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SYNOPTIC BIOLOGICAL SURVEY OF 14 SPRING-RUN STREAMS IN NORTH AND CENTRAL FLORIDA

II. SUBMERGED AQUATIC VEGETATION COMMUNITIES - ALGAE

by

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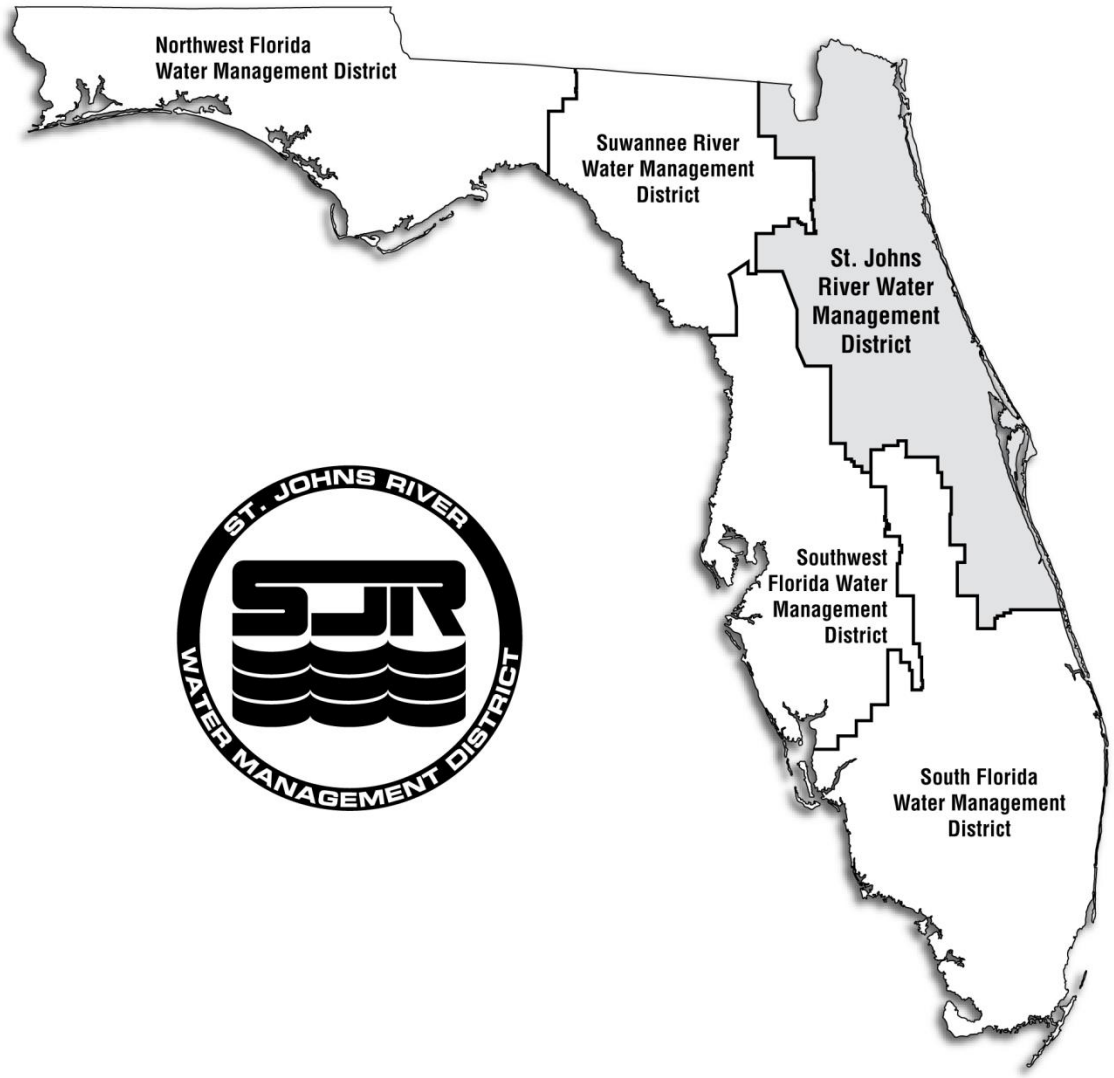

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EXECUTIVE SUMMARY

As part of a broader initiative to better understand, manage, and restore the springs of the St. Johns River, a short-term, synoptic biological study of 14 springs and their spring-run streams was undertaken by the St. Johns River Water Management District in 2015. This study quantitatively sampled physical-chemical characteristics and biological measures in these spring-run streams, including submerged macrophyte cover and dry weight; macro- and epiphytic algal cover, dry weight and ash-free dry weight; and vegetation-associated macroinvertebrate community richness, density, diversity, and biological characteristics. The submerged aquatic vegetation community (SAV — both macrophytes and algae) was a major focus of this study due to its prevalence in spring-run streams and because of the changes observed in this community in a number of springs over the past 50 years: a shift from a macrophyte-dominated to an algae-dominated community. This report presents the epiphytic and macroalgal species richness, cover and standing crop data and analyses from the springs synoptic sampling effort. Submerged macrophyte data were presented in a previous report (2019), and macroinvertebrate data and analyses will be presented in a subsequent report.

Six sampling events were conducted in 2015 to measure physical-chemical conditions (stream physical characteristics and *in situ* water quality). The spring-run streams and their headsprings exhibited a wide range of physicochemical characteristics, including channel width and depth, canopy cover, discharge, base water chemistry, and nutrient concentrations. Spring discharges ranged from small second-magnitude springs (Juniper) to some of the largest first-magnitude spring groups in Florida (Silver, Rainbow). Base water chemistry (concentration of dissolved solids such as calcium, chloride, etc. as measured by conductivity) ranged from near softwater, low ion springs (Juniper) to salt springs (Silver Glen). Nutrient concentrations (based on existing data, not collected in this study) also varied, from systems with low concentrations of nitrogen and phosphorus, indicating natural background water quality conditions (Juniper, Alexander), to systems with elevated concentrations of one or both nutrients (Silver, Wekiva).

Epiphytic algae and macroalgae were sampled in all 14 spring-run streams on two sampling events, in spring and fall 2015. Mean epiphytic algal taxa richness ranged from 2.7 to 5.7 in the spring and 3.0 to 7.3 in the fall. Highest mean taxa richness was seen in Alexander Springs Creek, Silver Glen Spring Run and Wacissa River. Most commonly occurring epiphytic algae were the cyanophyte *Microseira wollei*, the green alga *Cladophora glomerata*, and the diatom *Ulnaria* cf *ulna*. Mean macroalgal taxa richness (including attached epiphytic algae) ranged from 2.3 to 4.0 in spring and 2.0 to 4.0 in fall. Highest macroalgal taxa richness was seen in Rainbow River, Silver Glen Spring Run and Volusia Blue Spring Run. Most commonly occurring macroalgal taxa were the cyanophyte *M. wollei*, the green algal *Rhizoclonium heiroglyphicum*, and the filamentous diatom *Terpsinoe musica*.

Epiphytic algal and macroalgal abundance were measured using three measures: percent (%) cover, Chlorophyll a (Chl *a*), and Ash-Free Dry Weight (AFDW). Mean epiphyte cover

ranged from <1–100% in spring and 9.17-86.67% in fall; mean epiphytic Chl *a* ranged from 1.4–55.2 mg/m^2 in spring and from 0.4–17.5 mg/m^2 in fall; mean epiphytic AFDW ranged from 1.2–23.4 g/m^2 in spring and 0.05–17.6 g/m^2 in fall. Lowest algal standing crop as Chl *a* was generally seen in Juniper Creek and Weeki Wachee River and highest at Alexander Springs Creek, Rainbow River and Gum Slough. Lowest epiphyte standing crop as AFDW was generally seen at Juniper Creek and Weeki Wachee River and highest at Gum Slough and Rainbow River. Mean macroalgal cover ranged from 3–91% in spring and 10–70% in fall; mean macroalgal Chl *a* from 60.1–570 mg/m^2 in spring and 29.1-917 mg/m^2 in fall; mean macroalgal AFDW ranged from 7–142.8 g/m^2 in spring and 15.05–290.1 g/m^2 in fall. Highest macroalgal standing crop as Chl *a* and/or AFDW was generally seen at Alexander Springs Creek, Rainbow River and Silver Glen Spring Run in spring and Wakulla River and Weeki Wachee River in fall.

Algal community measures were compared among the 14 spring-run streams and with physical-chemical variables using the *BEST* procedure in the PRIMER software. The spring-run streams were grouped based on the composition of the algal community in terms of occurrence of the dominant algal taxa; few or no groupings of sites were seen based on algal abundance measures (both epiphytic and macroalgae). Physical-chemical variables that appeared to most influence algal abundance included current velocity, conductivity, and light regime (measured as some combination of tree canopy cover, water depth, turbidity, and/or stream width).

Comparison of the data collected in this study with historical data on algae from Florida spring-run streams indicates that the assemblage of species found today is very similar to that existing ~70 years ago, largely consisting of taxa from the Cyanophyta ("Blue-green" algae), Chlorophyta (Green algae), and Bacillariophyta (Diatoms). Very little historical quantitative data exist on algal abundance (cover, Chlorophyll a, dry weight and/or AFDW). Comparison of epiphyte dry weight data collected in this study with similar data collected in upper Silver River in the 1950s and in 2004 indicates a general increase in epiphytic algal standing crop compared to historical conditions. No data exist for macroalgal abundance decades ago, but comparison with data collected in 2003 was inconclusive due to differences in collection methodology.

There have been thresholds proposed for an undesirable abundance of attached algae in streams, most indicating a Chlorophyll a level >100-150 mg/m^2 or cover >20–40% constitutes a "nuisance condition." Half or more of the spring-run streams sampled in this study exceed one or both criteria either for epiphytic algae or macroalgae.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

The karst geology of Florida is the basis for the existence of perhaps the densest concentration of springs in the world (Florida Springs Task Force 2000). These aquatic resources have long captivated explorers, visitors, artists and scientists, ranging from Ponce de Leon's mythological search for the "fountain of youth" to the writer Marjory Stoneman Douglas' description of Florida springs as "bowls of liquid light." In Florida, there are two main types of springs; seep springs, which originate from shallow aquifers and vent springs which originate from deeper aquifers that are partially confined, resulting in groundwater that is under artesian pressure (Copeland 2003). Of the 1,089 individual springs currently mapped in Florida, most are fed by artesian flow from the Floridan Aquifer System, a large regional aquifer system that underlies all of Florida and parts of South Carolina, Georgia, and Alabama. Some Florida springs, particularly those in the Suwannee River Basin, reverse flow and are known as estavelles (Copeland 2003). When the rivers partially fed by these springs flood, the pressure from the overlying surface water overcomes the groundwater pressure head, and the springs reverse flow and take in surface (river) water.

Florida springs have long been classified by Mienzer's system of spring discharge, which is expressed in cubic feet per second (cfs) or a lesser unit of discharge as volume/unit time (Scott et al. 2004). First-magnitude springs are the largest, with a mean annual discharge of greater than 100 cfs (64.6 million gallons/day). Second-magnitude springs discharge between 10 and 100 cfs, and third-magnitude springs discharge between 1 and 10 cfs. The system goes down to eighth-magnitude springs with a discharge of <1 pint/minute (200 gal/day). Florida has 33 first-magnitude springs and spring groups (groups of spring vents that collectively discharge water and are in close proximity).

Springs are also classified by the composition of the ions and minerals dissolved in the spring water (Woodruff 1993, Slack and Rosenau 1979). Seep springs fed by shallow surficial aquifers are mostly softwater springs with very low concentrations of dissolved solids. Most vent springs discharge water containing dissolved calcium bicarbonate and other ions. This water is considered "hard" water (containing dissolved calcium carbonate) and originates from the carbonate rocks that comprise the Floridan Aquifer System. Some springs are a mixed or salt-water quality type, with higher concentrations of chloride and other dissolved solids. These are found in the St. Johns River valley and along the Gulf coast from Taylor County south to Hernando County. The existence of highly mineralized saline groundwater in the aquifer contributing to these springs is related to the depth of the water source in the aquifer and proximity to the coast. In the St. Johns River Valley, the saline groundwater is relict seawater left behind in the aquifer during periods of higher sea level in the Pleistocene Epoch. Along the Gulf Coast, however, this is due to recharge of saline water from the adjacent Gulf of Mexico.

The water discharged from Florida's springs historically had extremely low concentrations of nitrogen compounds, particularly nitrate-nitrite as nitrogen (NOx-N). Background concentrations are generally 0.05-0.1 mg/L, due to a lack of natural sources other than

atmospheric deposition (Scott et al. 2004). Background phosphorus concentrations (as total phosphorus, TP) have been moderate in some springs (0.04-0.06 mg/L TP) due to the existence of natural phosphate deposits in some geologic formations in portions of Florida (Scott et al. 2004). In general, spring ecosystems are adapted to naturally low nutrient concentrations and may suffer when these are increased (Brown et al. 2008).

