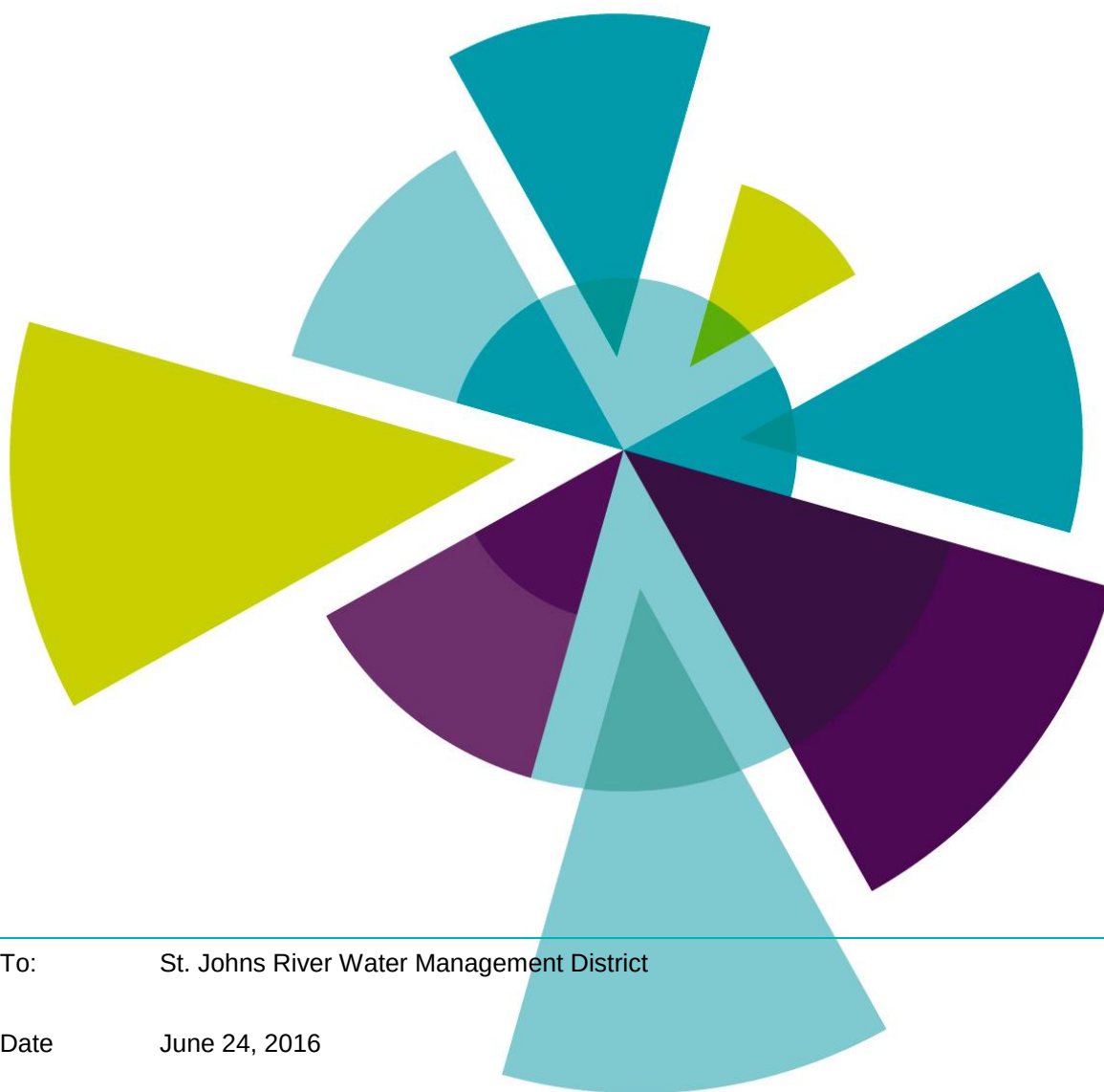




Synoptic Biological Monitoring of Springs Data Collection FINAL REPORT



To: St. Johns River Water Management District

Date June 24, 2016

From: Amec Foster Wheeler Environment & Infrastructure, Inc.

**SYNOPTIC BIOLOGICAL MONITORING OF SPRINGS DATA COLLECTION
FINAL REPORT**

Prepared for



St. Johns River Water Management District

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Amec Foster Wheeler Project No. 600397

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1.0 INTRODUCTION

The following is the Final Report detailing the project methods that Amec Foster Wheeler Environment & Infrastructure, Inc. (Amec Foster Wheeler) used to perform quality control and assurance, obtain work permits and site access, collect and analyze samples, and summarize and report the data. Additionally, this Final Report includes maps of all study sites in the spring-run streams (**Appendix A**).

Project objectives included developing a baseline set of biological community composition (i.e. abundance and biomass) and distribution data that can be used to assess current ecological conditions to compare to historical and future conditions in spring ecosystems. Specific objectives for this project that were accomplished include:

- Finalize Project Work Plan and obtain permits
- Conduct *in-situ* physical and chemical condition sampling
- Perform biological sampling
- Perform biological processing, measurements and taxonomic identification
- Deliver database

2.0 QUALITY CONTROL AND ASSURANCE

Amec Foster Wheeler's project approach includes a program to subject all field physicochemical and biological field sampling methods and laboratory SAV, algae and macroinvertebrate processing, analysis, and identification methods to Amec Foster Wheeler's field and laboratory QA/QC procedures. Specific Amec Foster Wheeler procedures or other accepted standard operating procedures (SOPs) to be used for each aspect of the project are described in detail in the **Project Work Plan – Section 2, Appendix B, and D**.

2.1 **Field Monitoring QA/QC Methods**

For the field sampling component, several SOPs such as SJRWMD's Field SOPs for Surface Water Sampling among others were kept on-hand with the field monitoring staff during mobilization and pre-event preparation. SOPs were followed during sampling. Pre and post-event instrument verification was conducted prior to commencing sampling and at the end of each sampling day.

United States Geological Survey (USGS) standard methods to obtain velocity profiles were followed according to Rantz et al. 1982 to the extent possible, although certain aspects of the procedures were modified to meet specific objectives of this project. Modifications to the standard protocol were approved by the District Project Manager during initial site visits. The flow meter unit that was used for this project is the SonTek FlowTracker Handheld Acoustic Doppler Velocimeter (ADV), which has a velocity accuracy of ± 0.008 ft/s (± 0.25 cm/s) or 1% of measured velocity. Quality assurance is provided by the equipment that has internal QA/QC checks that users interact with in real time and in post-processing. Further, the software for the ADV provides standard error measurements that were checked in the field, enabling re-measurements to be made prior to departure if necessary. The SonTek Flowtracker handheld unit provides an array of real-time quality checks during current measurement. One of these real-time feedbacks includes checking for interference by objects in the ADV beam, which is

extremely useful when attempting to obtain accurate spot velocities within dense SAV communities or in extremely shallow water (e.g. less than one-inch depth). If interference was detected, the technician would move the sensor or trim back the interfering object(s) incrementally until a valid reading was established. The SonTek unit also informs the user what the prevailing current angle is to the orientation of the beam, thus enabling the user to turn the equipment so it is facing the maximum vector, which is very useful for a spot-habitat study where local velocities within or over particular habitats are desired. Further, the software provides on-the-fly diagnostics regarding important variables related to assuring accurate measurements such as signal-to-noise ratio (SNR), velocity spikes, and standard error of velocity. The QA process in the field involved taking redundant measures when necessary and checking results for consistency. All of these values were checked before leaving the field.

The following documents and non-Amec Foster Wheeler SOPs were used to maintain a high level of accuracy in data collection to ensure sound QA/QC management practices were being followed:

Velocity Profile Measurements

- Rantz, S. E. et al. 1982. Measurement and Computation of Streamflow: Volume 1. Measurement of Stage and Discharge. United States Geological Survey Water-Supply Paper 2175.

Surface Water *In-situ* Sampling, Instrument Calibration and Verification

- SJRWMD. 2012. Field Standard Operating Procedures for Surface Water Sampling – Fiscal Year 2013. St. Johns River Water Management District, Palatka FL. 92 pp.

2.2 Laboratory QA/QC Methods

Amec Foster Wheeler's Biological-Toxicology Laboratory has a Taxonomy Laboratory Procedure Manual which outlines methods in general accordance with the following protocols, SOPs, and manuals:

- American Public Health Association 1992. Standard Methods for the Examination of Water and Wastewater, 18th edition.
- FDEP 2014. Standard Operation Procedures (SOPs) for biological communities sampling, algae (periphyton and phytoplankton) sample preparation and identification, and benthic macroinvertebrate sample preparation and identification. Florida Department of Environmental Protection.
- USEPA. 1999. Rapid Bioassessment Protocol for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. United States Environmental Protection Agency.

Sorting efficiency is evaluated on 10 percent of the samples sorted by an individual technician and macroinvertebrate identification efficiency is evaluated on 5 percent of the samples identified by an individual taxonomist. If the cumulative sorting or identification efficiency of an individual falls below 95%, precautionary measures are taken which involve retraining and demonstration of capabilities. If cumulative efficiencies fall below 90%, corrective actions are taken which involve re-sorting/re-identifying samples processed by this individual.

3.0 WORK OBJECTIVES

3.1 Obtain Site Access and Permits

As part of the **Task 1** planning effort to establish transects, Amec Foster Wheeler staff worked with land managers, District, state and federal staff to obtain permits and permissions to access and sample study sites by conducting wading, snorkeling, or SCUBA activities (**Project Work Plan – Appendices F and G**).

SCUBA Scientific Dive Operation and Safety Plans were developed and approved by District and State staff (**Project Work Plan – Appendix H**). Pre and Post Dive Plans were also submitted and approved to conduct SCUBA assisted sampling in Ichetucknee and Silver Rivers for the first biological event that took place in May 2015 and the second biological event that took place in September 2015 (**Project Work Plan – Appendix H**).

3.1.1 Sampling Transect Establishment

Preliminary sampling transect locations were finalized as part of the Project Work Plan. Approved and final station and transect station names with associated latitude and longitude coordinate locations are provided in **Table 1** and shown in **Figure 1**. **Appendix A** provides specific transect location maps of each of the sites that were finalized for the project. The naming convention used to create transect site names is as follows: 1) the first three (or four if necessary) letters of the site were used for each station; 2) transect 1, 2 or 3 begins with 1 as the most upstream; and 3) replicates were always positioned as having 1 as the left-most replicate while facing upstream. Photos representing examples of variability between transects in regards to stream morphometry and habitat availability are shown in **Figures 2a, 2b, and 2c**.

Table 1
Transect Sampling Stations and Locations

Sampling Station	Latitude DD	Longitude DD	Latitude DMS	Longitude DMS	Northing NAD 1983 HARN UTM Zone 17N	Easting NAD 1983 HARN UTM Zone 17N
ALE1	29.08259003	-81.57825003	29° 4' 57" N	81° 34' 42" W	3217273.979	443722.808
ALE2	29.07929	-81.56691997	29° 4' 45" N	81° 34' 1" W	3216902.994	444823.747
GUM1	28.95340999	-82.23836998	28° 57' 12" N	82° 14' 18" W	3203455.248	379323.771
GUM2	28.95974999	-82.23209001	28° 57' 35" N	82° 13' 56" W	3204151.360	379943.105
ICH1	29.9799	-82.7589	29° 58' 48" N	82° 45' 32" W	3317859.887	330310.547
ICH2	29.957241	-82.780301	29° 57' 26" N	82° 46' 49" W	3315380.366	328206.565
JUN1	29.18449004	-81.70372999	29° 11' 4" N	81° 42' 13" W	3228630.716	431577.905
JUN2	29.21174997	-81.65322002	29° 12' 42" N	81° 39' 12" W	3231622.716	436505.792
MAN1	29.48948003	-82.97798002	29° 29' 22" N	82° 58' 41" W	3263847.596	308239.758
RAI1	29.09076667	-82.42656667	29° 5' 27" N	82° 25' 36" W	3218882.283	361166.445
RAI2	29.06896667	-82.42753333	29° 4' 8" N	82° 25' 39" W	3216467.742	361043.101
ROC1	28.77171667	-81.50291667	28° 46' 18" N	81° 30' 10" W	3182797.912	450908.370
ROC2	28.7411	-81.46794002	28° 44' 28" N	81° 28' 5" W	3179391.985	454309.290
SILG1	29.24471	-81.64127001	29° 14' 41" N	81° 38' 29" W	3235268.245	437687.340
SIL1	29.21573333	-82.04845	29° 12' 57" N	82° 2' 54" W	3232342.547	398090.805
SIL2	29.21528333	-82.0417	29° 12' 55" N	82° 2' 30" W	3232286.843	398746.500
SIL3	29.20348333	-82.015	29° 12' 13" N	82° 0' 54" W	3230956.616	401330.587
VOL1	28.94707	-81.33972	28° 56' 49" N	81° 20' 23" W	3202168.952	466894.348
WAC1	30.327034	-83.987714	30° 19' 37" N	83° 59' 16" W	3358808.385	212727.146
WAC2	30.203283	-83.970364	30° 12' 12" N	83° 58' 13" W	3345042.229	214037.240
WAK1	30.234019	-84.294372	30° 14' 2" N	84° 17' 40" W	3349310.148	182926.854
WAK2	30.211438	-84.259876	30° 12' 41" N	84° 15' 36" W	3346710.115	186176.977
WEE1	28.51895	-82.573891	28° 31' 8" N	82° 34' 26" W	3155701.422	345988.143
WEE2	28.519443	-82.583234	28° 31' 10" N	82° 35' 0" W	3155768.081	345074.485
WEK1	28.71415	-81.45805	28° 42' 51" N	81° 27' 29" W	3176402.473	455263.512
WEK2	28.79926667	-81.4144	28° 47' 57" N	81° 24' 52" W	3185816.869	459559.543

Note: DD is coordinates in Decimal Degrees and DMS is Degrees, Minutes, Seconds. UTM coordinates in meters.

