TN analyses. After coring, the floc will be given 1 minute to settle, water will be decanted so that 3 cm of water remained above the top of the floc layer, then floc will be collected by stirring it into the water using a swirling motion and poured into a sample container. The location of each core will be recorded to ensure they are resampled in consecutive weeks.

Biofilm samples will be collected from periphytometers that consisted of 30, 48cm long, 3.2cm wide, 6 mil thick, plastic strips like those used by Chick et al. (2008). Periphytometers

will be placed in the center of the enclosures at the start of the experiment and samples collected by carefully removing 30 strips from the enclosure and placing them in a plastic bag before transporting the sample on ice for processing at FIU. To account for the effects of shading by the enclosure walls, 9 control periphytometers were placed surrounding the enclosure plot at the start of the experiment and were collected at the same time as those inside of the enclosures. In the lab, biofilm will be removed from enough plastic strips to achieve a total surface area of 2,400cm^2 for each enclosure. The resulting biofilm will be freeze-dried, then ground to fine powder using a mortar and pestle.

The biofilm on periphytometers may be exposed to more physical disturbances than the biofilm on the enclosure walls as the fish may use the periphytometers for cover, thus rubbing against them or creating flow as they move past them. For this reason, we will also collect biofilm from the enclosure walls during one of the experimental seasons to compare the biomass accumulation between these two substrates. To avoid confusion, the biofilm collected from the enclosure walls will from here on out be referred to as periphyton samples, whereas the biofilm collected from the periphytometers will be referred to ad biofilm. Four 20cm x 20cm squares of the enclosure wall material will be adhered to the inside of each of the four walls of the enclosures just beneath the surface of the water and directly above the substrate. One square from each depth will be collected from each wall every two weeks. One half of each square (200cm^2) will be scraped clean, and the periphyton collected. The resulting periphyton will be freeze-dried, then ground to fine powder using a mortar and pestle. Biofilm, periphyton and floc samples will be submitted to the SFWMD Nutrient Analysis Laboratory for quantification.

Excretion rates will be measured on aquatic fauna using short-term incubations based on the methods developed in Work Order: 4600003032-WO01 (i.e., methods comparable to Schaus et al. 1997, Torres and Vanni 2007, Capps and Flecker 2013). We will experimentally measure N and P excretion and egestion for three of the most abundant large-bodied species captured in our electrofishing surveys and the three most abundant small-bodied species collected during our throw trap surveys. Large-bodied species used in past work were Blue Tilapia, Vermiculated Sailfin Catfish, and Largemouth Bass. Small-bodied species used were Eastern Mosquitofish (*Gambusia holbrooki*), Bluefin Killifish (*Lucania goodei*), and Sailfin Molly (*Poecilla latipinna*). Total P (TP), total dissolved P (TDP), total N (TN), ammonium (NH_4-N) excretion rates will be measured via short-term incubations.

Twenty daytime and twenty nighttime 60-minute incubations will be conducted for each of the three small-bodied species. Each incubation will be carried out in a 25x50cm 2 mil polyethylene fish bag filled with 0.5L of STA water that will be prefiltered through a 0.7 µm glass fiber filter to remove phytoplankton and particulates. After filling with water, the 'pre-treatment' samples are collected via a 60-mL syringe (125mL TP/TN, 60mL TDP, 60mL NH4). The incubation chambers will then be populated with a minimum of 3 individuals that represent the size distribution of the species in the STAs as determined from the throw-trap surveys (Task 3). The number of individuals will vary across all twenty replicates (3-25 individuals) to achieve a range of incubation biomasses. Additionally, fishless control incubations will be conducted in parallel to the fish incubations at the beginning and end of each experimental day, and after every fifth replicate. The fishless controls will follow the same methods as the fish incubations except the chambers will contain no fish. Fishless controls will quantify any non-fish related nutrient transformations occurring in the chambers during the incubation period. After the incubation period, the 'post-treatment' samples will be collected (125mL TP/TN, 60mL TDP, 60mL NH4) via a 60-mL syringe.

Pre-treatment and post-treatment samples will be filtered immediately (0.45 µm) and acidified for TDP and NH4, and only acidified for TP/TN. Acidification will use a volume of sulfuric acid (H_2SO_4) sufficient to achieve a preserved sample pH between 1.3 and 2. All samples will be placed on ice and transported to the SFWMD Nutrient Analysis Laboratory within 24 h. Per capita fish excretion will be estimated as the difference in nutrient concentration between the fish incubations and the fishless controls.

Following completion of the small-bodied fish excretion samples, the same methods will be used to quantify excretion by Blue Tilapia, Largemouth Bass, and Vermiculated Sailfin Catfish in 10L incubation chambers. All samples will be placed on ice and transported to the SFWMD Nutrient Analysis Laboratory within 24 h.

Deliverables

The UNIVERSITY shall prepare a Draft Task Report that summarizes impacts of bioturbation in resuspending P from STA benthos. 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 4 Report. Based on the comments provided by the District, the UNIVERSITY shall provide the District with a Final Task 4 Report two weeks of receiving such comments.