Many Florida springs give rise to lotic (flowing water) ecosystems known as spring-run streams. The exceptionally clear water in these streams allows for the proliferation of dense beds of submerged aquatic vegetation (SAV). The SAV habitat (which includes submerged macrophytes and associated algal communities) found in spring-run streams are a major source of primary production, provide habitat for diverse macroinvertebrate and fish communities and provide food sources for freshwater turtles and the endangered Florida Manatee, *Trichechus manatus latirostris* (Odum 1957a, Walsh et al. 2009, Walsh and Williams 2003). The springs also provide a warm water winter refuge habitat for manatee populations. Many springs are also inhabited by endemic species, including certain species in the snail family Hydrobiidae ("silt snails") which are found nowhere else in the world (Thompson 1968). Similarly, the submerged cave systems associated with many springs support one or more species of cave crayfish (mostly species of *Procambarus*) which may only be associated with that particular spring cave system (Franz et al. 1994).

Florida's springs have been subjected to many of the same pressures which have affected other aquatic ecosystems in the state, primarily degradation of water quality and alterations in hydrology (Copeland et al. 2009). Groundwater quantity and quality are both affected by human activities that occur in the highly vulnerable karst areas of Florida. Many springs are discharging water with increased concentrations of nitrate. Nitrogen loading to the landscape in these springsheds comes from agricultural and urban development (MACTEC 2010, Katz et al. 1999). Increased nitrate concentration is one factor that may be contributing to ecological changes in these springs (Stevenson et al. 2007). In addition, many springs in Florida are exhibiting reduced discharge, leading to decreases in current velocity (Kaplan et al. 2017; King 2014). These changes in hydrology are the cumulative result of multiple factors, including changes in rainfall, drainage alteration, and groundwater withdrawals (Copeland et al. 2009). Florida's burgeoning human population, which now exceeds 20 million residents, is placing increasing demands on the state's groundwater resources, and spring ecosystems appear to be exhibiting responses to these demands.

PURPOSE AND OBJECTIVES

This study was conducted as part of a broader management initiative begun by the St. Johns River Water Management District (SJRWMD or the District) in 2013. Called the Springs Protection Initiative (SPI), the effort involved a combination of scientific studies and identification of projects to implement which, 1) reduce nutrient loading (particularly nitrogen) to the landscape of springsheds, and/or, 2) reduce groundwater withdrawal/ pumping. These projects were selected based on a combination of existing data and best professional judgement. As part of the science component of the SPI, District scientists

determined that a broad field study of the biology of multiple springs and their spring-run streams was needed. The data from this study would be useful to investigate patterns in vegetation communities and selected elements of the faunal communities and their relationships with physicochemical conditions.

A major focus of the SPI science component (SPIS) was to better understand the drivers (physical, chemical, and/or biological) which exert the greatest influence on the primary producer community structure (the submerged macrophyte and algal communities) in spring-run streams (Reddy et al. 2017). This was driven by the observation in many of these streams of proliferation of large mats of "nuisance" benthic algae, which either replaced the macrophytes, and/or substantially increased epiphytic algal biomass on the macrophyte leaves. Hypotheses advanced to explain these biological shifts include increased nitrate concentrations and loads discharged from the springs (Scott et al. 2004, Mattson et al. 2006, Stevenson et al. 2007), decreased spring flows resulting in reduced current velocity (King 2014, Kaplan et al. 2017), and reductions in algal grazer populations, possibly due to lower dissolved oxygen (DO) concentrations in the spring discharge (Heffernan et al. 2010, Liebowitz et al. 2014). Of broader note, Hudon et al. (2014) report that proliferation of nuisance benthic algae, particularly the filamentous cyanobacterium *Lyngbya wollei* (now called *Microseira wollei*), appears to be a growing phenomenon in freshwater ecosystems worldwide.

The specific objectives of this study were:

- Select a range of springs and their spring-run streams in which to conduct concurrent quantitative biological and physicochemical sampling
- Quantitatively sample macrophytes and algae to assess current ecological conditions; include quantitative sampling of one or more major groups of fauna
- Evaluate similarities and differences within and among the spring-run streams, both spatially and temporally

These data will form a baseline dataset for comparison with future sampling efforts, and to compare with similar biological data collected in prior studies of Florida spring-run streams.

DESCRIPTIONS OF SPRING-RUN STREAMS

In 2015, SJRWMD employed Amec Foster Wheeler (now Wood Environment and Infrastructure) to conduct an intensive, synoptic (short-term) biological survey in 14 spring-run streams in north and central Florida (Figure 1). Seven of these were in the St. Johns River Basin (northeast and east central Florida): Alexander Springs Creek, Volusia Blue Spring Run, Juniper Creek, Rock Springs Run, Silver River, Silver Glen Spring Run, and Wekiva River. Three spring-run streams were in west central Florida: Rainbow River, Gum Slough, and Weeki Wachee River. Four streams were in north Florida: Manatee Spring Run, Ichetucknee River, Wacissa River, and Wakulla River. These 14 streams were selected because all had a long term ($\geq$10 years) record of discharge and water chemistry. They were also chosen based

Figure 1. Map of the region showing the locations of the 14 study streams. Red lines show county boundaries.

on the personal knowledge of the senior author in consultation with other SJRWMD scientists, scientists with other water management districts and the Florida Department of Environmental Protection (FDEP). Brief descriptions of each spring-run stream and its headspring(s) isprovided below. A summary of some physicochemical characteristics of each headspring (and data sources) is presented in Table 1.

Alexander Spring Creek. Originates at Alexander Spring, a first-magnitude spring located in the Ocala National Forest in Lake County. Mean annual flow of Alexander Spring is 102 cfs (Appendix A) and the flow originates from a single main vent. The groundwater contributing area, or springshed (after Copeland 2003) is approximately 151.52 km^2 (Walsh et al. 2009). The spring-run stream flows 19.1 km from the headspring to the mainstem of the St. Johns River, the confluence with the river located near Lake Dexter. Alexander Spring base water quality has been characterized as a mixed spring (Woodruff 1993), with moderately high levels of dissolved ions and salts. Nutrient concentrations (nitrate-nitrite nitrogen, NOx-N, and total phosphorus, TP) in Alexander Spring are low and reflective of background conditions (<0.1 mg/L NOx-N and <0.06 mg/L TP). Human use of the recreational area at the headspring is high, particularly in the summer, but attendance figures (number of persons/day) were not available. Much of Alexander Spring Creek below the County Road (CR) 445 bridge is open to motorized boat traffic, but it is not heavily used due to very shallow depths. Use of the creek by canoes and kayaks is moderate.

Blue Spring Run. Originates at Volusia Blue Spring (called this because of the common use of this spring name throughout the state), located in Blue Spring State Park in Volusia County. Volusia Blue Spring is a first-magnitude spring, with a mean annual flow of 144 cfs (Appendix A), although mean annual flow is historically reported as 162 cfs (Scott et al. 2004). Spring flow and stage in the spring run are heavily influenced by backwater from the adjacent St. Johns River. The flow originates from a single main vent in the spring pool. The springshed area is approximately 270.09 km^2 (Shoemaker et al. 2004). The spring run flows 0.67 km to the mainstem of the St. Johns River. Volusia Blue Spring is characterized as a salt spring (Woodruff 1993), with high levels of dissolved sodium, chloride and other ions. The source of these is relict seawater in a groundwater zone beneath the St. Johns River corridor (Stringfield and Cooper 1951; J. Stewart, SJRWMD, pers. comm.). Nitrate concentrations in Volusia Blue Spring are elevated relative to background conditions (currently averaging 0.6-0.8 mg/L NOx-N). TP concentrations are slightly higher than background (averaging 0.07 mg/L P). Recreational use of the park is high, with an average annual attendance of 589,941 in 2016-17[1]. The spring run is closed to motorized boat traffic. Canoes and kayaks are permitted in the run during certain hours. The entire run and headspring are closed to all human use between November and March to permit manatee use as a warm water refuge.

Juniper Creek. Originates at Juniper Spring in the Ocala National Forest in Marion County. Juniper Spring is a second-magnitude spring, with a mean annual flow of 11 cfs (Appendix A). The flow originates from a single main vent and possibly one or more minor vents in the spring

[1] Attendance figures from this and subsequent descriptions are from: https://floridadep.gov/sites/default/files/Economic%20Impact%20Assessment%202016-2017.pdf

Table 1. Selected physicochemical characteristics of the headsprings of the 14 spring-run streams surveyed in this study. Data sources are indicated at bottom of the table. Period of record varies by spring and may not be current data. ND = not determined.

	Alexander	Blue	Juniper	Rock	Silver	Silver Glen	Wekiva
Mean Discharge[1] (cfs)	102	144	11	54	722	101	62
Total Length of Run (km)	19.1	0.7	16.3	14.5	8.5	1.1	25.5
Springshed area[1] (km^2)	151.5	270.1	ND	43.5	2,238	ND	81.8
Conductivity[2] (mean; µmhos/cm)	1,109	1,676	115	261	464	1,815	338
Total Dissolved Solids[2] (mean; mg/L)	593	914	66	148	273	1002	193
pH[2] (mean; units)	7.88	7.37	8.46	7.64	7.20	7.74	7.39
Alkalinity[2] (mean; mg/L as CaCO3)	86	144	47	97	198	69	129
Sodium[3] (total, mean; mg/L)	122	167	2.30	4.80	5.92	238	10.20
Chloride[2] (mean; mg/L)	252	379	5	9	11	437	16
Dissolved Oxygen[2] (mean; mg/L)	1.58	0.47	6.51	0.91	1.91	2.94	0.75
Total Phosphorus[4] (mean; unfiltered mg/L)	0.05	0.07	0.03	0.09	0.04	0.03	0.12
Orthophosphate[2] (mean; mg/L)	0.05	0.07	0.03	0.08	0.04	0.03	0.12
Nitrate-Nitrite N[2] (mean; mg/L)	0.04	0.51	0.10	1.29	1.14	0.06	1.00

1 – Appendix A or sources cited in text;
2 – Di and Mattson, unpublished report using data collected 2009-2013;
3 – from Scott et al. 2004 (single value sampled 2001 or 2002);
4 – calculated from data provided by SWFWMD (Rainbow, Gum, Weeki Wachee), SRWMD (Manatee, Ichetucknee, Wacissa), NWFWMD (Wakulla) and SJRWMD data (Alexander, Blue, Juniper, Rock, Silver, Silver Glen, Wekiwa)

Table 1. Continued.

	Rainbow	Gum	Weeki Wachee	Manatee	Ichetucknee	Wacissa	Wakulla
Discharge[1] (cfs)	687	81	171	181	326	439	417
Total Length of Run (km)	9.7	8.0	12.1	0.4	8.8	21.7	14.5
Springshed area[1] (km^2)	1,904	ND	622	ND	960	ND	5,180**
Conductivity[3] (µmhos/cm)	161	318	320	430	319	326	328
Total Dissolved Solids[3] (mg/L)	89	175	176	268	183	184	183
pH[3] (units)	7.95	7.57	7.70	7.04	7.91	7.40	7.20
Alkalinity[3] (mg/L as CaCO3)	67	129	147	198	154	163	146
Sodium[3] (total or unfiltered; mg/L)	2.33	3.40	3.78	3.78	2.12	2.94	4.99
Chloride[3] (total or unfiltered; mg/L)	3.9	6.0	6.7	7.2	3.6	5.1	7.8
Dissolved Oxygen[3] (mg/L)	6.61	1.81	1.30	1.60	3.52	0.90	2.39
Total Phosphorus[4] (mean; unfiltered mg/L)	0.03	0.03	0.01	0.03	0.03	0.04	0.03
Orthophosphate[4] (mg/L)	0.03	0.03	0.01	0.03	0.02	0.05	0.03
Nitrate-Nitrite N[4] (mg/L)	1.70	1.50	0.90	2.00	0.76	0.30	0.50

** - includes springshed area of Wakulla Spring, Spring Creek Spring group, and St. Marks River Rise

1 – Appendix A or sources cited in text;
2 – Di and Mattson, unpublished report using data collected 2009-2013;
3 – from Scott et al. 2004 (single value sampled 2001 or 2002);
4 – calculated from data provided by SWFWMD (Rainbow, Gum, Weeki Wachee), SRWMD (Manatee, Ichetucknee, Wacissa), NWFWMD (Wakulla) and SJRWMD data (Alexander, Blue, Juniper, Rock, Silver, Silver Glen, Wekiwa)

pool. The springshed area for Juniper Spring has not been determined to date. Two other springs contribute to Juniper Creek, Fern Hammock Spring, which flows into the creek downstream of Juniper Spring, and Sweetwater Spring, which flows into the creek near the State Road (SR) 19 crossing. Fern Hammock is a second-magnitude spring with a mean flow of 11 cfs (Appendix A). Sweetwater Spring is also a second-magnitude spring with a mean flow of 13 cfs (Appendix A). Juniper Creek flows 16.33 km from the headspring to a confluence with Lake George. Juniper and Fern Hammock are both calcium bicarbonate springs, while Sweetwater is a salt spring (Woodruff 1993). Nutrient concentrations (NOx-N and TP) in Juniper Spring are at or below background levels ($\leq$0.10 mg/L NOx-N; 0.05 mg/L TP). Visitor use of the recreational area at the headspring is moderate to high, but attendance figures were not available. The upper half of Juniper Creek (above the SR 19 crossing) is closed to motorized boat traffic but has moderate to heavy use by canoes and kayaks. The lower half of the creek is open to boat traffic, but shallow depths generally preclude most motorized craft from navigating all but the lower part of the creek, near the confluence with Lake George.