Figure 1
Overview Map of Final Sampling Stations and Transect Locations

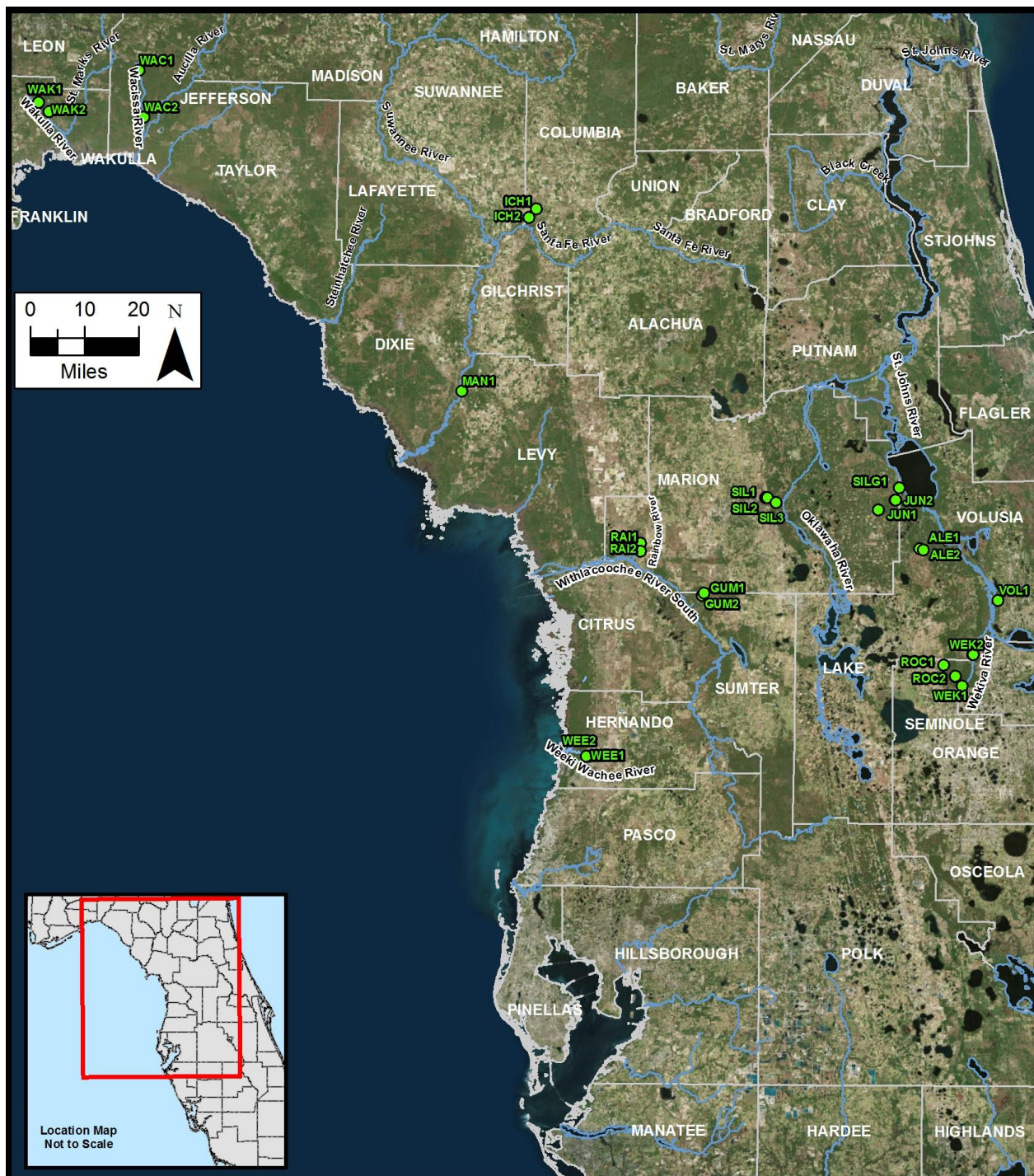


Figure 2a
Example of Habitat Variability of Transect Locations
Site Name: WAC2 – Downstream Wacissa River Transect



Figure 2b
Example of Habitat Variability of Transect Locations
Site Name: SIL3 – Downstream Silver River Transect



Figure 2c
Example of Habitat Variability of Transect Locations
Site Name: WEE1 – Upstream Weeki Wachee River Transect



3.2 Sample Collection and Analyses

The following sections and field SOPs (provided in the **Project Work Plan – Appendix B**) provide details on field sample collection and laboratory analytical methods that were employed during the project to achieve the stated objectives of collecting physicochemical and biological conditions in the spring runs. Copies of field data sheets used to collect data have been provided in the **Project Work Plan – Appendix C**.

3.2.1 Field Monitoring Activities – Sample Collection Methodology

Field monitoring activities included physiochemical and biological sampling. Amec Foster Wheeler conducted sample collection for six physicochemical sampling events spread across a range of river stages so that velocity measurements can be connected with flow gauges in each spring system. Amec Foster Wheeler also conducted biological sampling for two sampling events (coupled with two of the physicochemical events in May/June and September/October). Four of the 26 transect sampling locations (ICH2, SIL1, SIL2, and SIL3) were too deep to snorkel and required Amec Foster Wheeler SCUBA divers to safely collect biological samples. If the site was located within a state park, Amec Foster Wheeler coordinated access and permission to the site with the FDEP prior to each sampling event. The specific field monitoring objectives included

sample collection at each of the 26 transects, which are summarized below for the following physicochemical and biological parameters:

- *In-situ* surface water chemistry measurements to collect specific conductance, dissolved oxygen, pH, and temperature data using a multiparameter sonde, and using a portable turbidity meter to collect turbidity
- In-stream physical condition sampling of total water depth, height of SAV canopy, and canopy cover using a spherical densiometer
- Up to ten point velocity measurements across the transect and above benthic substrate quadrat locations using a SonTek FlowTracker-ADV (shown in **Figure 3**), and staff gauge readings if applicable
- SAV, macroalgal and epiphytic algal cover using transect/quadrat survey method on a percent cover and/or Braun-Blanquet scale
- SAV, macroalgal and epiphytic algal, and macroinvertebrate (collected from the SAV and macroalgal biomass samples) biomass sample collection using a custom made modified Hess sampler with a known sampling area (shown in **Figure 5**)
- Collection of composite whole SAV plant samples for morphometric measurements
- Macroalgal and epiphytic algal sample collection for physiological (chlorophyll content) and qualitative taxonomic analyses

Figure 3
Photo of SonTek Flow Tracker-ADV Unit Used to Collect Point Velocity Measurements
above Substrate and Velocity Profiles across the Channel



Table 2 provides the number of samples that were collected for each of the biological sampling, processing, and analysis components for the entire Project.

Table 2
Number of Replicates and Total Samples for Project

	Replicates per transect ¹	Total Replicates for 26 transects (Spring)	Total Replicates for 26 transects (Fall)	Total Samples for Both Events
Submerged Aquatic Vegetation				
Cover	5	130	130	260
Biomass Sample Collection	3	72	69	141
Morphometrics Processing ²	12	180	186	366
Dry Weight (Root/Shoot)	6	90	93	183
Dry Weight (SAV biomass)	3	72	69	141
Total SAV Related Samples	29	544	547	1091
Macroalgae				
Cover	5	130	130	260
Biomass Sample Collection	3	33	27	60
Dry Weight & AFDW (algal mat)	3	33	27	60
Chlorophyll a (algal mat)	3	33	27	60
Qualitative sample identification	3	33	27	60
Total Macroalgae Related Samples	17	262	238	500
Epiphytic Algae				
Cover	5	130	130	260
Dry Weight & AFDW (algal mat)	3	69	69	141
Chlorophyll a	3	72	69	144
Qualitative sample identification	3	72	69	144
Total Epiphytic Algae Related Samples	14	274	337	611
Benthic Macroinvertebrates				
Taxa classification and identification (SAV habitat)	3	72	69	141
Taxa classification and identification (macroalgal habitat)	3	33	27	60
Total Macroinvertebrates Related Samples	6	105	96	201
Grand Total for Sample Collection, Processing, and Analysis	66	1185	1218	2403

¹ Maximum number of replicates if all sample types (SAV and Macroalgae) and species (*Sagittaria kurziana* and *Vallisneria americana*) were found on each transect.

² The number of Replicates per transect shown for Morphometrics Processing is based on both *Sagittaria kurziana* and *Vallisneria americana* being present at the transect. This number varies across transects, and the subsequent columns are based on actual presence and abundance of these species.

3.2.1.1 *Physical and Chemical Sampling*

Physicochemical sampling consisted of *in-situ* physical and chemical sampling at each of the 26 transects on six individual sampling occasions throughout 2015. Two of those sampling events were concurrent with biological sampling events. The field parameters that will be collected by Amec Foster Wheeler staff during physicochemical sampling events are as follows:

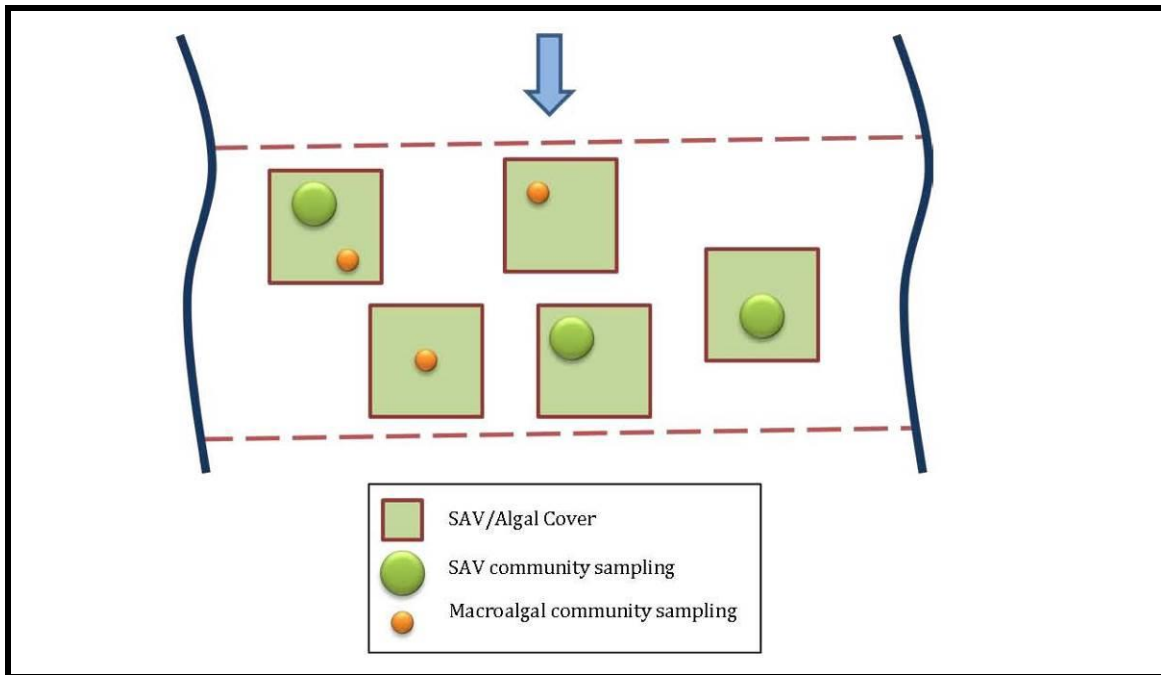
- Water depth (total, m), with a levelling rod or a wading rod
- Depth to top of SAV canopy (m), with a levelling rod or a wading rod
- Canopy cover over the stream channel (%), with a spherical densiometer (Model–C)
- Current velocity measurements at each Braun-Blanquet quadrat above benthic substrate (m/s), with a SonTek FlowTracker (velocity accuracy of ± 0.008 ft/s)
- Detailed current velocity profile measurements across the channel (m/s), with a SonTek FlowTracker (velocity accuracy of ± 0.008 ft/s)
- Water temperature ($^{\circ}\text{C}$), with a YSI- 5 series
- Specific conductance ($\mu\text{mhos/cm}$), with a YSI- 5 series
- pH (units), with a YSI- 5 series
- Dissolved O_2 (mg/L and % saturation), with a YSI- 5 series
- Turbidity (NTU), with a portable turbidimeter
- Staff gauge reading (if one is nearby the sampling transect)

SJRWMD's Field SOPs for Surface Water Sampling were followed to collect water chemistry measurements.

3.2.1.2 *Quantitative Submerged Aquatic Vegetation and Algal Sampling*

Biological monitoring transects were positioned perpendicularly to river flow for all biological samples collected as part of this project. Amec Foster Wheeler conducted quantitative SAV and benthic algal abundance (coverage and dry weight biomass) sampling within five randomly (for less than 12 m channel width) or systematically placed (for greater than 12 m channel width) sampling quadrats (replicates) along each transect. Biological samples were collected according to the scheme shown in **Figure 4** within each transect for both cover and biomass.

Figure 4
Diagram of Biological Sampling Quadrats within Transect



Cover

Five replicates of SAV, macroalgae, and epiphytes were visually estimated for coverage within a 1-m² quadrat on a percent coverage scale that was converted to a modified Braun-Blanquet scale. The modified Braun-Blanquet scale used for estimating coverage is shown below:

- 5 = >75-100% cover
- 4 = >50-75% cover
- 3 = >25-50% cover
- 2 = 5-25% cover
- 1 = <5% cover
- 0 = Bare sediment (no plant cover at all)

SAV Biomass

At or nearby three of the transect sampling quadrats, three above-sediment SAV replicate samples were collected for dry weight biomass with a modified Hess sampler as shown in **Figure 5**. The modified Hess sampler has a known area of 0.064 m². SAV biomass samples were collected by placing the sampler over an area of SAV, clipping the material above the sediment surface, and retaining it in the sampler netting. The SAV biomass samples collected were the same samples that were used to collect SAV-associated macroinvertebrates in the laboratory. The SAV samples were carefully transferred into large plastic bags and preserved fresh in cold storage (on ice) until the samples were transported to the laboratory for processing within the 24 hour holding time.

Algal Biomass

Benthic macroalgae mats and epiphytic algae were sampled from the stream bottom and SAV leaves, respectively, within each transect. Algal biomass sampling methods differ for the two types of algae.

Benthic macroalgae was collected within or nearby the cover quadrats with the modified Hess sampler mentioned previously (**Figure 5**). The macroalgal biomass samples were the same samples used to collect macroalgae-associated macroinvertebrates in the laboratory. The macroalgal biomass samples were carefully transferred from the sampler into plastic bags and preserved fresh in cold storage (on ice) until the samples were transported to the laboratory for processing within the defined 24 hour holding time.