Draft Task 4 Report
Final Task 4 Report

District Laboratory Services

Overview

The proposed project includes two phases: 1) descriptive sampling to document patterns of fish and macroinvertebrate abundance and biomass in STAs -1W, -1E, -2, and -3/4, and 2) two studies permitting those data to be interpreted with respect to phosphorus (P) cycles in the STAs. Two objectives within Task #4 of the Work Order will be used to translate fish biomass into phosphorus cycling. These objectives are: (1) quantify the potential of three benthic fish species to increase water column total nitrogen (TN) and total phosphorus (TP) via bioturbation, and (2) quantify the mass-specific N and P excretion rates of the six most abundant fish species in the STAs. The proposed sample budget for the number of analyses to be completed by the District's Nutrient Analysis Laboratory is detailed below.

The bioturbation objective of Task #4 will require approximately 552 TP and TN samples (1,104 Total Analyses). This assumes that TC data will be provided with TN data from solid samples and is not counted as an extra analyte. These samples will include 228 water samples (including equipment blanks), 216 dried floc samples, and 108 dried biofilm samples (scraped from periphytometers).

The nutrient excretion objective of Task #4 will require 606 excretion and equipment blank water samples analyzed for four analytes (TP, TDP, TN, NH_4; 2,424 Total Analyses).

In total, Task #4 will generate 1,158 samples (3528 Total Analyses) for submission to the District's Nutrient Analysis Laboratory for analysis.

A detailed outline of the necessary samples, analyses and approx. timeline can be found below.

Task #4 - Bioturbation Samples

- 3 Fish Species x 3 Densities x 3 Replicates x 4 Weeks x 2 Substrates (water, floc) x 2 Seasons (Winter, Spring) = 432 samples plus 24 equipment blanks analyzed for TP and TN = **912 Total Analyses**
- 3 Fish Species x 3 Densities x 3 Replicates x 2 Weeks (sampled biweekly over 4 weeks) x 2 Substrates (biofilm, periphyton [only in Spring 2019]) x 2 Seasons (Winter, Spring) = 162 analyzed for TP and TN = **324 Total Analyses**
- **Grand Total of 1,236 analyses**

  Winter 2018/19

  - 120 Water Samples (including equipment blanks) will be submitted over the period of one month during Winter 2018 (November/December 2018)
    - 1/4 of the water samples (30 samples) will be submitted weekly for duration of the 4-week experimental trial
  - 108 Floc Samples and 54 Biofilm Samples will be submitted ≈4-8 weeks after the completion of the experimental trial (tentatively end of February 2019) *All samples will be delivered dried and pulverized.

  Spring 2019

  - 120 Water Samples (including equipment blanks) will be submitted over the period of one month during Spring 2018 (Tentatively scheduled for April 2019)
    - 1/4 of the water samples (30 samples) will be submitted weekly for duration of the 4-week experimental trial
  - 108 Floc Samples, 54 Biofilm Samples, and 54 periphyton samples will be submitted ≈4-8 weeks after the completion of the experimental trial (tentatively June 2019) *All samples will be delivered dried and pulverized.

Task #4 - Excretion Samples

Water: 6 Species x 26 Incubations (20 Treatments + 5 Fishless Controls* + 1 Equipment Blank) x 2 (Before/After) x 2 (Day & Night) = (612 Samples + 12 Equipment Blanks*) x 4 Analytes (TP, TDP, TN, NH_4-N) = **2,496 Total Analyses**

*Assumes study will require 6 days and 6 nights to complete (1 days/nights per species) with 5 fishless controls and 1 equipment blank per day/night.

Winter (January) 2019

- All 624 Samples will be submitted over a one-month period
  - Daytime samples will be delivered Monday-Thursday prior to noon daily
  - Nighttime samples will be delivered Tuesday-Friday before noon each day

Task 5. 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 and 4, from the current contract, as well as from Tasks 4, 5 and 6 from Work Order: 9500006537, and provide meaningful interpretation of the data with emphasis on the implications of communities for short-term and long-term performance of the STAs.

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. Joel Trexler
Roles and Responsibilities:

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

Co-PI**:** Dr. Mark Barton
Roles and Responsibilities:

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

Lead Technician**:** Matthew Lodato
Roles and Responsibilities:

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

VIII. SCHEDULE OF ACTIVITIES

	2018				2019									
	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Nov
Throw-trap Survey														
Electrofishing Survey														
Bioturbation Experiment														
Excretion Experiment														
Sample Processing														
Data Analysis and Writing														
Task 1 Deliverable														
Task 2 Deliverable														
Task 3 Deliverable														
Task 4 Deliverable														
Task 5 Deliverable														

IX. PERMITS

Florida Fish and Wildlife Conservation Commission

Commissioners
Robert A. Spottswood
Chairman
Key West

Michael W. Sole
Vice Chairman
Tequesta

Joshua Kellam
Palm Beach Gardens

Gary Lester
Oxford

Gary Nicklaus
Jupiter

Bo Rivard
Panama City

Sonya Rood
St. Augustine

Office of the 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

Florida Fish and Wildlife Conservation Commission

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

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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

REFERENCES

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