Rock Springs Run. Originates at Rock Springs in Kelly Park, Orange County. Rock Springs is a second-magnitude spring, with a mean annual flow of 54 cfs (Appendix A). The flow emerges from two cave openings in a vertical rock face at the headspring. The springshed area of Rock Springs is approximately 43.51 km^2 (Walsh et al. 2009). A small spring known as Sulphur Spring contributes flow to the run downstream of Rock Springs. It is a fourth magnitude spring with a mean annual flow of 0.74 cfs (www.sjrwmd.com/waterways/springs/list/). Rock Springs Run flows 14.46 km to a confluence with the Wekiva River. Both Rock Springs and Sulphur Spring are calcium bicarbonate water chemistry types (Woodruff 1993), although the latter gets its name from the odor of hydrogen sulfide in the spring water. Rock Springs is characterized by elevated NOx-N ($\geq$2.0 mg/L) and somewhat elevated TP (0.08 mg/L). Recreational use of the spring is high, with an average monthly attendance of 54,373. Annual attendance over the period 1998-2005 ranged from 73,626-214,983 (201.7-589 persons/day; Wetland Solutions Inc. 2007). Rock Springs Run is closed to motorized boat traffic but has moderate to heavy use by canoes and kayaks.

Silver River. The Silver River is a tributary of the Ocklawaha River. The headspring area of the river is known as the Silver Springs group (after Copeland 2003), because it consists of at least 30 mapped, named spring vents (Munch et al. 2006). Historically, Silver Springs was the largest inland spring in the state by discharge, with a mean annual flow of 820 cfs (Scott et al. 2004). Based on current data, the mean average flow of the Silver Springs group is 722 cfs (Sutherland et al. 2017). About half of this flow is discharged from the main headspring, known as Mammoth Spring or Silver Spring. Flow in the Silver River is influenced by backwater effects during high stage on the Ocklawaha River (Baird et al. Unpublished Report). The springshed area of the springs group is listed as 2,238 km^2, which constitutes the "1,000-year capture zone" as delineated by groundwater modeling (Munch et al. 2006). The Silver River runs 8.5 km to the Ocklawaha River confluence. The Silver Springs group is a calcium bicarbonate water chemistry type (Woodruff 1993). Nitrate concentrations discharged from the springs group are elevated (averaging 1.1-1.3 mg/L NOx-N). TP is at background

concentration (0.04 mg/L P). Since the 1920s, the headspring area of Silver Springs has been a tourist attraction, one of the main features being glass-bottom boat rides to view the underwater communities, accompanied by narration from the boat captain (which continues today). The Silver River is now part of Silver River State Park and the Ocklawaha River Aquatic Preserve. Total annual attendance at the park in 2016–2017 was 480,272. The Silver River is open to motorized boat traffic up to the headspring and also is used heavily by canoes and kayaks.

Silver Glen Spring Run. Originates at Silver Glen Springs in the Ocala National Forest in Marion County. Silver Glen is a first-magnitude spring with a mean annual flow of 101 cfs (Appendix A). Since 2010, the flow of the spring has rarely reached over 100 cfs (SJRWMD unpublished data), and historically the mean annual flow of the spring has been listed as 110.5 cfs (Scott et al. 2004). The flow emerges from two vents, the main vent (Silver Glen) and a secondary vent known as the "Natural Well". Flow and water level in the spring and spring run are influenced by backwater from the adjacent St. Johns River. The springshed area of Silver Glen Springs has not been determined to date. The run flows for 1.13 km to a confluence with Lake George. Silver Glen Spring is characterized as a salt spring due to high levels of dissolved solids (Woodruff 1993). Nutrient concentrations in Silver Glen Springs are at or below background levels (<0.1 mg/L NOx-N; <0.06 mg/L TP). Recreational use of the headspring and run is very high. Boat traffic is permitted, and large numbers of motorized boats use the spring run, with no restriction on size or draft. A rope barrier prevents boats from entering the headspring pool. Attendance figures were unavailable.

Wekiva River. Originates at Wekiwa Springs (the spring spelling is different from the river) in Wekiwa Springs State Park, Orange County. The Wekiva River mainstem and all or portions of the tributaries are part of the Wekiva River Aquatic Preserve. Wekiwa Springs is a second-magnitude spring with a mean annual flow of 62 cfs (Appendix A). The flow originates primarily from a single main vent but there is a secondary vent in the spring pool that occasionally exhibits flow. Flow and water level are occasionally affected by backwater effects during high stage on the St. Johns River (SJRWMD unpublished data). The springshed area of Wekiwa Springs is approximately 81.84 km^2 (Walsh et al. 2009). The Wekiva River runs 25.47 km to its confluence with the St. Johns River downstream of Lake Monroe. The river receives inflow from three major tributary streams; Rock Springs Run, the Little Wekiva River, and Blackwater Creek. All of these tributaries receive some of their flow from a number of smaller springs, ranging from second to sixth magnitude. A total of 31 named springs contribute flow to the Wekiva River and its tributaries. Wekiwa Spring is a calcium bicarbonate water chemistry type. Nutrient concentration in the spring are elevated relative to background conditions; NOx-N has been as high as >2 mg/L and TP concentrations average 0.12 mg/L. Recreational use of Wekiwa Spring is high, with an annual state park attendance in 2016–2017 of 399,040. Annual visitor attendance over the period 1993–2006 ranged from 94,962–166,738 (260.2–456.8 persons/day; Wetland Solutions Inc. 2007). The Wekiva River below the Rock Springs Run confluence is open to boat traffic, but shallow depths and abundant woody snags restrict boat use to smaller vessels.

Rainbow River. Originates from a complex of multiple spring vents known as the Rainbow Springs group. The river is located in western Marion County, near the city of Dunnellon, and

is a tributary of the southern Withlacoochee River. Total length of the river is 9.7 km. The Rainbow Springs group is a first-magnitude springs group, with a median flow of 687 cfs (SWFWMD 2015). Flow in the lower Rainbow River is influenced by backwater effects during high stages on the Withlacoochee River (SWFWMD 2015). Historically, the springs group was the overall third largest spring in Florida by discharge. The springshed of the springs group encompasses about 1,904 km^2 (SWFWMD 2015). The base water chemistry of the Rainbow Springs group is a calcium bicarbonate type (Woodruff 1993). Nitrate concentrations are elevated, averaging over 2 mg/L NOx-N. Phosphorus levels are at background concentrations (<0.06 mg/L TP). The headspring area and part of the upper Rainbow River are within Rainbow Springs State Park, and the entire Rainbow River is a state-designated Aquatic Preserve. Annual attendance in the park in 2016-17 was 316,796 persons. Historically the springs were privately owned and operated as a tourist attraction, featuring "submarine boat" tours of the headspring area. The Rainbow River is open to boat traffic and there are many private residences on the river, but the headspring area is closed to motorized boat traffic and only canoes and kayaks are allowed.

Gum Slough. Originates at the Gum Springs group, a complex of at least 6–7 spring vents (Scott et al. 2004). The land surrounding the springs and much of the slough is in private ownership. The headsprings and slough are in Sumter County and the slough discharges to the southern Withlacoochee River upstream of the Rainbow River confluence. Total length of the slough is about 8 km. The Gum Springs group is a second-magnitude springs group with a mean annual flow of 81 cfs (King 2014). The base water quality of the springs is a calcium bicarbonate water quality type. As reported in King (2014), the headsprings exhibit elevated nitrate concentrations (1.4 mg/L NOx-N). Phosphorus concentrations are below background concentrations (<0.03 mg/L TP).

Weeki Wachee River. Originates at Weeki Wachee Spring in Hernando County. The spring is a first-magnitude spring, with a mean annual flow of 171 cfs (SWFWMD 2017). The Weeki Wachee springshed encompasses 622 km^2 (SWFWMD 2017). The Weeki Wachee River is about 12 km in length and discharges to the Gulf of Mexico near Bayport. The lower part of the river is affected by tidal fluctuation from the adjacent Gulf of Mexico. The base water chemistry of Weeki Wachee Spring is a calcium bicarbonate water quality type (Woodruff 1993). Nitrate concentrations in Weeki Wachee Spring are elevated ($\geq$0.9 mg/L NOx-N). TP concentrations are very low (`0.01 mg/L). The headspring and upper river are part of Weeki Wachee Springs State Park. Historically the headspring was privately owned and operated as a tourist attraction, the main draw being an underwater theatre where visitors would watch performances featuring women portraying mermaids and other characters. The state park continues to operate the underwater show today, along with pontoon boat tours on the river. Annual attendance at the park in 2016–2017 was 418,844. Downstream of the headspring/state park there are many private residences and subdivisions along the river, and it receives heavy recreational use by boats, canoes and kayaks.

Manatee Spring Run. Manatee Spring is located in Manatee Springs State Park, near the city of Chiefland in Levy County. The spring is a first-magnitude spring with a historic mean annual

flow of 181 cfs (Scott et al. 2004). The spring run is 0.37 km in length and discharges to the lower Suwannee River. During low river flows in the Suwannee, water levels in the spring are affected by tidal fluctuation. The springshed area of Manatee Spring has not been determined because it is difficult to delineate it from the adjacent Fanning Springs springshed. Manatee Spring is a calcium bicarbonate water quality type (Woodruff 1993). Nitrate concentrations are elevated (≥2.0 mg/L NOx-N). TP concentrations are below background (<0.06 mg/L). The spring run is closed to motorized boat traffic, but canoes and kayaks are allowed on the spring run. The state park experiences heavy recreational use by swimmers, snorkelers, and divers. Annual attendance in 2016–2017 was 308,175.

Ichetucknee River. Originates at the Ichetucknee Springs group; a complex of seven named springs. The springs and river are at the border of Suwannee and Columbia Counties, near the town of Fort White. The springs group and the upper half of the Ichetucknee River are within Ichetucknee Springs State Park. The mean annual flow of the springs group is 326 cfs (Katz et al. 2009). About half of that flow comes from Ichetucknee Spring (second-magnitude; mean flow 45 cfs) and the Blue Hole or Jug Spring (first- magnitude; mean flow 144 cfs). The springshed area encompasses 960 km^2 (Katz et al. 2009). The Ichetucknee River flows for 8.8 km to the lower Santa Fe River, a tributary of the middle Suwannee River. The springs of the Ichetucknee group all exhibit a calcium bicarbonate water quality type (Woodruff 1993). Nitrate concentrations in most of the springs in the spring group are elevated (>0.50 mg/L NOx-N). TP concentrations are within the background range (0.04-0.06 mg/L TP). The upper half of the river within the state park is closed to motorized boat traffic, but is heavily used for tubing, swimming, snorkeling, and canoeing/kayaking, particularly between Memorial Day and Labor Day. Total annual attendance in the park in 2016-17 was 416,892. The lower half of the river is bordered by private residences with docks and boats are permitted to access this part of the river.

Wacissa River. Originates at the Wacissa Springs group, a complex of at least 16 known springs (Hornsby and Ceryak 2000). The springs and river are in Jefferson County. Much of the land around the river is state-owned as part of the Aucilla Wildlife Management Area. The Wacissa River is a tributary of the Aucilla River and runs 21.7 km from the headsprings group to the Aucilla River confluence. The mean annual flow of the springs group is 439 cfs, making it the fourth largest spring in the state by discharge (Hornsby and Ceryak 2000). The springshed area has not been determined. The base water chemistry of the springs comprising the springs group is a calcium bicarbonate type. Nitrate concentrations in many of the springs are somewhat elevated over natural background (varying from 0.2-0.4 mg/L NOx-N), although not as much as seen in many of the other spring-run streams in this study. TP concentrations are at background levels (<0.06 mg/L). The river is mainly accessed from a county park at the headspring group and at the Goose Pasture public recreation area on the river, but attendance figures were not available.