The epiphyte biomass samples were obtained by collecting several healthy (non-necrotic) leaf blades of varying age and maturity of the dominant SAV species within each transect. At least three leaves were scraped for biomass sample; however, additional quantities of leaves sometimes were needed to provide enough material to accurately determine ash free dry weight (AFDW) of the epiphytic algae sample. Leaves were preserved on ice until the samples were transported to the laboratory. Epiphytic material was obtained by carefully scraping the epiphytes from the SAV leaves. One site, Juniper Springs Run, had little SAV along transect 1 and the SAV leaves had very little epiphytic fouling. The leaves did not produce sufficient epiphytic material for obtaining dry weight (DW) or AFDW. Therefore, replicates at that site (JUN-1) will not have analytical results for epiphyte related data.

In addition to the algae biomass samples, sufficient macroalgae and epiphytic material needed to be obtained to perform algal community measurements, with at least three replicate samples for each measurement. Algal community measurements for each replicate include dominant taxa composition, dry weight, ash-free dry weight, and chlorophyll-a (mg/m^2 , and corrected for phaeophytins). Epiphytic material was quantified using the SAV leaf area. The community measurements chlorophyll a (mg/m^2 , and corrected for phaeophytins) and qualitative taxonomic identification for both macroalgal and epiphytic algal samples were performed by Amec Foster Wheeler's subcontractor's AEL and GreenWater, respectively. Further detail on the measurements are provided in the laboratory component of this task.

Figure 5
Photo of Custom-Made Modified Hess Sampler Used to Collect SAV and Benthic Macroalgal Samples



Morphometrics

In addition to the biomass samples, six whole plants of meadow-forming SAV, specifically *Sagittaria kurziana* and *Vallisneria americana* (if both were present at the site), were collected as a composite sample across the transect. An effort was made to capture the variability of water depth, light availability, and current velocity within the transect. Each whole plant sample required a minimum of five shoots, including a rhizome, roots, and leaves. In some cases, it was not possible to obtain a plant with all five shoots without uprooting a large area of the SAV meadow in order to obtain an entire plant. In those cases, smaller plants of two to three shoots were obtained. In those cases, up to twelve whole plants were obtained. Refer to **Table 2** for the number of replicates that were collected and processed for SAV morphometrics. The SAV samples were carefully transferred into plastic bags and preserved fresh in cold storage (on ice) until the samples were transported to the laboratory for morphometric processing.

3.2.1.3 *Quantitative Benthic Macroinvertebrate Sampling*

This task consisted of sampling macroinvertebrates from the SAV and macroalgal habitats that were collected for their respective biomass measurements. Methods regarding sampling procedure, number of replicates, and preservation for sampling of those habitats is provided in **Section 3.2.1.2**. Methods for processing, analysis, and calculation of community measurements for the macroinvertebrate samples is provided in the laboratory component part of this task below.

3.2.2 Laboratory Component - Sample Processing and Analysis Methodology

Amec Foster Wheeler conducted laboratory biological sample processing and analyses for the vast majority of the activities necessary to meet the laboratory processing and analysis objectives specific to the laboratory component of the project. Amec Foster Wheeler's in-house biological laboratory staff conducted the following tasks:

- SAV biomass sample processing, sorting to species, taxonomic identification

- SAV morphometric analyses (including dry weight of whole plants and above/below ground fractions)
- Macroalgal biomass sample processing for dry weight and ash-free dry weight (per benthic area)
- Epiphytic biomass algae dry weight and ash-free dry weight (per leaf area)
- Macroinvertebrate processing, taxonomic identification from both SAV and macroalgal habitats, macroinvertebrate community measurements

Amec Foster Wheeler's project partner, GreenWater, Inc., conducted all algal taxonomy activities for macroalgae and epiphytic algae. GreenWater's algal taxonomy laboratory performed the following specific tasks:

- Epiphytic algae processing (carefully scraping algae from known area of SAV leaves) for qualitative sample taxonomic identification
- Macroalgal processing for qualitative sample taxonomic identification

Amec Foster Wheeler laboratory staff conducted the chlorophyll extraction procedure and transported samples for final chlorophyll a (and chlorophyll a corrected for phaeophytins) analysis to Advanced Environmental Laboratories (AEL). AEL provided chlorophyll pigment analytical results on a mass per extraction volume (mg/m³) basis for macroalgae and epiphytic algae samples. Detailed laboratory processing and analytical methodology SOPs were developed specifically by Amec Foster Wheeler Laboratory for this project and can be found in the **Project Work Plan – Appendix D**, along with the chain of custody (COC) forms and bench sheets that were used to conduct appropriate QA/QC measures and are provided in the **Project Work Plan – Appendix E**.

3.2.2.1 *Laboratory Processing of Biological Samples*

Samples collected from each transect provided type, density, morphometric, and community measurements for SAV, algae, and macroinvertebrates. The Laboratory SOPs (**Project Work Plan – Appendix D**) have outlined the laboratory processes in a way that demonstrates requirements for preservation, holding time, and any potential for dual uses (and therefore increased processing efficiency) of samples that follow a sample through the processing steps and usages.

After each daily sampling event, field-collected samples were transported to the lab on ice for immediate processing, preservation, and/or storage at appropriate temperatures. **Table 3** provides the type and number of samples for processing that were returned to the laboratory per transect. The quantities may not reflect the total number of replicates as some samples were further divided in the laboratory.

Table 3
Parameters Obtained per Sample Type

Type of Sample	Sample Type Name	Replicate Quantity	Purpose	Parameter
SAV leaves per known area	Sample A	3	Dual	- Macroinvertebrate Community Measurements - SAV DW
Macroalgae per known area	Sample B	3	Dual	- Macroinvertebrate Community Measurements - Macroalgal DW & AFDW - Chlorophyll-a
Macroalgae per known area	Sample C	3	Single	- Qualitative Identification - Semi-quantification per bottom area
SAV Plants	Sample D	3	Dual	- Epiphyte Qualitative Identification - Epiphyte Semi-quantification per SAV leaf area
SAV Plants	Sample E	3	Dual	- Epiphyte DW & AFDW per SAV leaf area - Epiphyte Chlorophyll-a per SAV leaf area
SAV Plants	Sample F	6-12	Dual	- Leaf Count, Leaf & Rhizome Measurements - Above- & Below-ground DW (3)

Note: DW = Dry Weight; AFDW = Ash Free Dry Weight

SAV Biomass

Samples containing SAV leaves per known area were processed as described above to remove macroinvertebrates. SAV leaves from which all silt, sand, macroinvertebrates, and epiphytes were removed were sorted by species, dried to constant weight at 100°C, and dry weight (DW) in grams was determined. Dry weight was used as part of the macroinvertebrate density calculations.

SAV Morphometrics

SAV samples containing six to 12 whole plants of dominant SAV species were held at 4°C until ready for processing. Only those plants that had a minimum of five whole shoots were used. However, if there were not enough plants with at least five shoots available, plants with four or fewer shoots were measured. For each set of whole plants, the following measurements were recorded: 1) number of leaves per shoot; 2) Leaf length (cm); 3) Leaf width (mm); and 4) Internodal distance (length of rhizome between shoots, cm).

Above- and Below-ground Biomass

Three of the plants above were used to obtain shoot and root/rhizome dry weights. Above-ground material of each plant was severed from the below-ground roots and rhizomic material.

Above- and below-ground material was dried to constant weight at 100°C, and dry weight in grams determined.

Macroalgae Biomass

Samples containing macroalgae per known area were processed similarly as described for SAV biomass above to remove macroinvertebrates. Macroalgal filaments from which all silt, sand, and macroinvertebrates have been removed were separated into two aliquots: one dime-sized aliquot and the remainder. The dime-sized aliquot was weighed and frozen for later processing for chlorophyll-a. The remaining larger aliquot was dried to constant weight at 100°C, and dry weight determined. AFDW was obtained after combustion of DW material in a 500 °F muffle furnace for 6 hours. Dry weight and AFDW was recorded per area (g dry weight/m² of bottom area and g AFDW/m² of bottom area, respectively). Dry weight was used as part of macroinvertebrate density calculations.

Epiphytic Algae Biomass

Epiphytic material was carefully scraped from leaves of dominant SAV until a designated weight was obtained that represents the minimum amount of wet weight material necessary to obtain measurable values of AFDW material. Prior to commencement of the project, Amec Foster Wheeler staff determined the minimum mass of DW of epiphytic material per composited leaves that produced ash weights with detectable values (refer to **Project Work Plan – Appendix D** for details). In order to minimize processing but ensure the appropriate amount of epiphytic material was obtained, the pre-determined mass was at least doubled to take into account varying proportions of epifauna or other combustible particulates that may be encountered with epiphytic material.

Epiphytic material was dried to constant weight at 100°C, and dry weight was determined. AFDW was obtained after combustion of DW material in a 500 °F muffle furnace for six hours. Dry weight and AFDW were recorded per composited leaf area (g dry weight/m² of leaf area and g AFDW/m² of leaf area, respectively).

Macroalgal Chlorophyll-a Processing

Aliquots of macroalgal chlorophyll a samples were obtained as described above and were frozen for holding until processing. Algal filaments of the aliquot were subject to physical rupture by the grinding-settling method with 80% acetone to extract pigments into solution (Su et al. 2010). Ground samples in acetone solution were allowed to settle in the dark for 24 hours at 4°C. Supernatant containing the pigments were shipped to the analytical lab (AEL) for chlorophyll-a and chlorophyll-a corrected for phaeophytins analysis. Chlorophyll-a and chlorophyll-a corrected are reported as milligrams (mg) chlorophyll-a/m² of bottom area. The following equations were used to determine chlorophyll-a and chlorophyll-a corrected as milligrams (mg) chlorophyll-a/m² of bottom area:

$$\text{Chlorophyll-a mg/m}^2 = \left(\frac{\text{Chl-a aliquot (mg/m}^3\text{)} \times \text{extraction volume (m}^3\text{)}}{\text{Sampled area (m}^2\text{)}^*} \right) \times \text{Ratio}$$

$$*\text{Sampled area} = 0.064 \text{ m}^3$$

Where Ratio equals the total weight of macroalgae (g) in a replicate divided by the weight of the dime-sized aliquot (g) used for chlorophyll-a analysis.

$$\text{Ratio} = \text{macroalgae (g)} / \text{macroalgal aliquot (g)}$$

Epiphytic Algae Chlorophyll-a Processing

The remaining leaves from shoots, processed as part of the epiphytic biomass samples described above, were processed to obtain known areas of epiphytic material for chlorophyll a and chlorophyll a corrected analysis. Methods for chlorophyll a extraction were followed as described for macroalgal samples. Chlorophyll a and chlorophyll a corrected are reported as mg chlorophyll a/m² of leaf area. The following equation was used to determine chlorophyll-a and chlorophyll-a corrected as milligrams (mg) chlorophyll-a/m² of leaf area:

$$\text{Chlorophyll-a mg/m}^2 = \frac{\text{Chl-a (mg/m}^3\text{)} \times \text{extraction volume (m}^3\text{)}}{\text{Sampled leaf area (m}^2\text{)}}$$

Semi-Qualitative Macroalgae Taxonomy

Samples containing macroalgal mats harvested from a known bottom area were sent to GreenWater Laboratories for processing and identification by an algal taxonomist. Samples were processed following Biggs and Kilroy (1994) and as described below:

Samples or subsamples were poured into a vessel and all debris removed (invertebrates, leaves, wood debris, moss, etc.). Sample and DI water was added to a blender and blended for at least 30 seconds (stopping every 10 seconds to free filaments that have caught on the blades) or until the mixture was free of obvious clumps of material. If the sample contained large amounts of filamentous algae, sharp scissors were used to cut filaments into smaller pieces prior to blending. Homogenized sample was poured into a stoppered bottle and shaken thoroughly to mix sample. Aliquots were taken and preserved with glutaraldehyde. One aliquot was placed into a settling chamber and allowed to settle for at least 15 minutes. If the material was too dense to be analyzed then a new sample was made by diluting the sample material with DI water. If there was not sufficient algae present in the material then a larger volume of sample was settled to achieve the desired algal density.

Samples were identified using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics and epifluorescence. The entire sample was scanned at 100X and a list of algal species present was generated. Higher magnification was used as necessary to aid in identification. Identifications were taken to the lowest practicable level. To estimate relative abundance of cell numbers the scale in **Table 4** was used:

Table 4
Scale of Cell Numbers Relative Abundance

Abundance Rating	Description of Rating	Estimated % Of Cells Observed
Rare (R)	Only one or two cells observed during entire scan	0-1
Frequent (F)	More than one cell is observed, but they appear sporadically	1-5
Common (C)	Individual cells appear in several fields of view	5-20
Abundant (A)	One or two cells appear in most fields of view	20-40
Very Abundant (VA)	Multiple cells appear in most fields of view	40-70
Dominant (D)	Cells greatly exceed those of other algae in numbers	70-100

Using the cell abundance rating and taking the relative size of the different algae into consideration, the relative percentage of total biovolume of the dominant algae (three to five taxa) was estimated (Ponander and Winter 2002). Photomicrographs were taken of the dominant algae in each sample. Specimen photos were submitted to the District's FTP website.

Epiphytic Algae Taxonomy

Samples containing whole SAV shoots harvested from the transects were sent to GreenWater Laboratories. Samples were processed following Sagan (2003) and Biggs and Kilroy (1994) and as described below.

SAV shoots were rinsed to remove silt, sand, and other non-epiphytic material. Macroinvertebrates were removed by hand if present. Leaves representing different age classes of leaves were selected from the interior portion of the shoot rosette, middle portion of rosette, and outer portion. The inner and outer leaves are comprised of youngest and oldest shoot leaves, respectively. Such subsampling is important in order to capture the different epiphytic community structure or species that colonize new versus older leaves (Burkholder and Wetzel 1989). Studies have also found that epiphyte biomass is higher on older leaves than on younger leaves (Bulthuis and Woelkerling 1983; Borum et al. 1984; Heijs 1984; Borum 1987; Horner 1987).

Selected leaves from each sample were gently scraped with a soft spatula or brush to remove attached epiphytes and the scrapings composited. The base, middle, and near the top of both sides of each leaf were scraped from *Sagittaria* and *Vallisneria* leaves. Scraped areas of leaves were examined to ensure that all or most of the algal epiphytes were collected without excessive damage to the algal cells and that excessive amounts of host plant tissue were not being inadvertently collected. The area of all sides of the leaves from which material was scraped was measured. Epiphytic material was scraped into a blender into which DI water was added and blended. The remaining processing, algal identification, and semi-quantitation methods are the same as described above for macroalgal samples.

Macroinvertebrate Processing and Taxonomy

Samples containing SAV leaves and macroalgae per known area were processed within 24 hours of sampling to prevent death and decomposition of the macroinvertebrates within the habitat samples. FDEP SOP (FS7400) for Benthic Macroinvertebrate Sampling dictates that

samples may be stored on ice (with no addition of formalin) if they will be processed within 24 hours of sampling. Macroinvertebrates attached to or collected with SAV or macroalgae were removed from samples and identified to the lowest practical taxonomic level (LPTL) using the following the steps below.

Samples received at the Amec Foster Wheeler Taxonomy Lab were logged-in and processed in general accordance with FDEP SOP for Invertebrate Core/Grab/Dredge Sample Prepared (IZ-04). Samples were rinsed over a U.S. #30 sieve to catch invertebrates. SAV leaves/macroalgal filaments were gently scraped by hand and gently rinsed into the sieve. Sieve contents (macroinvertebrates) were transferred to appropriate containers and preserved with 10% buffered formalin and tinted with rose Bengal according to FDEP SOPs (FS 7400) and held for future sorting and identification.

Macroinvertebrate sorting was conducted as follows. Invertebrate samples were emptied into a U.S. #30 mesh sieve over a discard bucket to catch waste formalin. The sieved samples were thoroughly rinsed with tap water. The remaining material was transferred to white trays for sorting under a dissecting microscope (approximately 10X magnification). All organisms were picked from the sample material and placed in a vial filled with 80% ethanol. QA/QC checks were be completed on at least 10% of sorted aliquots.

Depending on the amount of material in the sample, the material was transferred to petri dishes in separate aliquots for sorting. In the case of excessively large samples, the sample was subsampled, and the subsampling methodology is clearly described in association with the data and appropriate notes are included in the laboratory benchsheets (e.g., divisible by 2, 4, etc.). The data provided in the Data Bioloader reflects the values corrected for subsampling.

Amec Foster Wheeler experienced taxonomists then identified the organisms in each sample according to FDEP SOP IZ-06. Organisms in each sample were identified to lowest practical taxonomic level (LPTL) and the identifications and enumeration are noted on benchsheets. Midges and worms were separated from the remainder of the sample for mounting and further identification under compound magnification. Midges and worms were mounted in general accordance with FDEP SOP IZ-08 and identified to LPTL and identification and enumeration are noted on benchsheets. Amec Foster Wheeler's extensive collection of taxonomic keys and reference specimens for invertebrates from Florida streams were used throughout the project to aid in identification. If an organism was found within the samples that was not already represented in our voucher reference collection, the individual was placed in a labeled vial in 95% ethanol and maintained for expert verification. Data was compiled to calculate macroinvertebrate community measurements as described below.

The following data was determined from the macroinvertebrate samples collected from each of the in-stream substrates (by replicate):

- Taxa richness – all macroinvertebrates collected were identified to the lowest practical taxonomic level using up to date taxonomic guides.
- Population density (as # individuals/g plant dry weight biomass, and as # individuals/m² of sampled area) by species – this was adapted after the methodology of Strayer and Malcom (2007) and Menzie (1980): the raw invertebrate abundance from each sorted sample was divided by the SAV total dry weight in the sample to get a value of # invertebrates/g of plant material. In addition,, the raw invertebrate abundance from each sorted sample was divided

by sampled area to obtain # invertebrates /m²). Population density of invertebrates in benthic algal mats was calculated in the same way.

- Diversity – the Shannon-Wiener index and the Margalef's species richness index was calculated for each replicate sample.
Evenness – Pielou's Evenness was calculated for each replicate sample.
 - Shannon's Index ($H'(\log_e)$) = $-\sum P_i * \log_e(P_i)$; where P_i = proportion of individuals found in the i^{th} species
 - Margalef's species richness index (d) = $(S-1)/\log_e(N)$
 - Pielou's Evenness (J') = $H'/\log_e S$
- Functional Feeding Group categorization – for each invertebrate taxon, a functional feeding group (FFG) category was associated with it in the database developed. These may be the FFG designations used by the FDEP (<http://www.dep.state.fl.us/labs/cgi-bin/sbio/database.asp>), Warren et al. (2000), Merritt and Cummins (1996), or other sources as determined by the Contractor and District staff.
- Life Habit categorization – for each invertebrate taxon, a Life Habit category as defined by Merritt et al. (1996) was associated with it in the database developed.
- Taxa identified by FDEP as “long-lived” (<http://www.dep.state.fl.us/labs/cgi-bin/sbio/database.asp#lists>) were identified in the database developed
- Taxa identified by FDEP as “sensitive” and “very tolerant” were identified in the database developed (<http://www.dep.state.fl.us/labs/cgi-bin/sbio/database.asp#lists>).

3.3 Data Summary and Reporting

3.3.1 Data Collection Summary

In-situ physical and chemical data was collected at each transect during four physicochemical sampling events that occurred throughout the course of the project. Two biological sampling events also occurred, during which *in-situ* physical and chemical data was collected as well as biological samples.

Samples and data were collected during two biological events which occurred during the Spring and Fall of 2015. During the spring biological event, samples were collected in the field from May 2015 to July 2015 and were processed in the lab from May-2015 to October-2015. During the fall biological event, samples were collected in the field from September 2015 to October 2015 and were processed in the lab from September-2015 to March-2016. **Table 5** summarizes which biological sample types were present at each transect and then processed in the lab (refer to **Table 3** for the descriptions of each sample type).

Table 5
Types of Biological Samples Collected in the Field and Processed in the Lab

Sampling Station	Sample A		Sample B/C		Sample D/E		Sample FS		Sample FV	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
ALE1	X	X	X	SNF	X	X	SNF	SNF	X	X
ALE2	X	X	X	X	X	X	SNF	SNF	X	X
GUM1	X	X	SNF	X	X	X	X	X	SNF	SNF
GUM2	X	X	SNF	SNF	X	X	X	X	X	X
ICH1	X	X	SNF	SNF	X	X	X	X	SNF	SNF
ICH2	X	X	SNF	SNF	X	X	SNF	SNF	X	X
JUN1	X	X	SNF	SNF	X	X	SNF	SNF	X	SNF
JUN2	X	X	SNF	SNF	X	X	SNF	SNF	X	X
MAN1	SNF	SNF	X	X	SNF	SNF	SNF	SNF	SNF	SNF
RAI1	X	X	SNF	SNF	X	X	X	X	SNF	SNF
RAI2	X	X	X	X	X	X	X	SNF	X	X
ROC1	X	X	X	SNF	X	X	SNF	X	X	X
ROC2	X	X	SNF	SNF	X	X	SNF	X	X	X
SIL1	X	X	SNF	SNF	X	X	X	X	SNF	SNF
SIL2	X	X	SNF	SNF	X	X	X	X	SNF	SNF
SIL3	X	X	SNF	SNF	X	X	X	X	X	X
SILG1	X	X	X	SNF	X	X	SNF	SNF	X	X
VOL1	SNF	SNF	X	X	SNF	SNF	SNF	SNF	SNF	SNF
WAC1	X	X	SNF	SNF	X	X	X	X	X	X
WAC2	X	X	SNF	SNF	X	X	X	X	SNF	SNF
WAK1	X	X	X	X	X	X	X	X	SNF	SNF
WAK2	X	X	X	X	X	X	X	X	X	X
WEE1	X	X	X	X	X	X	X	X	X	X
WEE2	X	X	X	X	X	X	SNF	X	X	X
WEK1	X	X	SNF	SNF	X	X	SNF	SNF	X	X
WEK2	X	X	SNF	SNF	X	X	SNF	SNF	X	X

Notes: "SNF" = Sample Not Found, which means that the sample type was not present at the transect or that there was not enough of that sample type to collect three replicates. An "X" indicates that three replicates were collected at that transect for that sample type. "Sample FS" refers to Morphometric samples consisting of *S. kurziana*. "Sample FV" refers to Sample F's consisting of *V. americana*.

3.3.2 Database and Reporting

Amec Foster Wheeler transmitted the following documents to the District's FTP website or by email to the District Project Manager for review and approval: final database, Amec Foster Wheeler lab bench sheets, Amec Foster Wheeler field sheets, AEL lab data sheets and reports, and GreenWater Laboratories lab data sheets and specimen photos.

All data, calculations, and metadata for the field sampling events and laboratory processing of samples were entered into the Data Bioloader database approved by the District. The Data Bioloader database includes data related to the following aspects of the project:

- Field data including sample locations and physical, chemical, and velocity profiles values
- Biological analytical data including SAV community measurements, quantitative algal values, SAV morphometric values, qualitative algal data, and benthic macroinvertebrate data
- Metadata

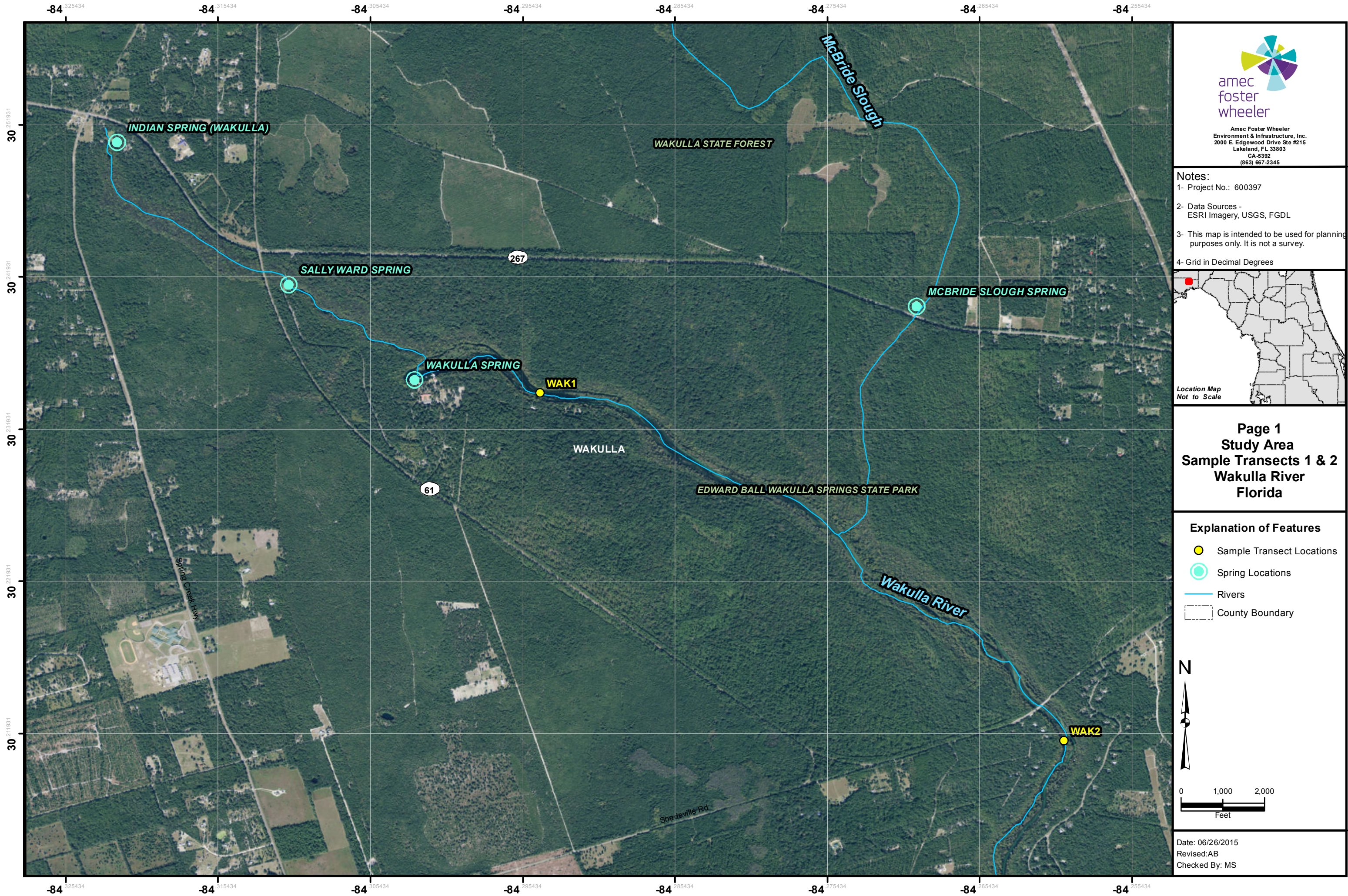
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Appendix A

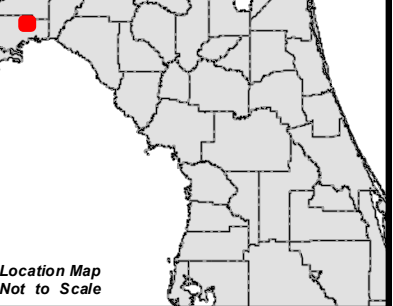
Site Maps



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- Notes:
- 1- Project No.: 600397
 - 2- Data Sources - ESRI Imagery, USGS, FGDL
 - 3- This map is intended to be used for planning purposes only. It is not a survey.
 - 4- Grid in Decimal Degrees