Wakulla River. The Wakulla River begins at Wakulla Spring. The spring and the upper third of the Wakulla River are within Wakulla Springs State Park. The springs and river lie entirely within Wakulla County. The river runs 14.5 km to its confluence with the St. Marks River near where it empties into the Gulf of Mexico near the town of St. Marks. The mean annual flow of

Wakulla Spring is 417 cfs (K. Coates, NWFWMD Pers. Comm.). The springshed area cannot be delineated from the overlapping springsheds of the Springs Creek Springs group on the coast and the St. Marks River Rise (K. Coates, NWFWMD Pers. Comm.). The overall area of these is 5,180 km^2. The base water chemistry of Wakulla Spring is a calcium bicarbonate type. Nitrate concentrations are elevated over background ($\geq$0.5 mg/L NOx-N), although nitrate concentrations have been decreasing over the past decade with the implementation of improved domestic wastewater effluent disposal practices in the upper springshed (K. Coates, NWFWMD Pers. Comm.). TP concentrations are below natural background (<0.06 mg/L). Annual attendance at the state park in 2016–2017 was 239,270.

METHODS

SAMPLING STATIONS

Figure 1 shows the locations of the 14 spring-run streams in this study. Two sampling locations were established at 10 of these streams, consisting of a transect across the stream channel from bank-to-bank and perpendicular to the channel thalweg. One transect was established upstream, close to the main headspring or headspring group. The other transect was established at a downstream location in the spring-run stream proper. Three transects were established on the Silver River (upstream, mid-reach, and downstream) to help support other scientific work being conducted on that stream. On the three shorter spring runs (Manatee Spring, Volusia Blue Spring, and Silver Glen Spring), a single transect was established downstream of the headspring in the run itself. The locations of the transects were not established randomly; they were selected based on the occurrence of beds of SAV (macrophytes and algae) and professional judgement. Table 2 presents descriptive and location data on the transects in the study and the site abbreviations used in subsequent tables, figures, and appendices. Appendix B presents maps showing the transect locations and the locations of related long-term ambient water quality sampling stations.

FIELD METHODS

A detailed summary of all methods used in this study was presented in Amec Foster Wheeler (2016). A general summary of the methodology is presented in this report. Field methods and QA/QC followed Standard Operating Procedures (SOPs) of SJRWMD and U.S. Geological Survey (Amec Foster Wheeler 2016). Physicochemical data (current velocity, *in situ* water chemistry, and stream channel characteristics such as depth and tree canopy cover) and biological data (algal taxonomic composition and abundance data) were collected at each sample transect in 2015 on six separate sampling dates. Biological sampling was conducted concurrently on two of these sampling dates in spring (May-June) and fall (September-October).

Physicochemical Sampling

Physicochemical sampling was conducted along a tag line stretched across the stream channel along with a measuring tape. Current velocity was measured and recorded with a SonTek FlowTracker handheld Acoustic Doppler Velocimeter (ADV) at up to 10 individual locations across the stream channel at depths above the top of the SAV canopy. *In situ* water quality was measured using a multi-parameter sonde and a hand-held turbidity meter at a mid-stream point on the transect. Chemical measurements were taken using a YSI Series 5 multi-parameter probe. The following variables were measured at each transect:

- Total water depth
- Height of the macrophyte canopy (as total depth minus depth to the top of the canopy)

Table 2. Location data and description of the sampling transects in this study.

Station ID	Latitude (decimal degrees)	Longitude (decimal degrees)	Description
ALE1	29.08259003	-81.57825003	Alexander Springs Creek near headspring
ALE2	29.07929	-81.56691997	Alexander Springs Creek downstream of County Road 445
GUM1	28.95340999	-82.23836998	Gum Slough near headspring group
GUM2	28.95974999	-82.23209001	Gum Slough between Gum Springs 3 & 4
ICH1	29.9799	-82.7589	Ichetucknee River downstream of Blue Hole Spring
ICH2	29.957241	-82.780301	Ichetucknee River above U.S. 27
JUN1	29.18449004	-81.70372999	Juniper Creek near headspring
JUN2	29.21174997	-81.65322003	Juniper Creek downstream of State Road 19
MAN1	29.48948003	-82.97798002	Manatee Spring Run downstream of headspring
RAI1	29.09076667	-82.42656667	Rainbow River near headsprings group
RAI2	29.06896667	-82.42753333	Rainbow River downstream of K.P. Hole park
ROC1	28.77171667	-81.50291667	Rock Springs Run downstream of King's Landing
ROC2	28.7411	-81.46794002	Rock Springs Run near Indian Mound camp site
SIL1	29.21573333	-82.04845	Silver River in headspring group (near Christmas Tree Spring)
SIL2	29.21528333	-82.0417	Silver River at USGS gauge/1,200 meter station
SIL3	29.20348333	-82.015	Silver River near SJRWMD minimum flows and levels transect 5
SLG1/SILG1	29.24471	-81.64127001	Silver Glen Spring Run downstream of headspring
VOL1	28.94707	-81.33972	Volusia Blue Spring Run downstream of headspring
WAC1	30.327034	-83.987714	Wacissa River near headspring group
WAC2	30.203283	-83.970364	Wacissa River at Goose Pasture
WAK1	30.234019	-84.294372	Wakulla River near headspring
WAK2	30.211438	-84.259876	Wakulla River downstream of County Road 365
WEE1	28.51895	-82.573891	Weeki Wachee River near headspring
WEE2	28.519443	-82.583234	Weeki Wachee River downstream
WEK1	28.71415	-81.45805	Wekiva River near headspring (downstream of lagoon)
WEK2	28.79926667	-81.4144	Wekiva River upstream of State Road 46

- Tree canopy cover (using a Model-C spherical densiometer)
- Current velocity
- Surface water elevation, if a staff gauge was present at the sampling transect
- Conductivity (specific conductance)
- Dissolved oxygen (DO)
- pH
- Water temperature
- Turbidity (hand-held turbidimeter)

Aquatic Vegetation Sampling

Sampling of submerged aquatic vegetation (macrophytes and algae) was conducted along a belt transect straddling the tag line along which physicochemical data were collected (Figure 2). The belt transect "straddled" the measuring tape and tag line along which the physicochemical measurements were taken. Macrophyte and algal cover was measured in five (5) 1 m^2 quadrats as described below. Quantitative macrophyte samples (with associated epiphytic algae) and macroalgae mats were sampled with a modified Hess-type sampler; three (3) replicate macrophyte and three (3) replicate macroalgae samples were collected at each biological sampling event (spring and fall 2015).

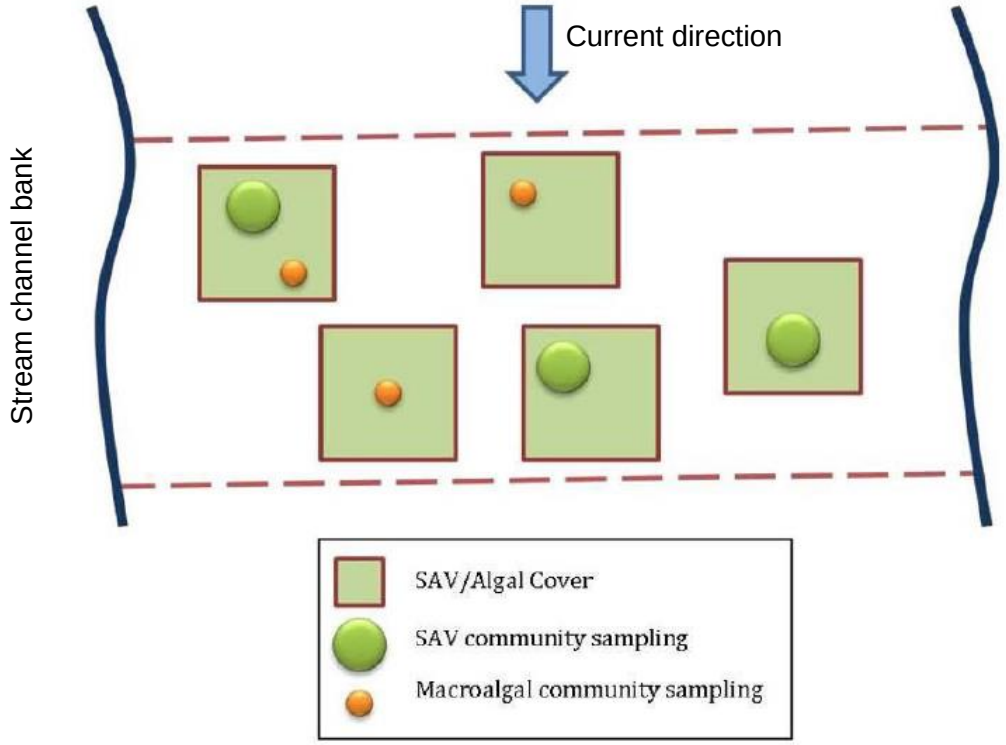


Figure 2. Schematic diagram showing the arrangement of replicate samples for SAV cover and standing crop ("community") sampling. Source: Amec Foster Wheeler 2016.

Transects and SAV sampling quadrats were placed where beds of SAV (macrophytes and/or algae) were present in locations that appeared to us to be representative of the reach/area in which we located the transect. Replicate samples for SAV cover and standing crop were also taken non-randomly (generally systematically across the stream channel from bank-to-bank); samples were collected where SAV was present.

Algal cover was sampled semi-quantitatively by estimating coverage (as % cover) in a 1 m^2 quadrat divided into 100 10 X 10 cm sections to enable accurate estimation of vegetation coverage. Epiphytic algal cover was determined by visually assessing algal coverage on the macrophyte vegetation in the quadrat. Macroalgal mat cover was assessed separately by visually estimating total coverage of filamentous algal mat in the quadrat.

Macroalgal mats were sampled using the modified Hess sampler; the sampler was placed over a mat and all algae within the sampler was collected. Samples were stored in plastic bags and preserved on ice until processed in the laboratory within 24 hours of collection. Epiphytic algae were sampled by collecting healthy (non-necrotic) leaf blades of the dominant macrophytes present in the sampling quadrats with attached epiphytic algae. Leaves were stored in plastic bags, preserved on ice, and processed within 24 hours of collection. At each sampling event, sufficient material was collected to enable quantitative measurements of algal species composition and abundance (chlorophyll a, ash-free dry weight).

LABORATORY METHODS

In the laboratory, all collected algae samples were cleaned of organic detritus, silt, and sand and all macroinvertebrates were sorted from the vegetation and preserved for subsequent analysis of the SAV-associated invertebrate community (to be described in a separate report).

Algal Species Richness and Composition

Macroalgal taxonomic analyses were conducted by GreenWater Laboratories. A qualitative sample of macroalgal mat after being cleaned was homogenized in a blender with deionized water to break up clumps. Homogenized material was shaken in a bottle and an aliquot preserved in glutaraldehyde. A subsample of this aliquot was placed in a settling chamber, allowed to settle for 15 minutes, and examined under a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics and epifluorescence. The settled subsample was scanned at 100X to develop a list of algal taxa present (identified to lowest practical taxonomic level). Algal relative abundance was estimated using the following scale:

Abundance rating	Description	Estimated % relative abundance
Rare (R)	1 or 2 cells observed	0-1
Frequent (F)	>1 cell observed but appear sporadically	1-5
Common (C)	Individual cells seen in several fields of view	5-20
Abundant (A)	1-2 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 all other algae in numbers	70-100

For taxonomic composition of epiphytic algae, samples of macrophyte blades/shoots were cleaned of silt, sand, and invertebrates as described above. Replicate samples were also taken and processed separately. Leaves/shoots of different age classes were selected for analysis (Amec Foster Wheeler 2016). Attached algae was gently scraped from leaves using a soft spatula or brush and the area of leaf scraped was measured. Scraped material was homogenized and analyzed as described above for macroalgae samples.

Algal Abundance

Macroalgal mat samples collected with the Hess sampler were processed in two ways. A dime-sized subsample of macroalgae was removed from the replicate sample and frozen for analysis of Chlorophyll *a* (hereafter "Chl *a*"). The remainder of the sample was dried to constant weight at 100 ^{0}C and weighed. The samples were then combusted in a muffle furnace at 500 ^{0}C for 6 hours for determination of ash-free dry weight (AFDW). Dry weight and AFDW data were converted to per unit area based on the area sampled by the Hess sampler. For analysis of Chl *a*, the dime-sized subsample was analyzed by grinding/settling and extraction in 80% acetone (Amec Foster Wheeler 2016). Chl *a* and corrected Chl *a* (subtracting phaeophytins) were determined by a subcontractor, AEL Laboratories. Chl *a* data were converted to per unit area using the equation:

$$\text{Chl } a \text{ (as mg/m}^2\text{)} = \frac{(\text{Chl } a \text{ subsample [mg/m}^3\text{] x extraction volume [m}^3\text{])}}{\text{Sampled area (m}^2\text{)}} \text{ X Ratio}$$

The "ratio" multiplier was calculated as the total mass of macroalgae (g) divided by the mass of the dime-sized subsample (g) used for Chl *a* analysis for each replicate sample

Epiphytic algae samples were obtained by carefully scraping adequate amounts of epiphytic algae from the surfaces of the dominant macrophytes in the replicate Hess samples and supplemental leaf material collected from the quadrats. The leaf area scraped was measured. Replicate samples were processed separately. One subsample of epiphytic algae was dried to constant weight at 100^0 C, and weighed, then the sample was combusted in a muffle furnace at 500^0 C for 6 hours to determine ash-free dry weight (AFDW). A second subsample of epiphytic algae was treated as described above for the macroalgae samples. Pigment was extracted by the grinding/settling method in 80% acetone and Chl *a* and corrected Chl *a* were determined. Chlorophyll a per unit area was determined as follows:

$$\text{Chl } a \text{ (mg/m}^2\text{)} = \frac{(\text{Chl a [mg/m}^3\text{] x extraction volume [m}^3\text{])}}{\text{Sampled leaf area (m2)}}$$

STATISTICAL ANALYSIS METHODS

All data summary and analysis were performed by District staff (RAM, DLH, and MQG). Physicochemical and vegetation data were summarized in tabular and graphical form, using Minitab™ version 18 software and program routines written by M.Q. Guyette in the R package. Due to the non-random placement of transects and sample sites within transects and the non-independence of transects within streams and sample sites within transects, statistical analysis using conventional statistics (both parametric and/or nonparametric) were considered not appropriate. Consequently, the purpose of our analyses was to indicate general trends and relationships, rather than indicating statistically significant differences. Graphical and tabular summaries of the data were used to compare epiphytic and macroalgal species composition and abundance among spring-run streams and to compare algal abundance and physicochemical characteristics. The physical, chemical and biological data from spring and fall sampling events are presented separately. In all cases, corrected Chl *a* data were used for presentation and analysis.