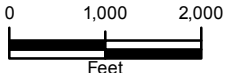


Page 1
Study Area
Sample Transects 1 & 2
Wakulla River
Florida

Explanation of Features

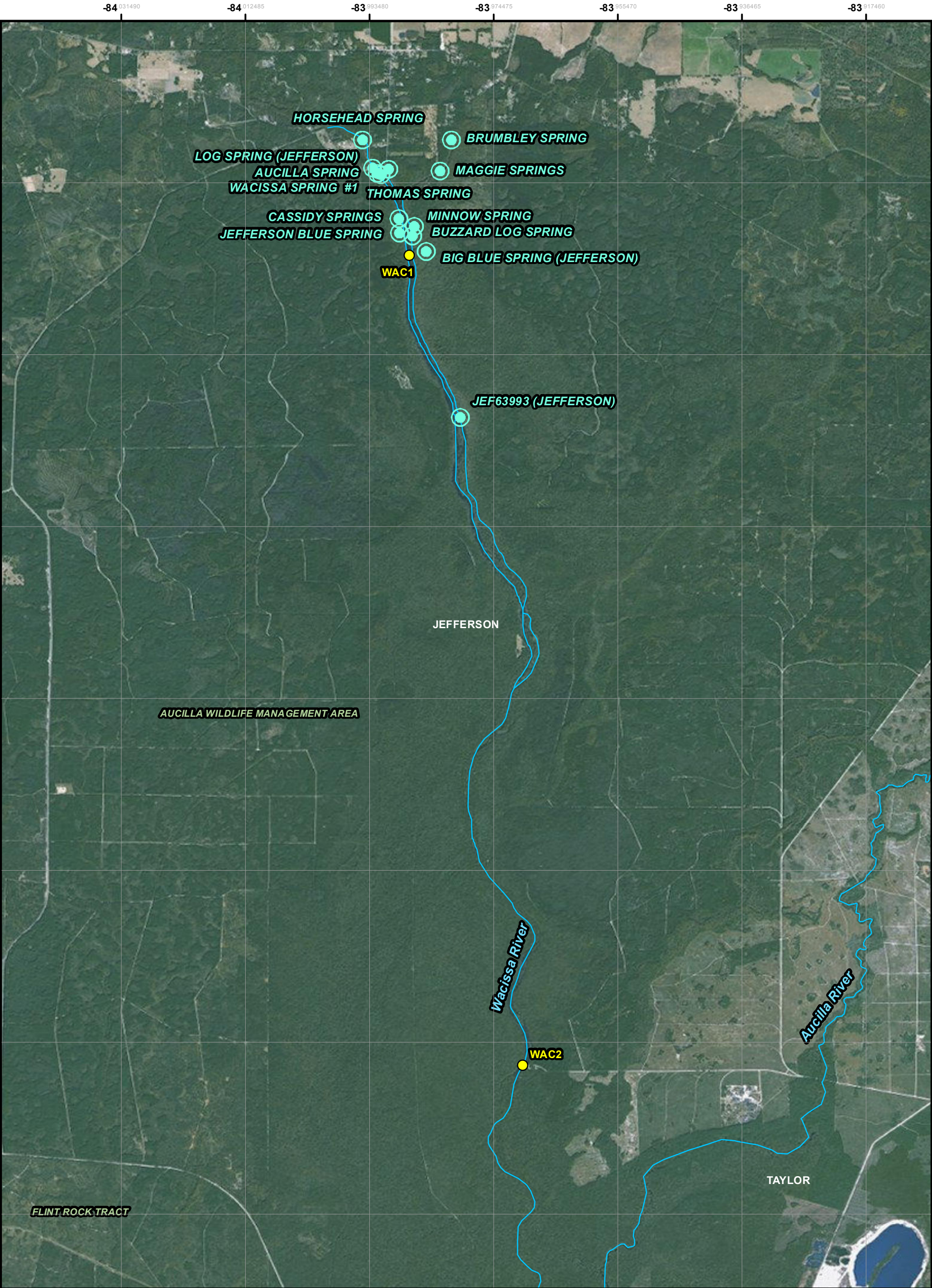
- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary

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Explanation of Features

Sample Transect Locations

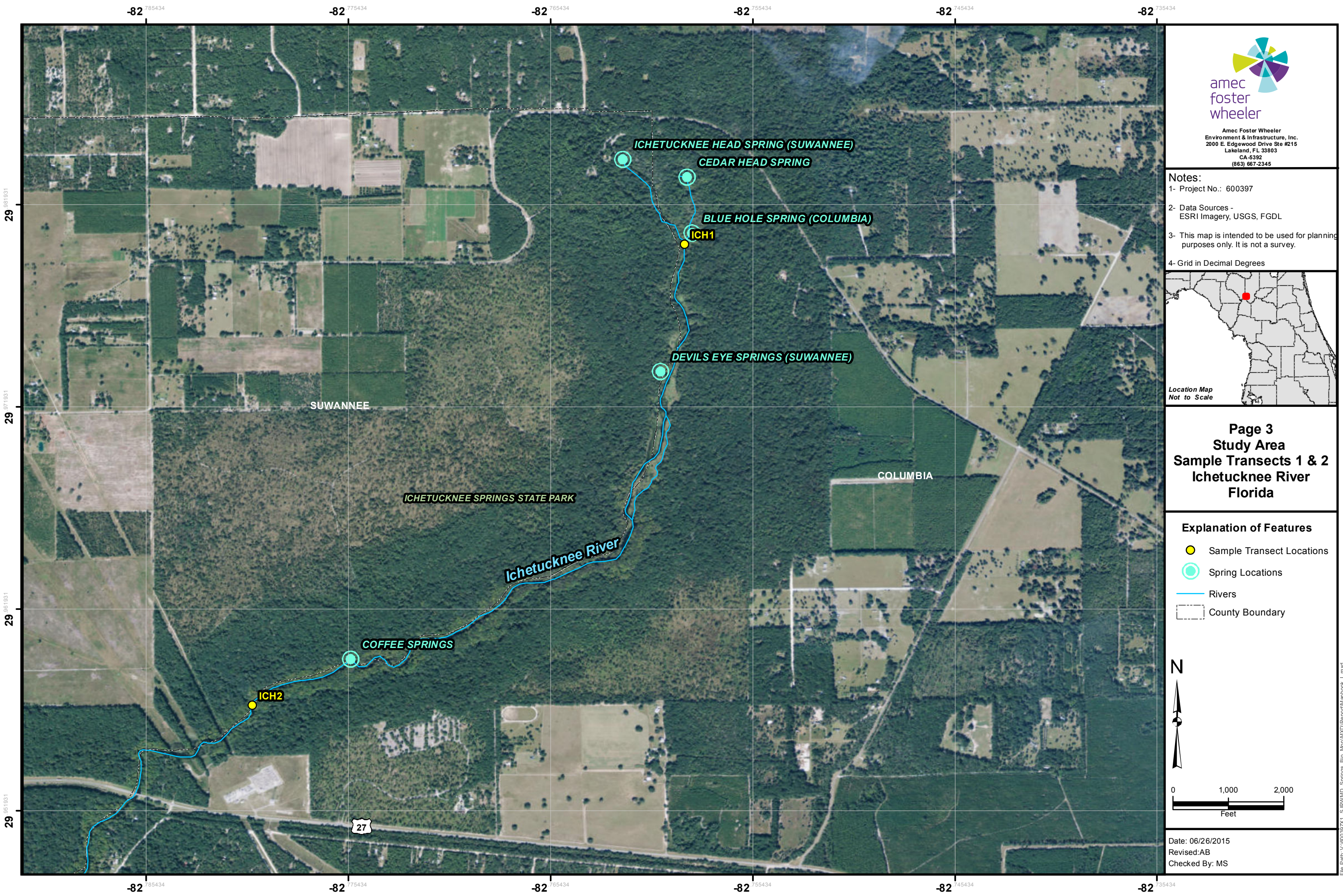
Spring Locations

Rivers

County Boundary

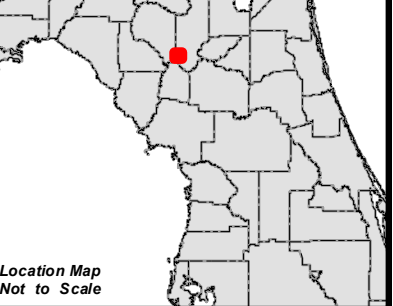


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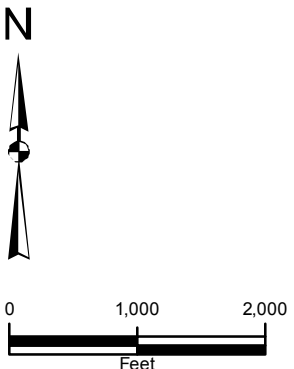
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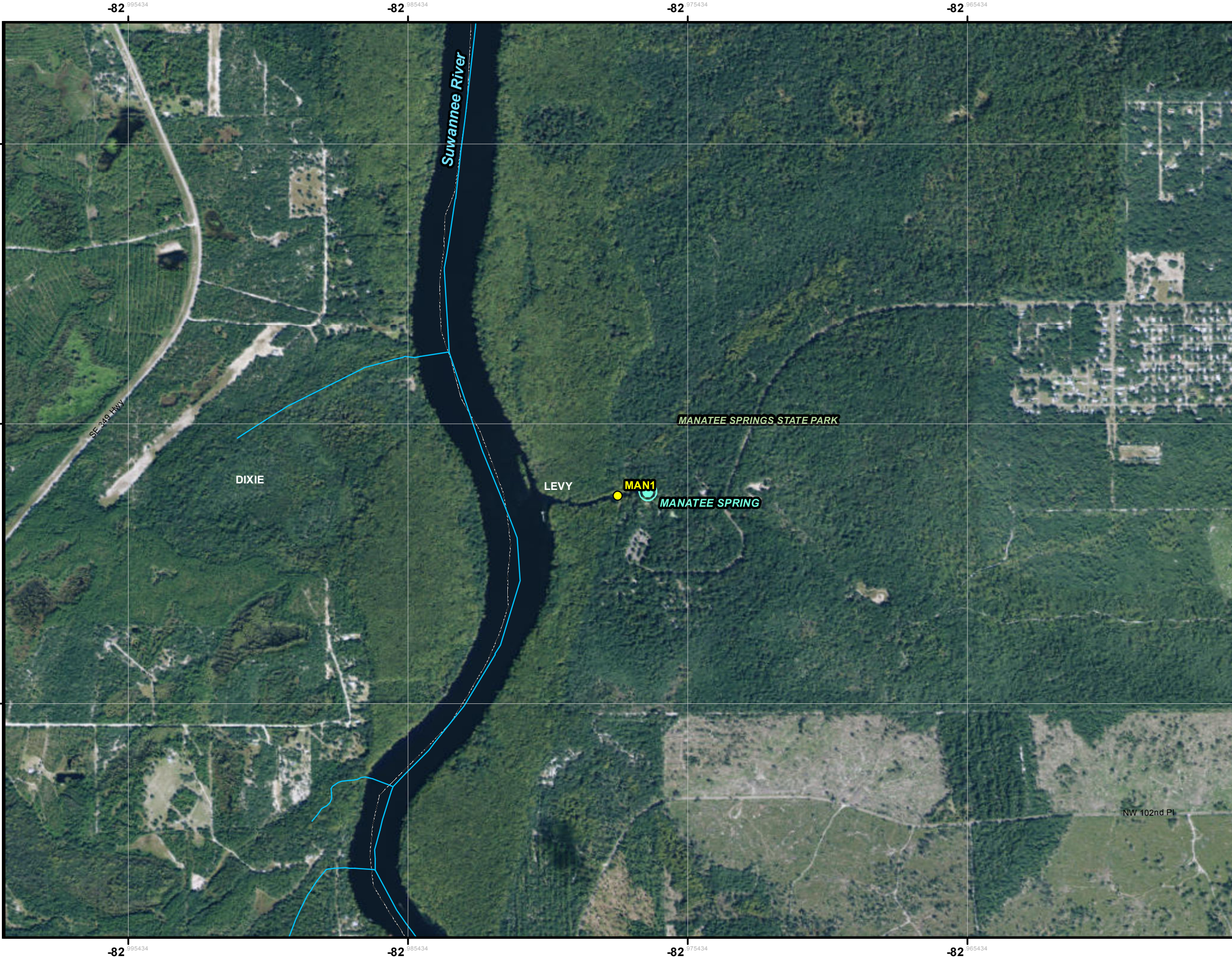
Page 3
Study Area
Sample Transects 1 & 2
Ichetucknee River
Florida

- Explanation of Features**
- Sample Transect Locations
 - Spring Locations
 - Rivers
 - County Boundary



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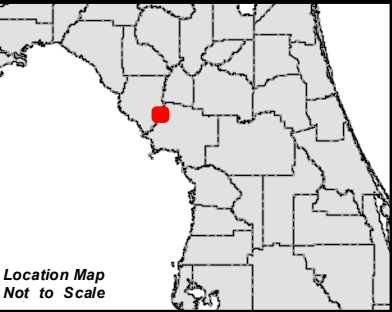


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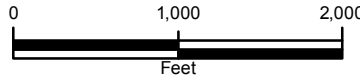
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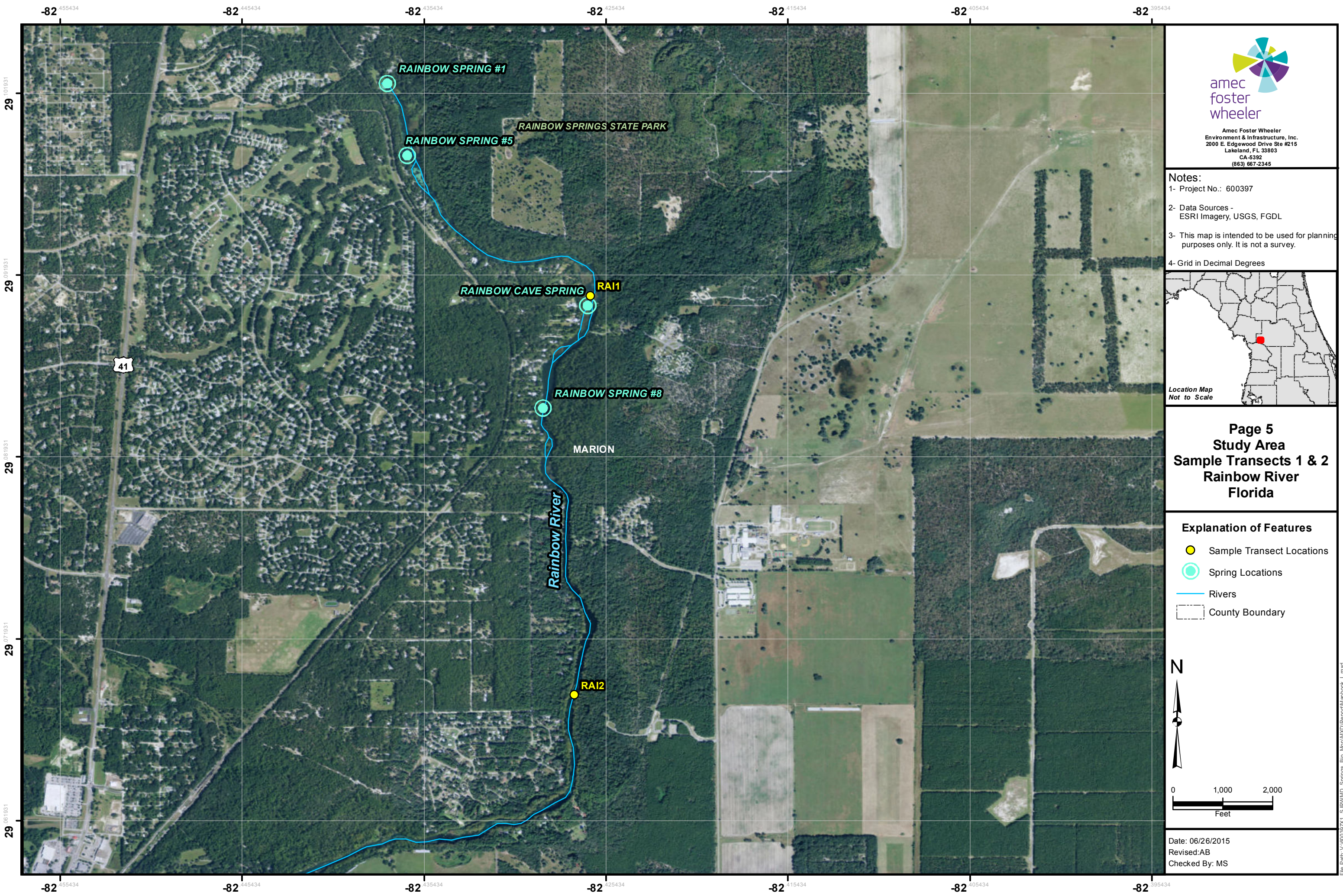
Page 4
Study Area
Sample Transect 1
Manatee Springs Run
Florida

Explanation of Features

-  Sample Transect Locations
-  Spring Locations
-  Rivers
-  County Boundary

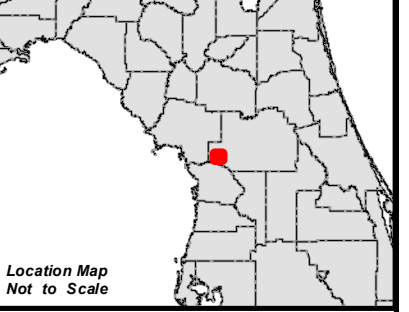


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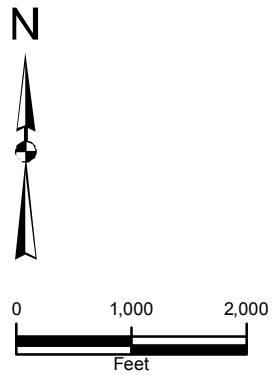
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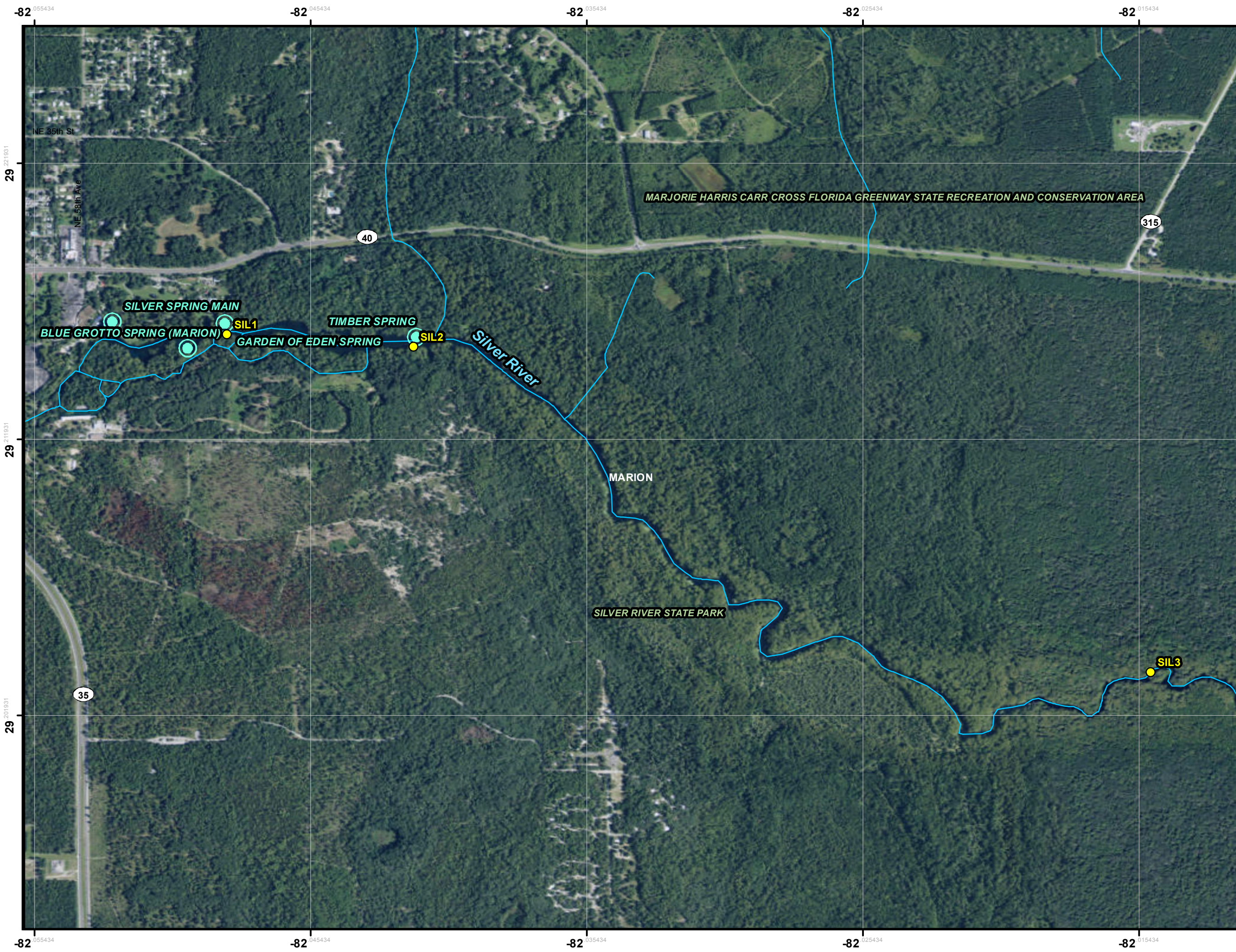
Page 5
Study Area
Sample Transects 1 & 2
Rainbow River
Florida

- Explanation of Features**
- Sample Transect Locations
 - Spring Locations
 - Rivers
 - County Boundary



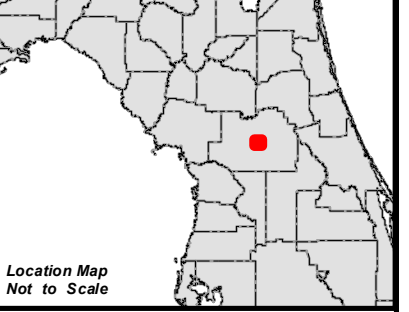
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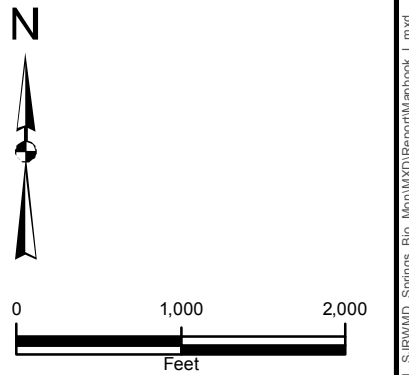
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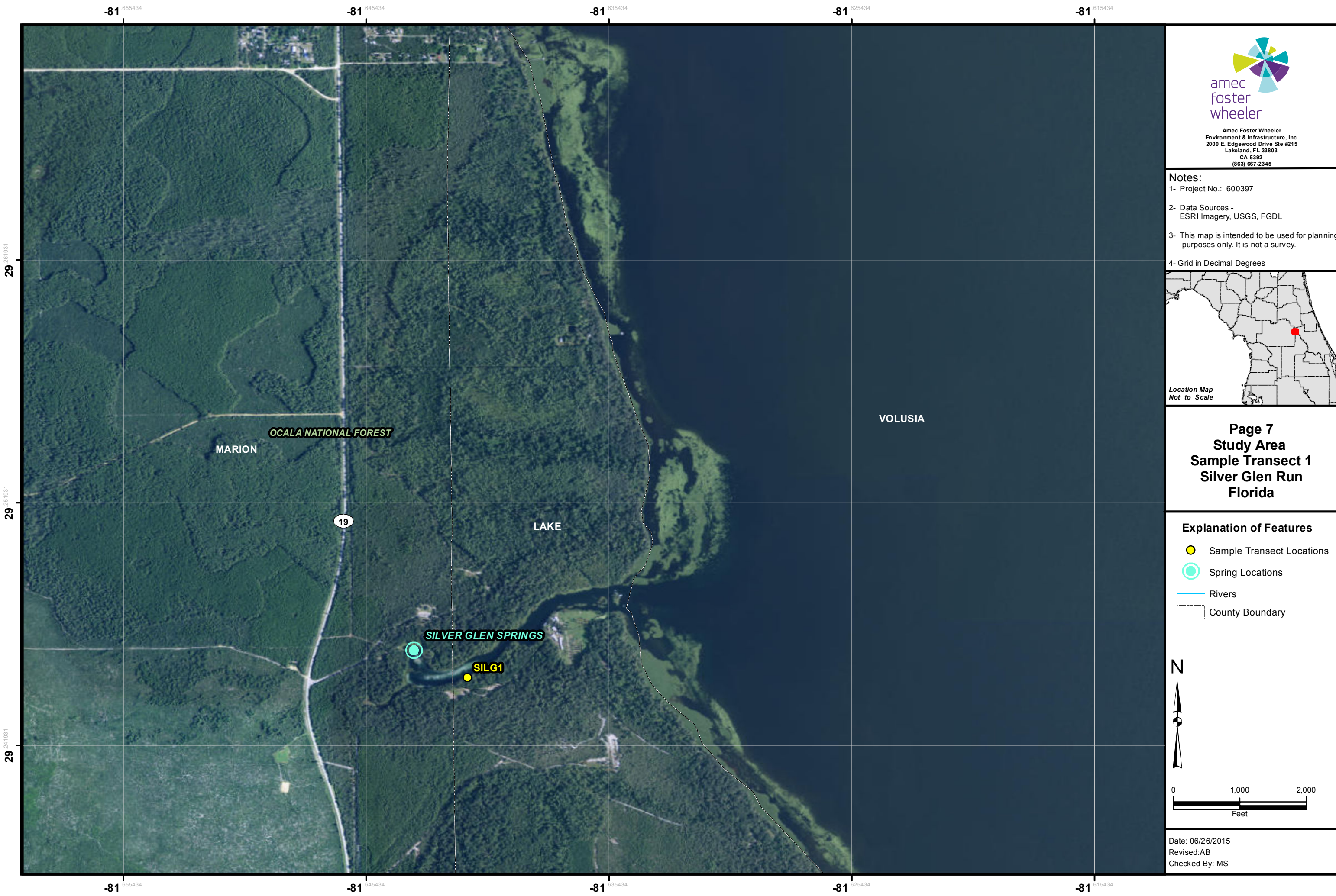
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Study Area
Sample Transects 1,2 &3
Silver River
Florida

- Explanation of Features**
- Sample Transect Locations
 - Spring Locations
 - Rivers
 - County Boundary



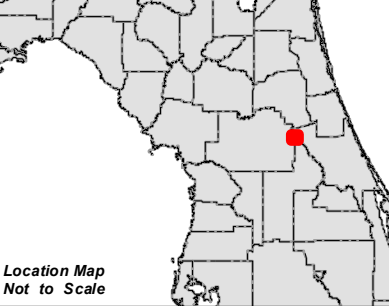
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- Notes:
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 - 2- Data Sources - ESRI Imagery, USGS, FGDL
 - 3- This map is intended to be used for planning purposes only. It is not a survey.
 - 4- Grid in Decimal Degrees