Multivariate analyses of the physicochemical and vegetation data were conducted using the PRIMER™ software (Clarke and Gorley 2015), which was developed to specifically deal with species-by-sample data in the assessment of biological changes in response to changes in the abiotic environment (Clarke 1993). These permutation tests were conducted in an exploratory fashion to look for patterns in the data. Transects (e.g., upstream, downstream) within streams were analyzed separately. However, site-specific data was averaged within each transect (the means of cover and dry weight, rather than individual replicate samples). Data for spring and fall sampling events were analyzed separately to avoid seasonal differences that might overwhelm inter-transect differences. Physicochemical variables were log-transformed and normalized prior to analysis and Euclidian distance was used to calculate resemblance matrices to test for similarities among transects. Algal cover and dry weight were (log+1)-transformed prior to analysis and the Bray-Curtis similarity index (Bray and Curtis 1957) was used to calculate resemblance matrices to test for similarities among transects.

The following analyses were performed:

Principal Components Analysis (*PCA*) was used to orthogonally transform the set of physicochemical variables into a smaller set of linearly uncorrelated axes to look for similarities among transects. Orthogonal axes are created based on how much of the variability between transects in the physicochemical variables is captured by the combination of the original variables, with the most variability captured in the first axis, the second axis accounting for the greatest amount of the remaining variability, and so on until most of the variability between transects is accounted for. When the axes are plotted against one another, transects with similar values for the suite of physicochemical variables will occur close together.

Cluster Analysis (*CLUSTER*) was used to search for similarities among transects based on physicochemical or algal compositional differences. Simultaneously, a Similarity Profile test

(*SIMPROF*) was used to assess the significance of cluster groups. *SIMPROF* runs permutations of the algal community or physicochemical composition at each node in the cluster to determine whether there is any evidence of multivariate structure within the group. If multivariate structure is detected, then the transects within the group at that node are considered significantly different from the other transects.

Analysis of Similarity (*ANOSIM*) was used to determine whether there were any differences between spring-run streams that flow into the St. Johns River (SJR) and the other streams (O), and between upstream and downstream transects based on their physicochemical or algal community composition. ANOSIM was also run comparing low and high NOx springs (< and $\geq$ 0.465 mg/L NOx-N; defined in discussion below) and low and high velocity springs (< and $\geq$ 0.22 m/second; defined in discussion below). The test statistic is "R" and a significant difference is a permutation probability (P_{perm}) <0.05. When differences were found between groups (i.e., SJR vs O or upstream vs downstream), a Similarity Percentages (*SIMPER*) routine was used to pinpoint which variables or species accounted for those differences.

The Bio-Env Stepwise procedure (*BEST*) was used to determine if there was a correlation between the distribution of stream sites based on the composition of the algal community and the distribution of sites based on the physicochemical variables collected at each site. The test compares resemblance matrices based on algal and physicochemical similarities and determines what combination of physicochemical variables accounts for the pattern in algal species composition among transects. The test statistic is "R" and there is no test for significance. For purposes of this analysis, weak or low correlation was R $\leq$ 0.3, moderate correlation was R >0.3 to <0.7 and high correlation was $\geq$0.7.

RESULTS AND DISCUSSION

PHYSICOCHEMICAL DATA

Table 3 shows the physicochemical data collected at the transects in spring and fall 2015. Channel width at each transect was generally similar in the spring and fall; variation is likely due to changes in water levels in the stream channel or sampling in a slightly different location. Tree canopy cover was variable, with generally higher tree cover associated with narrower stream channels. Current velocity likewise exhibited considerable variation; in some systems the downstream transect had higher velocities (Wekiva River), but in others the upstream transects were higher (e.g., Gum Slough). Water temperatures were consistent both among and within spring-run stream systems, varying from ~20–24 ^{0}C across all transects, and generally being similar at both upstream and downstream transects in all streams and in both spring and fall sampling episodes. In many cases the fall water temperature was slightly cooler than the spring. The more northern springs (WAC and WAK) generally had lower mean water temperature than the springs further south. Highest conductivity was measured at the downstream Juniper Creek site (JUN2) and the Silver Glen Run transect (SLG1), although Juniper Spring (JUN1) is a softwater spring (conductivity <200 µmhos/cm). Like water temperature, pH was very consistent among and within all stream systems; pH was generally circumneutral to slightly alkaline. Higher pH values (>8) appeared to generally be associated with high (supersaturated) dissolved oxygen (DO) concentrations, suggesting an effect of plant photosynthesis. DO was generally lower at upstream sites, nearer to the headspring discharge, but two upstream sites exhibited particularly high DO concentrations (JUN1 and RAI1). Turbidity was uniformly very low among and within the streams. The highest single turbidity was a value of 9.66 NTU at the Silver Glen Run transect in the fall. This may be due to recreational use of the spring on that day causing an increase in suspended sediments.

Principal Components Analysis showed that the same five variables accounted for over 80% of the variation among the transects across seasons: stream width, canopy cover, pH, DO, and current velocity accounted for 83.6% of the variation in the spring season, and 86.9% of the variation in the fall. Cluster analysis (Figure 3) showed no significantly different clusters based on water quality in both seasons, but in general spring-run streams on the St. Johns River mainstem (SJR) clustered together and "Other" streams (O - not on the mainstem of the St. Johns) also tended to cluster, especially in the spring. However, ANOSIM showed significant differences between SJR spring-run streams vs. O streams ($r=0.300$; $P_{perm}=0.001$ in spring; $r=0.245$; $P_{perm}=0.007$ in fall). There were also significant upstream versus downstream differences in spring ($r=0.167$, $P_{perm} = 0.019$). The SIMPER analysis indicated that conductivity, turbidity, current velocity, and water depth were main factors separating SJR streams from O streams in both seasons. The SJR streams generally had higher conductivity and turbidity, while O streams had greater water depth and/or current velocity (depending on season). The average dissimilarity between SJR and O transects was ≥ 20%, regardless of season. SIMPER analysis of the upstream versus downstream differences seen in the ANOSIM

Table 3. Physicochemical measurements collected at the sampling transects in spring and fall 2015. ND = no data.

TRANSECT	Channel Width (m)	Canopy Cover (%)	Current Velocity (m/sec)	Water Temp (^{0}C)	Conductivity (umhos/cm)	pH	Dissolved O_2 (mg/L)	Turbidity (NTU)
ALE1 sprg	41.0	1.5	0.05	24.35	1,172	7.56	2.64	0.17
ALE1 fall	47.0	8.3	0.06	24.13	1,073	7.42	2.28	0.57
ALE2 sprg	64.0	0	0.09	26.28	1,164	8.28	5.95	0.07
ALE2 fall	64.0	0	0.15	24.30	1,080	7.92	5.59	1.40
GUM1 sprg	15.0	62.5	0.14	23.55	363	7.24	5.62	0.27
GUM1 fall	9.0	58.5	0.15	23.24	355	7.48	6.02	0.61
GUM2 sprg	20.0	66.8	0.13	23.42	356	6.60	4.99	0.73
GUM2 fall	18.0	64.8	0.07	23.23	364	7.65	4.96	0.92
ICH1 sprg	13.7	54.8	0.10	21.67	312	7.28	3.70	0.27
ICH1 fall	13.7	52.8	0.24	21.76	287	7.18	2.80	0.31
ICH2 sprg	21.9	43.5	0.16	23.71	320	7.21	9.56	0.87
ICH2 fall	21.3	38.3	0.28	22.04	304	7.26	4.54	1.14
JUN1 sprg	6.0	41.8	0.32	23.13	143	7.17	8.17	1.45
JUN1 fall	ND	ND	ND	ND	ND	ND	ND	ND
JUN2 sprg	20.0	2.3	0.34	23.20	2,050	7.42	6.72	1.41
JUN2 fall	17.0	6.25	0.08	23.76	1,940	8.00	7.75	0.97
MAN1 sprg	29.0	14.5	0.00	22.31	524	7.06	1.48	0.79
MAN1 fall	26.0	36.3	0.07	22.54	534	7.26	1.37	0.19
RAI1 sprg	33.5	1.0	0.23	23.39	259	7.75	7.51	0.88
RAI1 fall	29.0	4.0	0.18	23.41	284	7.36	7.91	0.52
RAI2 sprg	51.8	5.0	0.17	23.63	265	8.01	8.85	0.75
RAI2 fall	42.7	2.5	0.20	24.27	283	7.89	10.70	0.35
ROC1 sprg	17.7	51.8	0.18	23.71	266	7.88	4.74	0.68
ROC1 fall	19.2	30.5	0.13	24.08	273	7.47	8.77	0.18
ROC2 sprg	11.6	16.0	0.13	24.44	271	7.93	7.15	1.51
ROC2 fall	9.1	31.0	0.44	23.00	350	6.97	6.55	1.04
SIL1 sprg	30.5	16.0	0.15	23.58	441	7.34	3.61	0.98
SIL1 fall	36.6	1.25	0.14	23.49	456	6.95	3.20	0.20

Table 3. Continued.

TRANSECT	Channel Width (m)	Canopy Cover (%)	Current Velocity (m/sec)	Water Temp (^{0}C)	Conductivity (umhos/cm)	pH	Dissolved O_2 (mg/L)	Turbidity (NTU)
SIL2 sprg	54.9	1.5	0.24	24.12	430	7.87	5.94	0.61
SIL2 fall	54.9	0	0.20	23.60	434	7.09	3.85	0.44
SIL3 sprg	31.4	36.0	0.19	24.73	446	7.47	7.86	1.29
SIL3 fall	27.4	26.3	0.14	23.89	393	6.65	4.32	2.28
SLG1 sprg	64.0	5.5	0.04	24.03	2,013	8.18	5.10	0.79
SLG1 fall	64.0	0	0.04	23.45	1,897	7.96	4.07	9.66
VOL1 sprg	23.2	54.3	0.07	23.27	1,934	7.38	0.52	0.18
VOL1 fall	25.9	52.5	0.06	23.10	2,348	7.3	0.42	0.09
WAC1 sprg	54.9	1.0	0.10	20.72	223	7.47	5.64	0.88
WAC1 fall	54.9	1.3	0.14	20.71	279	7.34	4.02	0.38
WAC2 sprg	77.7	1.5	0.19	26.69	295	8.20	10.17	1.15
WAC2 fall	73.2	0	0.20	23.41	304	8.17	9.85	0.43
WAK1 sprg	57.9	1.5	0.06	21.16	286	7.69	4.26	1.09
WAK1 fall	62.2	0	0.22	20.67	308	7.30	2.48	0.38
WAK2 sprg	25.6	21.5	0.07	23.19	298	8.04	8.78	2.38
WAK2 fall	24.4	11.5	0.22	21.36	308	7.58	5.43	1.47
WEE1sprg	29.0	26.8	0.10	23.85	325	7.62	2.07	0.88
WEE1 fall	21.3	78.3	0.15	23.84	343	7.52	2.25	0.22
WEE2 sprg	13.7	32.3	0.39	24.38	325	7.78	4.52	0.62
WEE2 fall	15.2	49.3	0.42	24.32	341	7.74	5.06	0.17
WEK1 sprg	21.3	8.5	0.07	24.57	357	7.93	2.51	0.88
WEK1 fall	15.2	10.0	0.06	24.05	358	7.17	2.29	0.41
WEK2 sprg	35.1	0.8	0.18	25.39	356	7.66	5.84	3.03
WEK2 fall	36.6	1.0	0.12	22.69	353	7.06	4.80	2.13