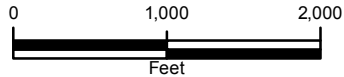


Location Map
Not to Scale

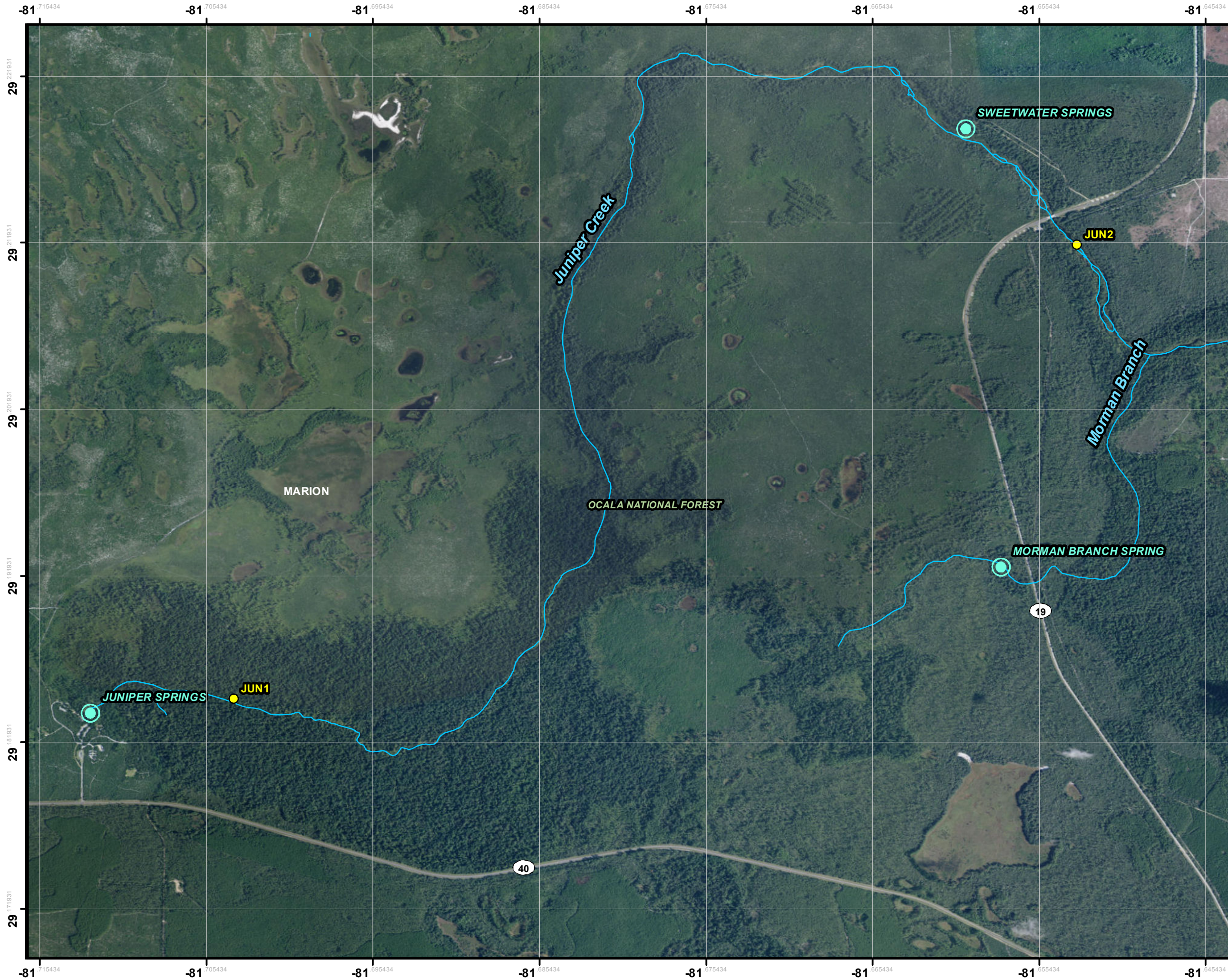
Page 7
Study Area
Sample Transect 1
Silver Glen Run
Florida

Explanation of Features

- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary



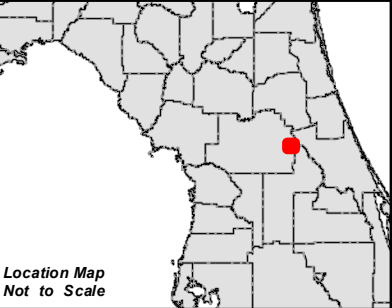
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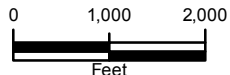
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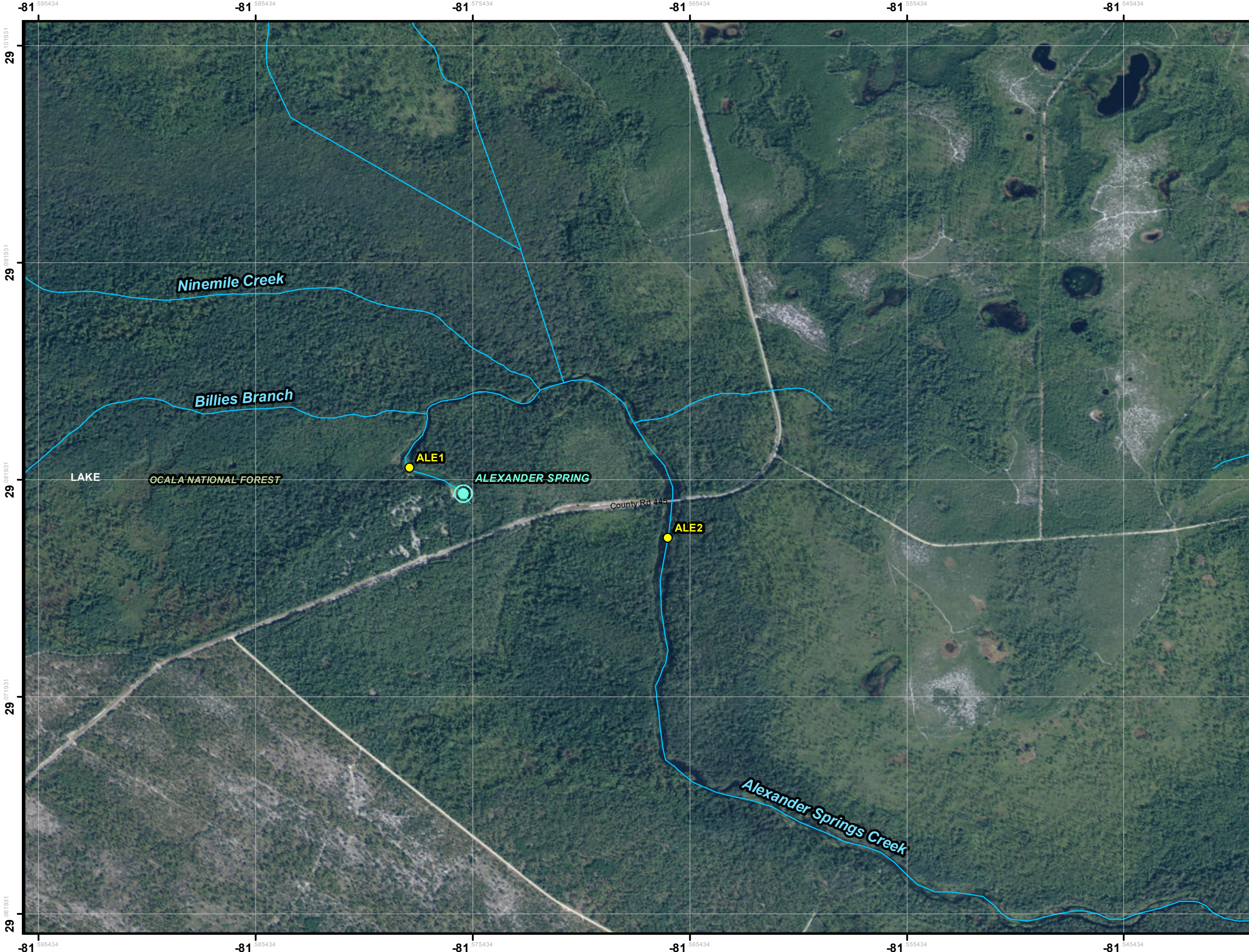
Page 8
Study Area
Sample Transects 1 & 2
Juniper Springs Run
Florida

Explanation of Features

- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary

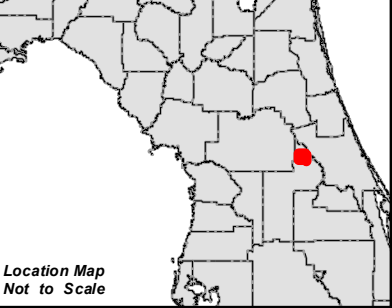


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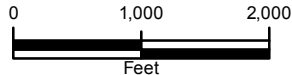


Location Map
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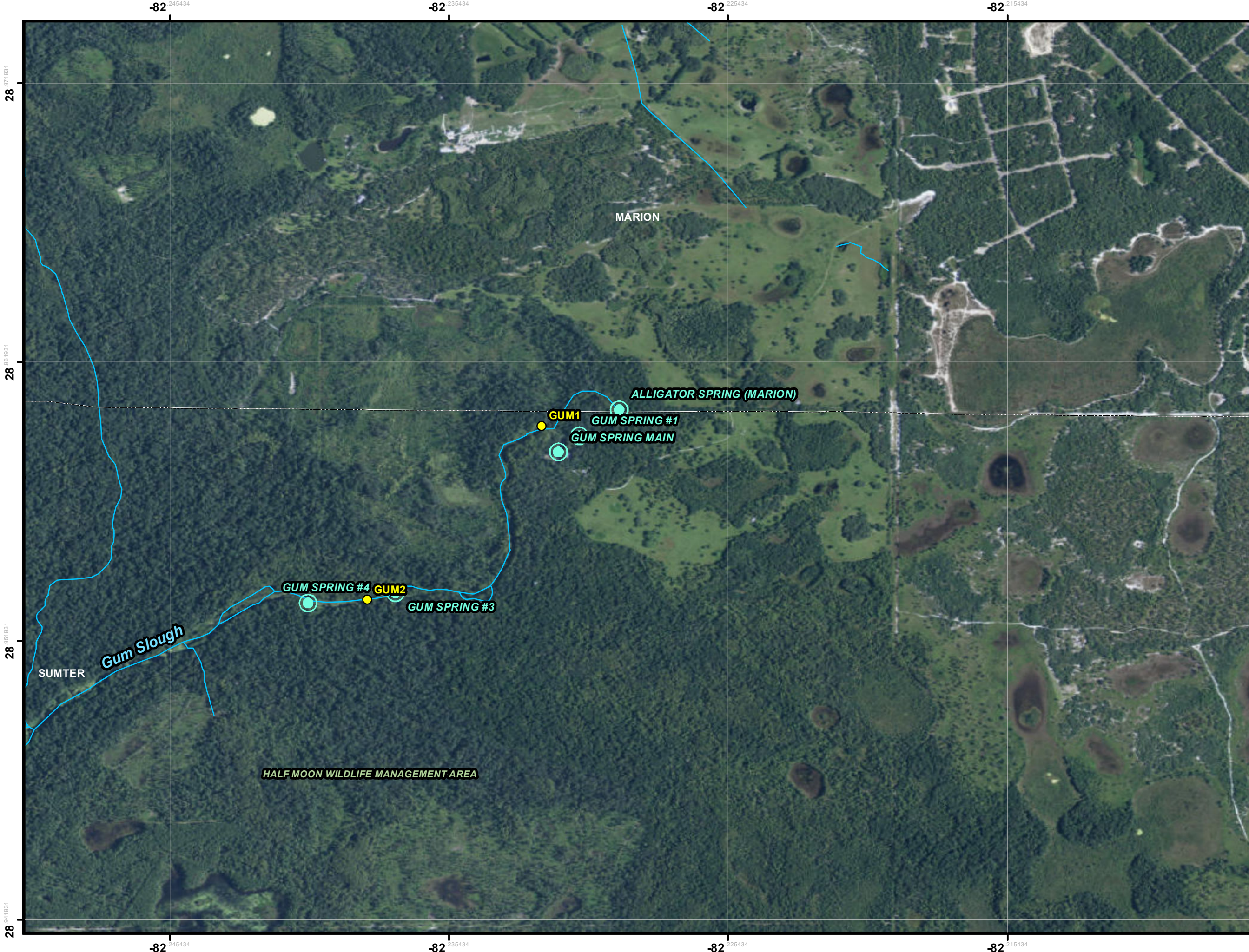
Page 9 Study Area Sample Transects 1 & 2 Alexander Springs Run Florida

Explanation of Features

- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary



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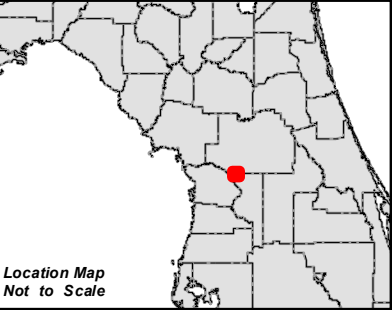


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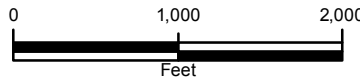
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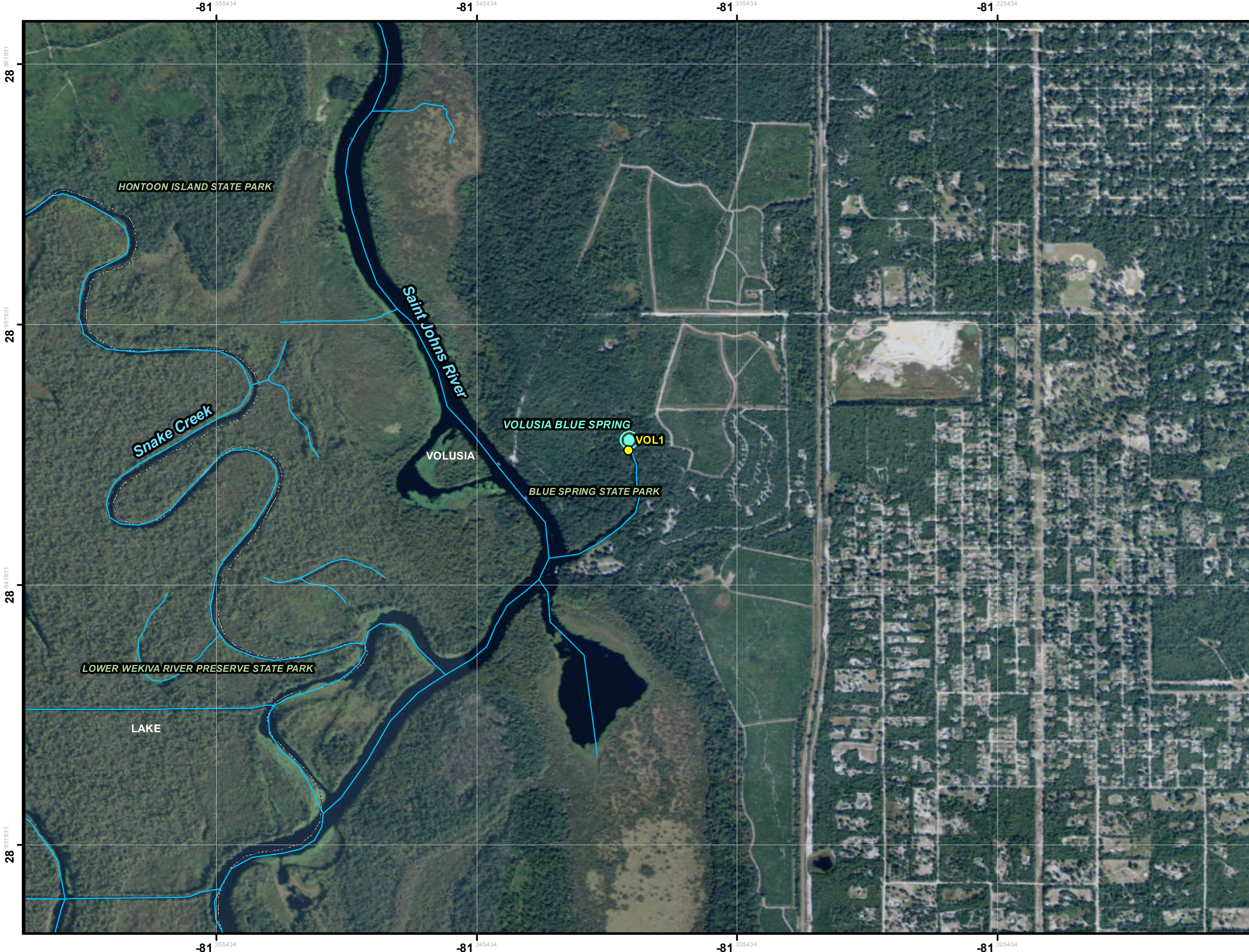
Page 10
Study Area
Sample Transects 1 & 2
Gum Slough Springs Run
Florida