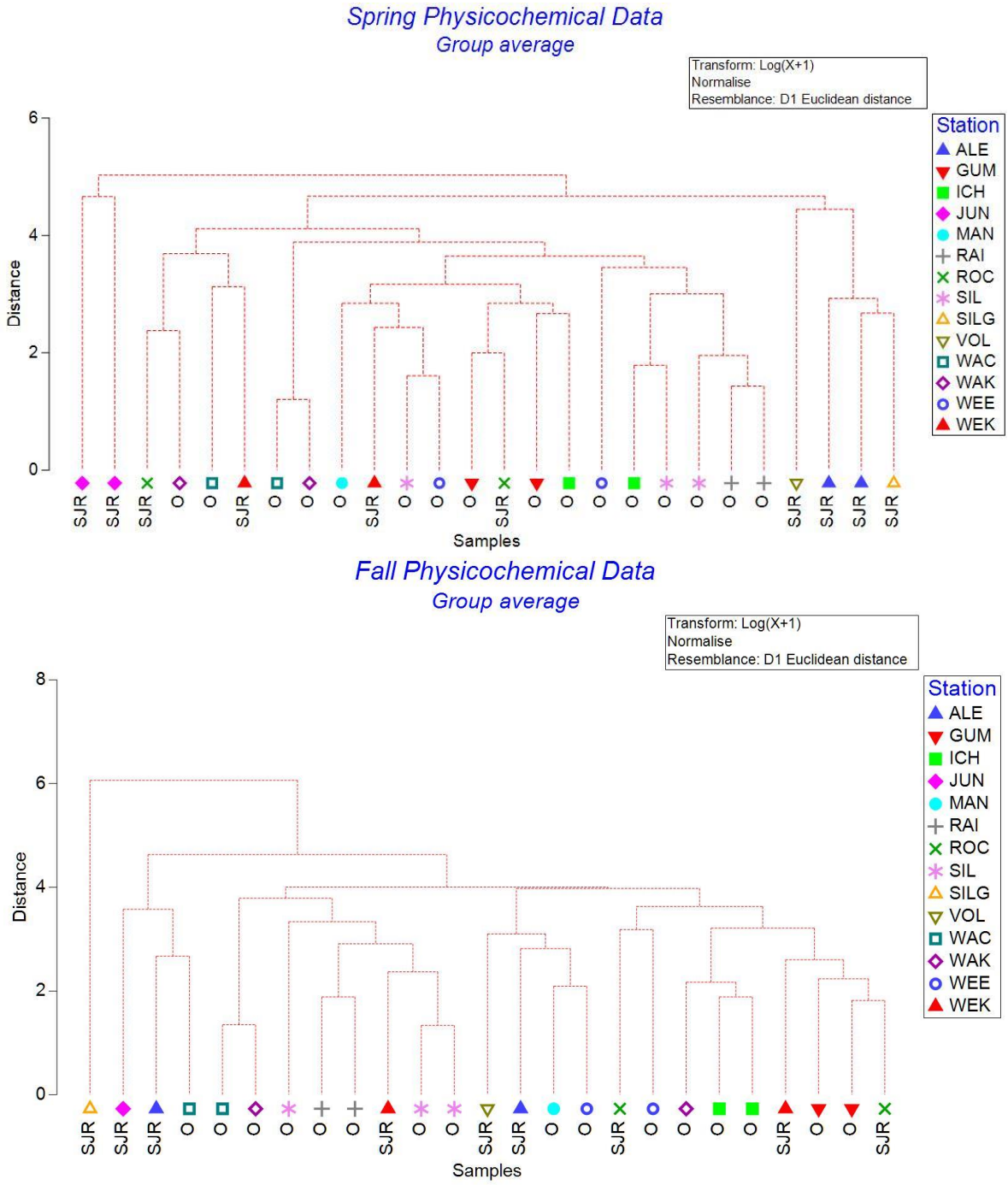


Figure 3. Cluster analysis of the physicochemical data at the spring-run stream transects. Each symbol is an individual transect on a stream. No significant differences among clusters were detected. See Table 2 for definitions of site abbreviations.

on the spring physicochemical data, showed that downstream sites had higher DO, water temperature, turbidity, current velocity, pH and conductivity with an average 19.30% dissimilarity between upstream and downstream sites.

ALGAE DATA

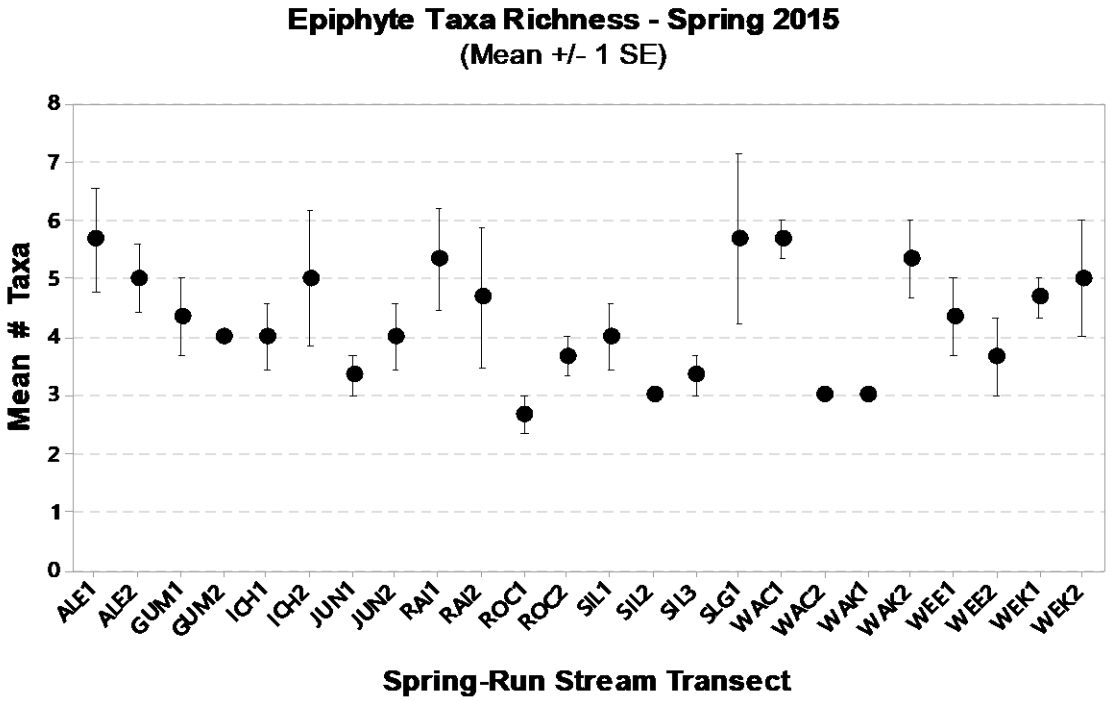
Hereafter, "Epiphytic Algae" refers to the algal epiphytes growing attached to the leaf blades of vascular macrophytes. "Macroalgae" refers to the various taxa of filamentous, mat-forming algae and also includes the epiphytes growing attached to the macroalgal filaments, which will sometimes be referred to as "macroalgae and attached epiphytes."

Epiphytic Algae

Taxa Richness and Composition

Spring 2015. A total of 39 taxa of epiphytic algae were collected on the submerged macrophytes (primarily blades of *Vallisneria* and *Sagittaria*) in spring (Appendix C). Taxa consisted of blue-green algae or Cyanophyta (20 taxa), green algae (Chlorophyta-8 taxa), diatoms (Bacillariophyta-9 taxa), and red algae (Rhodophyta-2 taxa). Commonly occurring algal taxa (found at most of the spring-run stream sites) included the green alga *Cladophora glomerata*, the diatoms *Ulnaria* cf *ulna* and unidentified species of pennate diatoms, and the red alga *Batrachospermum* sp. Species of *Cladophora* are common filamentous green algae found attached to various substrata in streams worldwide (Wehr and Sheath 2003). *Ulnaria* is a diatom taxon formerly synonymized with *Synedra* and is a common taxon in the attached periphyton communities in streams worldwide (Wehr and Sheath 2003). *Batrachospermum* is one of the few strictly freshwater species of red algae and is often found in spring run streams (R. Mattson pers. obs.). The estuarine green alga *Ulva* sp. was collected in Silver Glen Run (SLG1), a saline spring (see Introduction).

Highest total algal taxa richness ($\geq$10 taxa) was exhibited at ALE1, ALE2, RAI1, SLG1 and WAK2 (Appendix C). Highest mean taxa richness (>5 algal taxa) was seen at ALE1, RAI1, SLG1, WAC1 and WAK2 (Figure 4). Cluster analysis of the epiphyte taxa composition had to be conducted using the individual/raw samples, because there was no way to combine them. This indicated three (3) significant groupings of sites (Figure 5). These groupings were based on the presence/contribution of *Ulnaria* (a diatom), *Stigeoclonium* (a green algae) and *Microseira* (a cyanobacteria) in the samples. The small cluster (on the far left of the plot), mainly consisted of samples from the higher-conductivity SJR spring-run streams (Alexander and Silver Glen Springs) which contained greater abundances of *Stigeoclonium*. The smallest cluster represented samples from upstream sites on Gum Slough and the Rainbow River and contained greater abundances of *Microseira*. The largest cluster contained an assortment of samples from springs on the St. Johns River SJR) and other springs (O) not associated directly with the St. Johns River and had greater abundances of *Ulnaria*. ANOSIM analysis of the sites showed significant differences between (SJR and O sites ($r=0.619$, $P_{perm} = 0.001$). SIMPER analysis revealed that the difference was due to the greater abundance of *Ulnaria* in SJR sites.



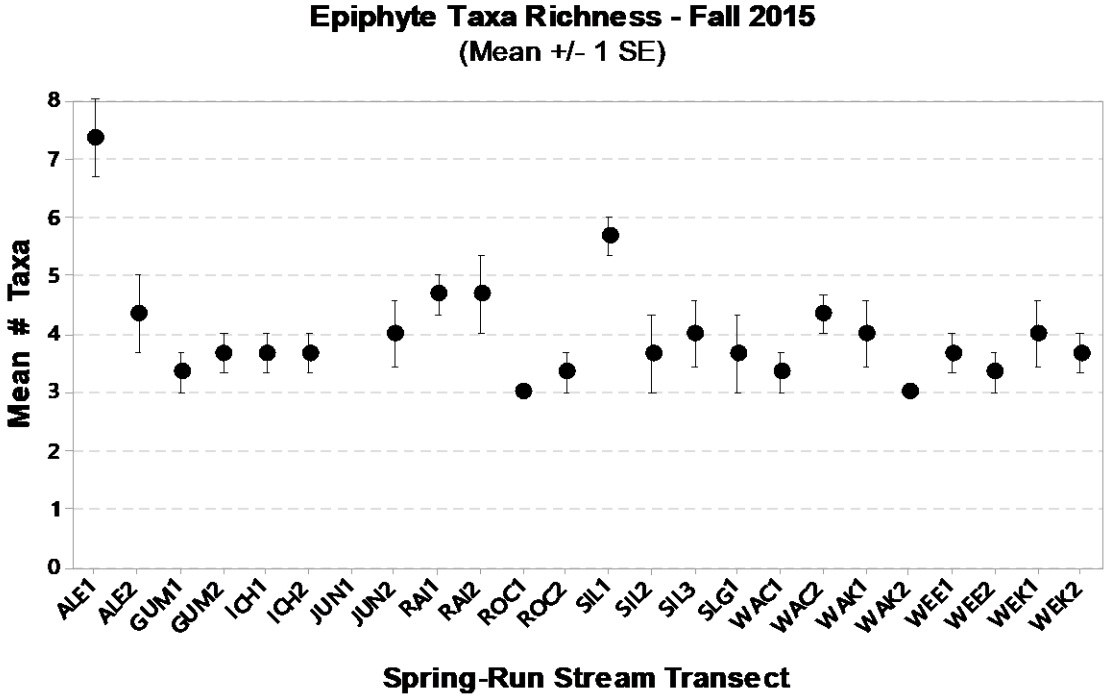


Figure 4. Mean epiphytic algal taxa richness in spring and fall 2015 at the sampling transects. No macrophytes were collected at JUN1 in the Fall (hence no epiphytic algae). See Table 2 for definitions of site abbreviations.

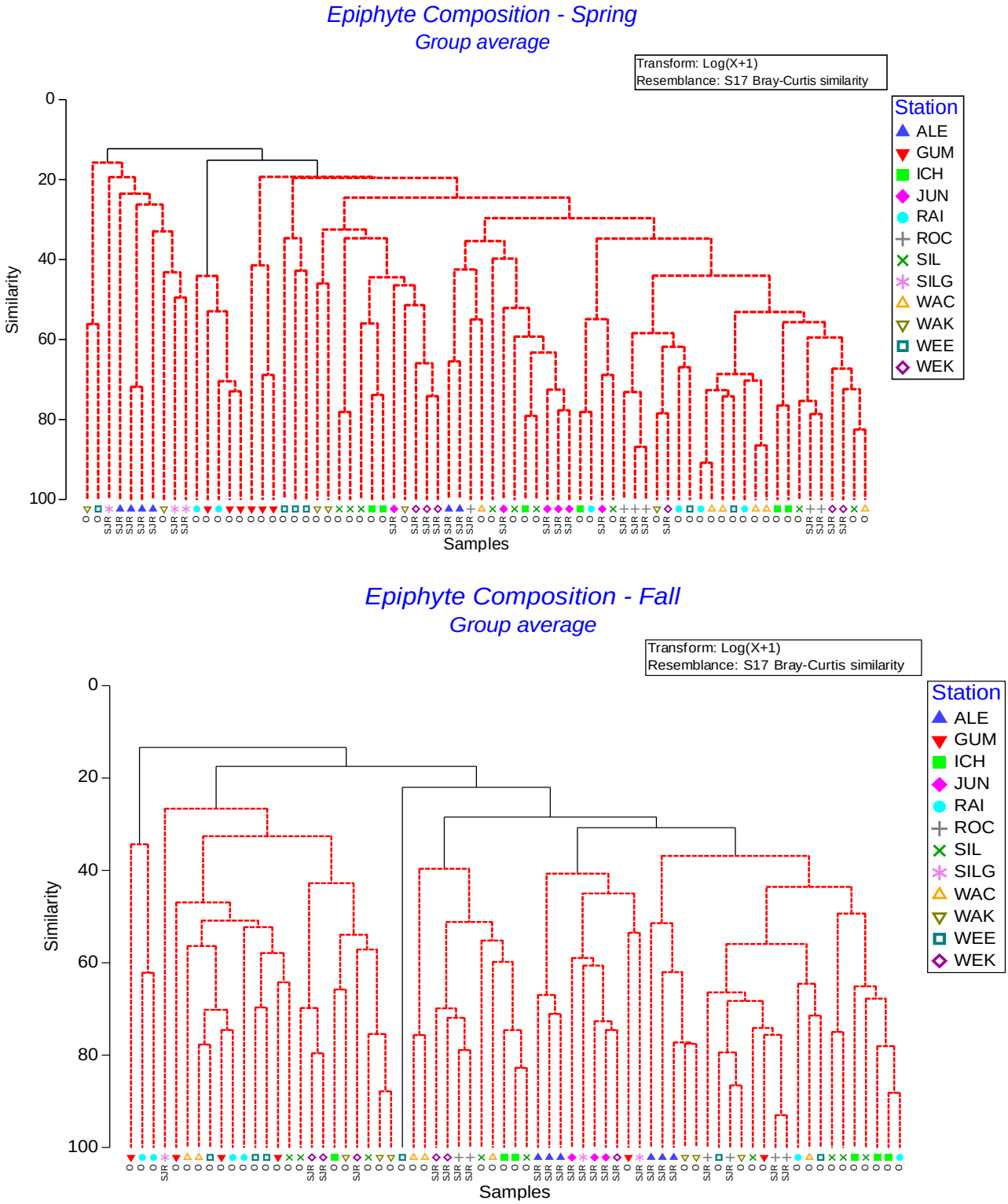


Figure 5. Cluster analyses of the epiphyte algal taxa composition raw samples. Clusters connected with a solid line are significantly different. See Table 2 for definitions of site abbreviations.

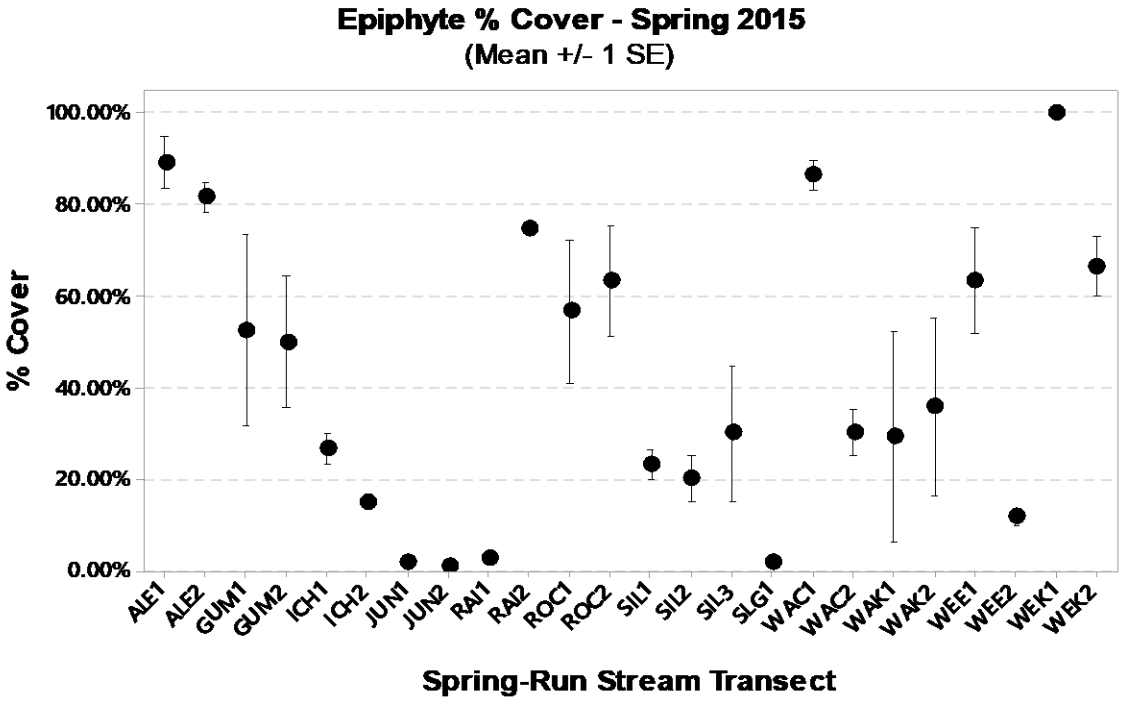
Fall 2015. In fall 2015, a total of 31 algal taxa were collected (Appendix C). These included the same major groups seen in the spring, Cyanophyta (12 taxa), Chlorophyta (6 taxa), Bacillariophyta (10 taxa) and Rhodophyta (3 taxa). No macrophytes were present at JUN1 in the fall, and thus no epiphytic algae were present. The most commonly occurring algal taxa were the diatoms *Ulnaria* cf *ulna* and unidentified species of pennate diatoms. *Cladophora* and *Batrachospermum* were not as commonly collected in the fall compared to spring. The red algal genus *Polysiphonia* was collected at the lower Juniper Creek site (JUN2), a relatively "saline" site, with high conductivity (Table 3). Some members of this algal genus are commonly found in low salinity, upper-estuarine areas (Dawes 1974).

Highest total algal taxa richness (≥10 taxa) was exhibited at ALE1 and SIL1 (Appendix C). Highest mean taxa richness (>5 taxa) was seen at ALE1 and SIL1 (Figure 4). Mean algal taxa richness was generally lower in the fall versus the spring at many sites (≤4 taxa). Results of cluster analysis of the taxa composition samples indicated five significant groups (Figure 5). A small cluster consisting of three samples from Rainbow River and Gum Slough (on the far left) was broken out from all other sites based on its abundance of *Rhizoclonium hieroglyphicum*. A single upstream sample from Weeki Wachee was pulled out from all the other samples possibly based on the occurrence of *Batrachospermum*. A medium sized grouping of ten (10) samples, mainly from SJR sites, contained high abundances of pennate diatoms and *Terpsinoe musica,* while the other medium sized group of 11 samples had high abundances of pennate diatoms and *Heteroleibleinia*. Of the remaining two large groupings, one cluster of 21 samples almost entirely from O sites had greater abundances of *Cladophora* and the other grouping of 23 samples from a mixture of SJR and O sites had high abundances of pennate diatoms and *Ulnaria* cf. *ulna*. ANOSIM analysis of the sites showed significant differences between SJR and O sites ($r=0.105$, $P_{perm} = 0.006$). SIMPER analysis revealed that the difference was due to the greater abundance of *Ulnaria* in O sites and greater abundances of pennate diatoms at SJR sites.

Cover

Spring 2015. Mean percent cover of epiphytic algae on the submerged macrophytes in spring 2015 exhibited wide variation (Figure 6). Highest mean epiphyte cover (>80%) was seen at both sites on Alexander Springs Creek (ALE1 and 2) and the upstream sites on the Wacissa and Wekiva Rivers (WAC1 and WEK1, respectively). Lowest mean epiphyte cover (generally <5%) was seen at JUN1 and 2, RAI1, and SLG1. Cluster analysis indicated no significant groupings of sites based on percent cover. However, ANOSIM analysis indicated a significant difference ($r=0.27$, $P_{perm} = 0.01$) in percent cover between SJR and O sites, with O sites having a greater percent cover of epiphytic algae.

Fall 2015. Mean epiphytic algal percent cover in fall also displayed considerable variation (Figure 6). Highest mean cover (>80%) was seen at ALE1, RAI1, and WEE1. The upstream Rainbow Springs transect (RAI1) exhibited very low mean epiphyte cover in the spring but was among the highest transects in fall. Relatively high mean fall cover (75%) was also seen at ICH1. Lowest mean cover was exhibited at JUN2 and WEE2 (<12%). JUN1 did not support



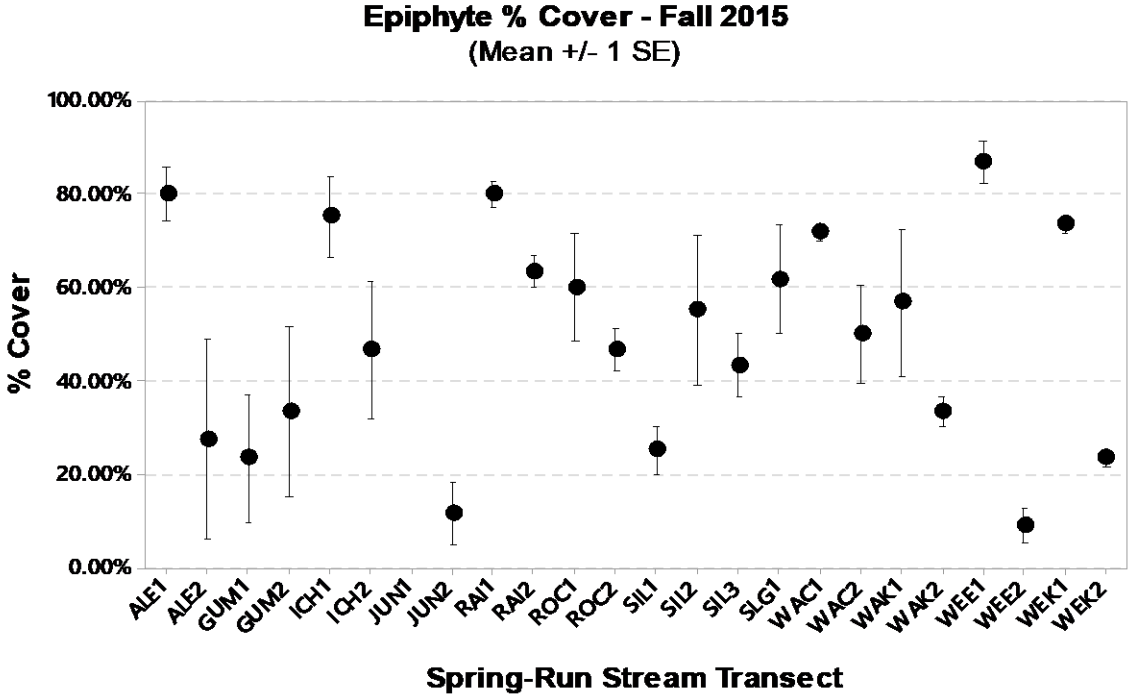


Figure 6. Mean epiphytic algal cover (%) in spring and fall 2015 at the sampling transects. Macrophytes were not present at JUN1 in the fall. See Table 2 for definitions of site abbreviations.

macrophytes in the fall, hence no epiphytic algae. A few sites exhibited lower mean algal cover in the fall versus the spring (ALE2, GUM1 and 2, and WEK2), while a number of other sites exhibited increased epiphytic algal cover in the fall (ICH1 and 2, JUN2, RAI1, SIL2, SIL3, SLG1, WAC2, and WAK1). Cluster analysis indicated no significant groupings of sites based on percent cover. ANOSIM analysis revealed no significant difference in percent cover between SJR and O sites in the fall.