Explanation of Features

- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary

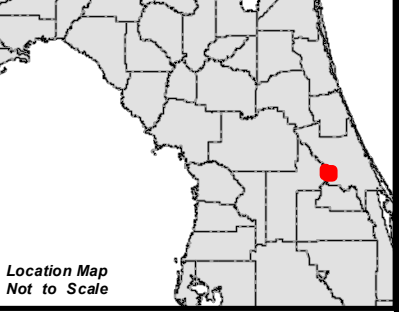


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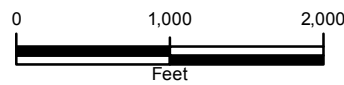


Location Map
Not to Scale

Page 11
Study Area
Sample Transect 1
Volusia Blue Run
Florida

Explanation of Features

- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary

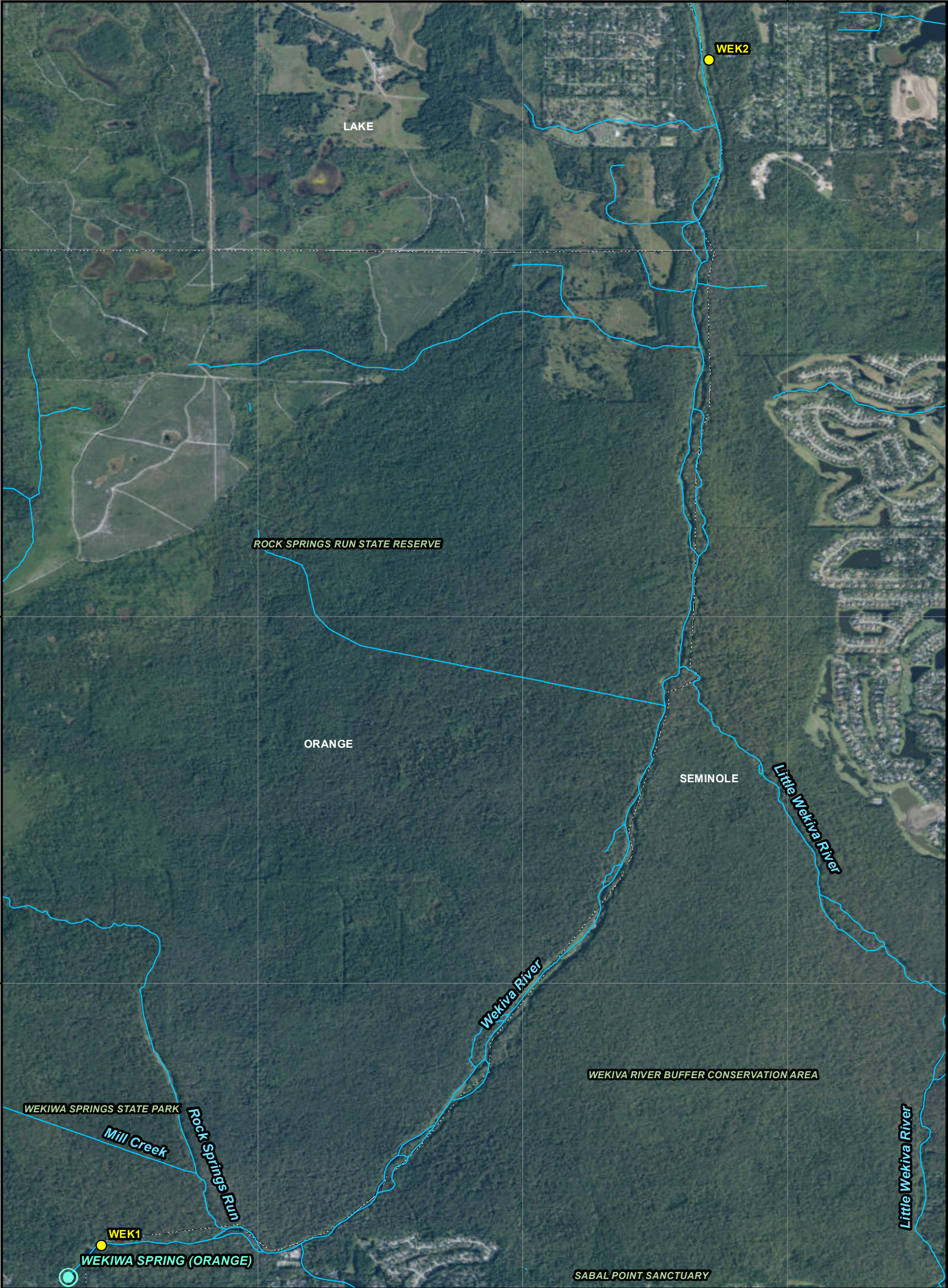


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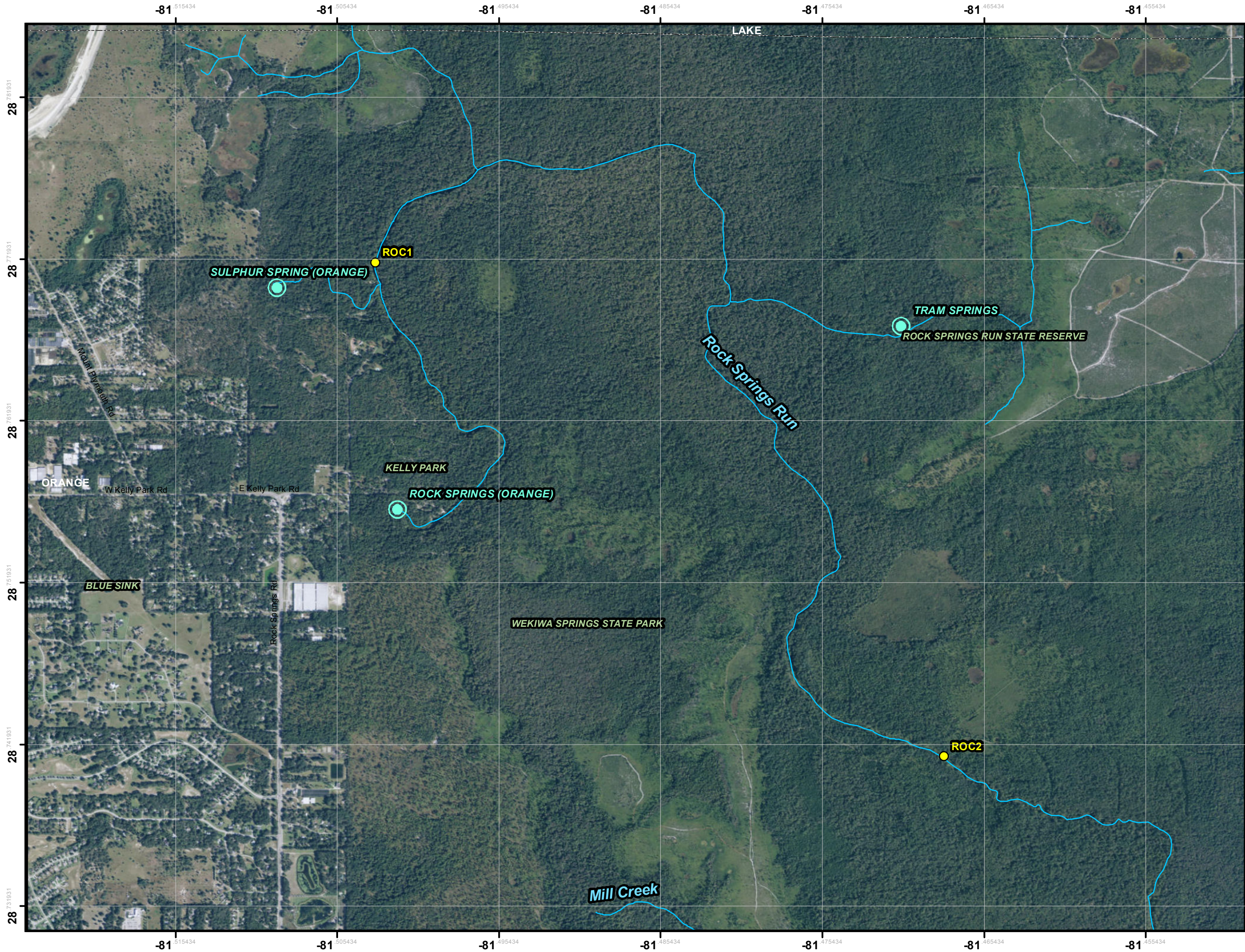
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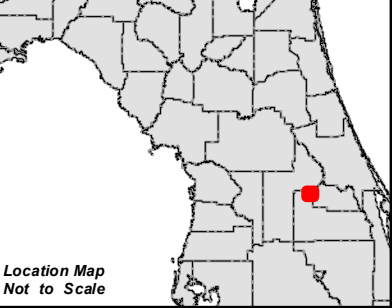


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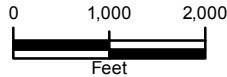


Location Map
Not to Scale

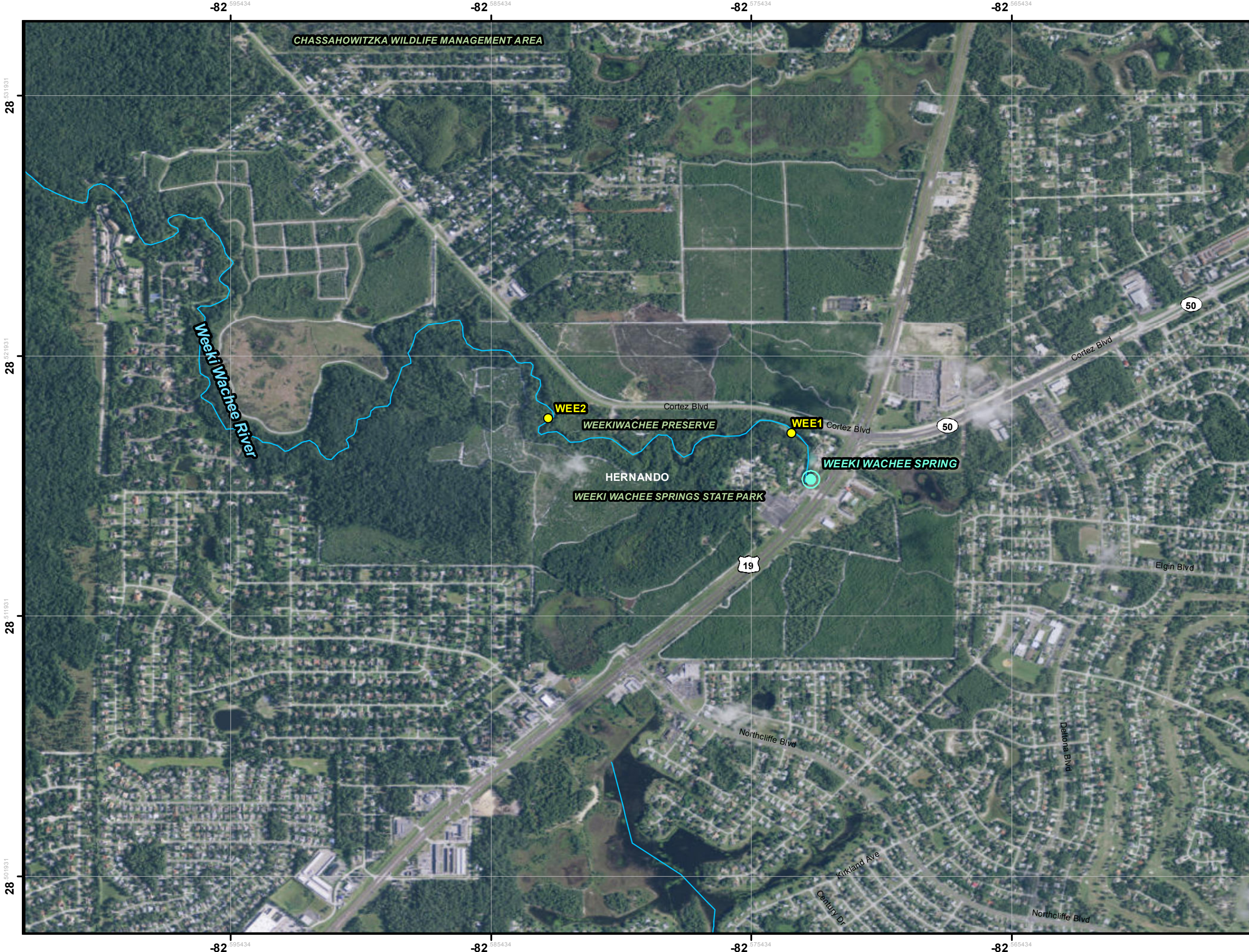
Page 13
Study Area
Sample Transects 1 & 2
Rock Springs Run
Florida

Explanation of Features

- Sample Transect Locations
- Spring Locations
- Rivers
- County Boundary



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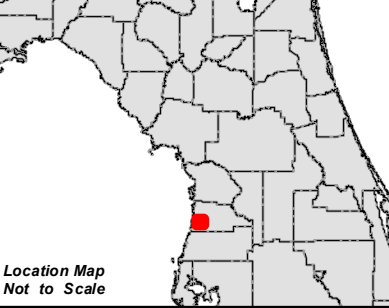


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Location Map
Not to Scale

Page 14
Study Area
Sample Transects 1 & 2
Weeki Wachee River
Florida

Explanation of Features

-  Sample Transect Locations
-  Spring Locations
-  Rivers
-  County Boundary

N



0 1,000 2,000
Feet

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