Chlorophyll a

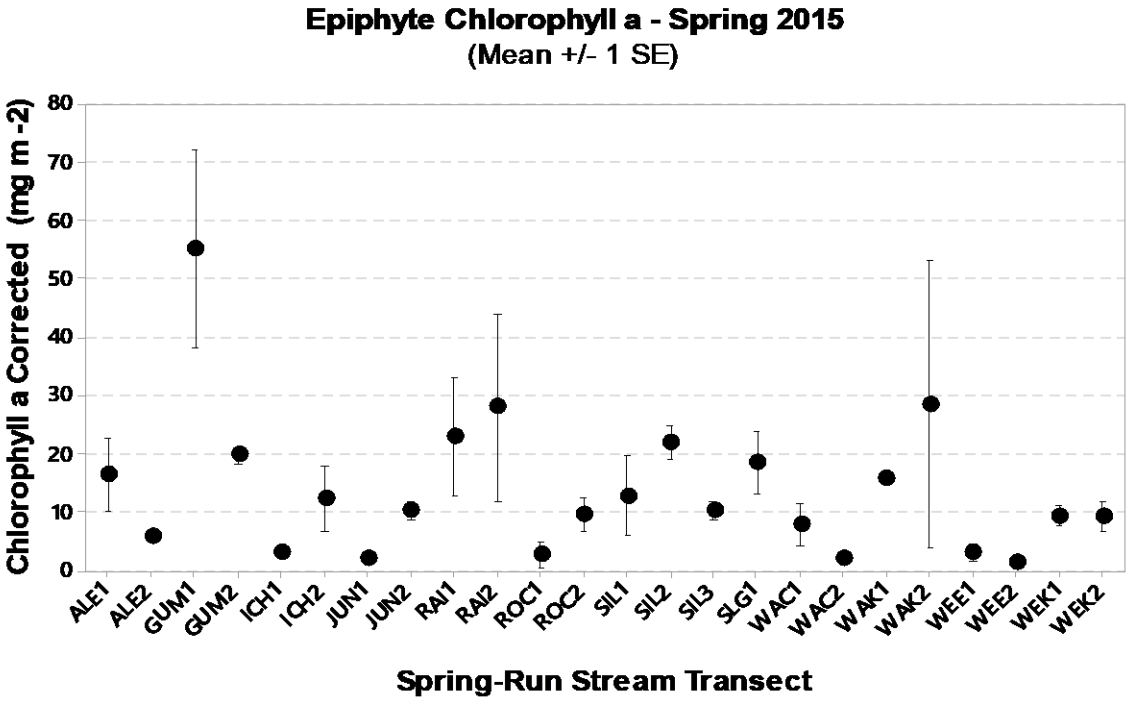
Spring 2015. Highest mean Chl *a* densities ($\geq$20 mg/m^2) in the spring were exhibited at GUM1, RAI1, RAI 2, SIL2, and WAK2 (Figure 7). Lowest mean densities (<5 mg/m^2) were seen at ICH1, JUN1, ROC1, WAC2, WEE1 and WEE2 (Figure 7). Cluster analysis showed no significant groupings of samples based on epiphytic Chl *a* densities in the spring. In addition, ANOSIM analysis revealed no significant difference between samples from SJR or O sites.

Fall 2015. Overall, mean Chl *a* densities were reduced at many sites in the fall; no transect equaled or exceeded 20 mg/m^2 Chl *a* (Figure 7). Highest mean Chl *a* densities in the fall ($\geq$10 mg/m^2) were seen at ALE1, GUM1, ICH1, RAI2, WAC1, WAC2, and WEK1. GUM1 and RAI2 were the sites that had high mean Chl *a* densities in both spring and fall. ICH1 and WAC2 exhibited very low mean Chl *a* densities in the spring, but among the highest in the fall, indicating considerable seasonal variability in mean epiphytic algal abundance at some spring sites. Cluster analysis showed no significant groupings of samples based on epiphytic Chl *a* densities in the fall. However, ANOSIM analysis revealed significant differences (R=0.121, P_{perm} = 0.012) in Chl *a* densities from SJR versus O sites. SIMPER analysis revealed that densities were higher in samples from O sites.

Ash-Free Dry Weight

Spring 2015. Highest mean AFDW ($\geq$10 g/m^2) was at GUM1, ICH1, and RAI2 (Figure 8). Lowest mean AFDW (<2 g/m^2) was at JUN2, SIL3, and WEE2 (Figure 8). Cluster analysis showed no significant groupings of sample sites based on epiphyte AFDW in the Spring. ANOSIM analysis revealed no significant differences in AFDW from samples based on location (SJR versus O).

Fall 2015. Like Chl *a*, mean AFDW at most sites was lower in fall 2015, both in terms of the mean values and the range from minimum to maximum (Figure 8). GUM1 was the only site with a mean AFDW >10 g/m^2. Relatively high mean AFDW (~9 g/m^2) was also seen at RAI2 and WEK1 (Figure 8). GUM1 and RAI2 also exhibited highest mean AFDW in the spring as well as the fall, although mean AFDW at ICH1 was lower in the fall versus spring. Cluster analysis showed no significant groupings of sampling based on epiphyte AFDW in the Fall. ANOSIM analysis revealed no significant differences in AFDW from samples based on location (SJR versus O).



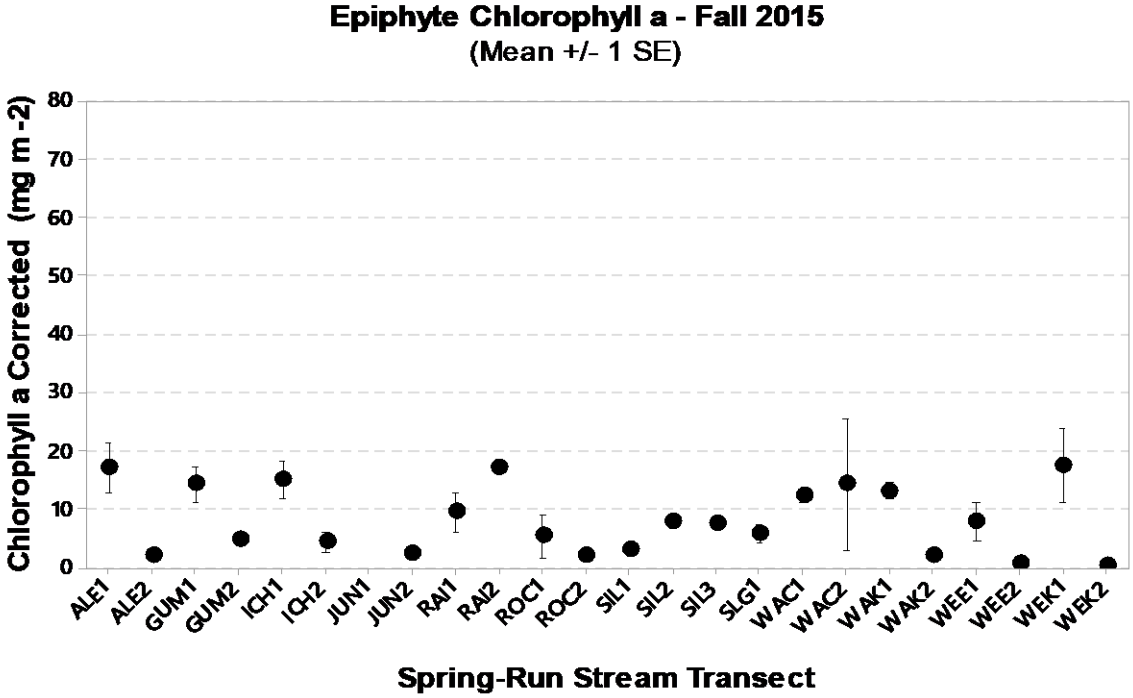
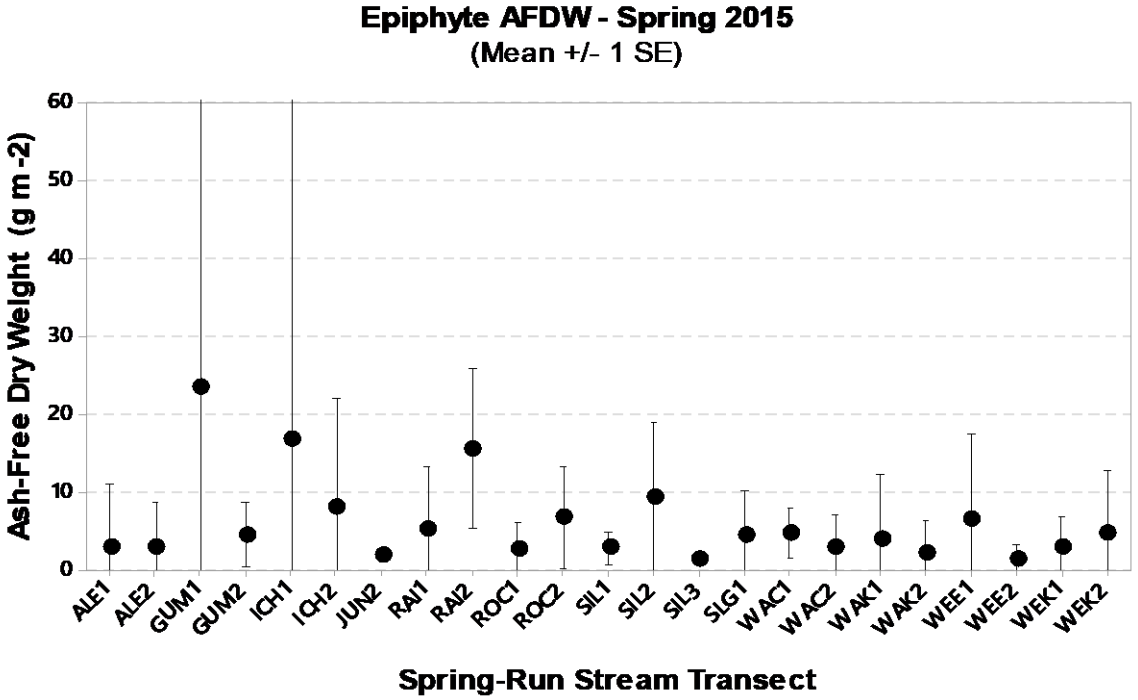


Figure 7. Mean epiphytic algal Chlorophyll a (mg/m^2) in spring and fall 2015. No macrophytes were collected at JUN1 in fall 2015, hence no epiphytic algae. See Table 2 for definitions of site abbreviations.



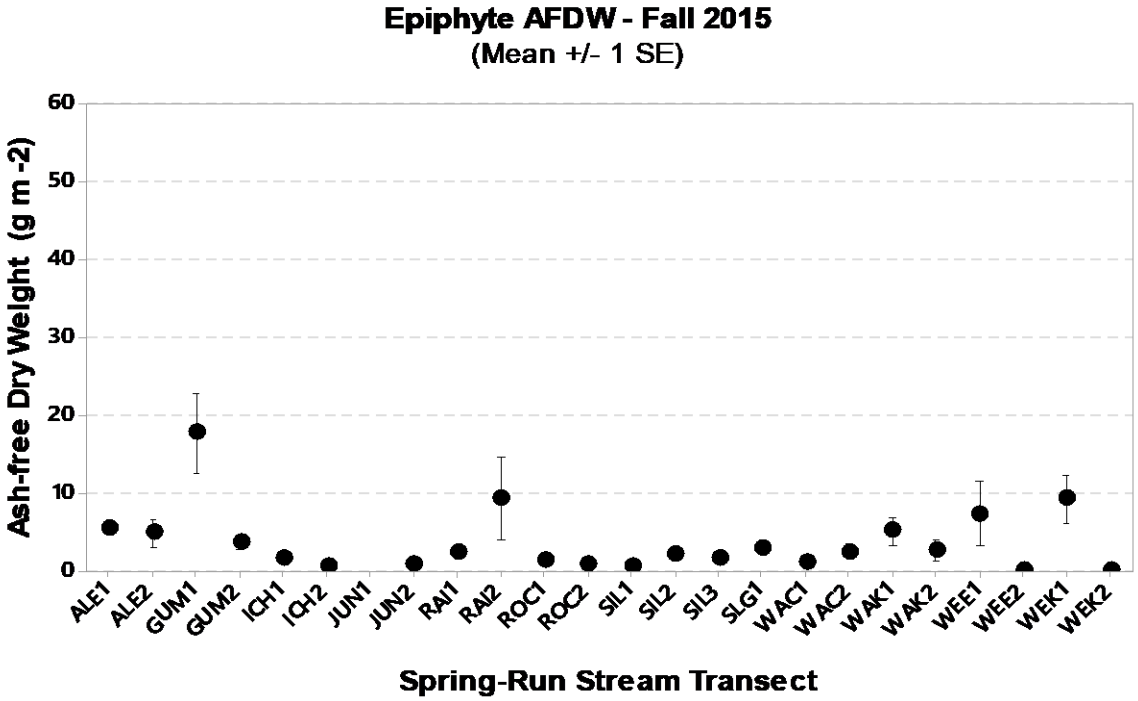


Figure 8. Mean epiphytic algal Ash-Free Dry Weight (g/m^2) in spring and fall 2015. See Table 2 for definitions of site abbreviations.

Macroalgae

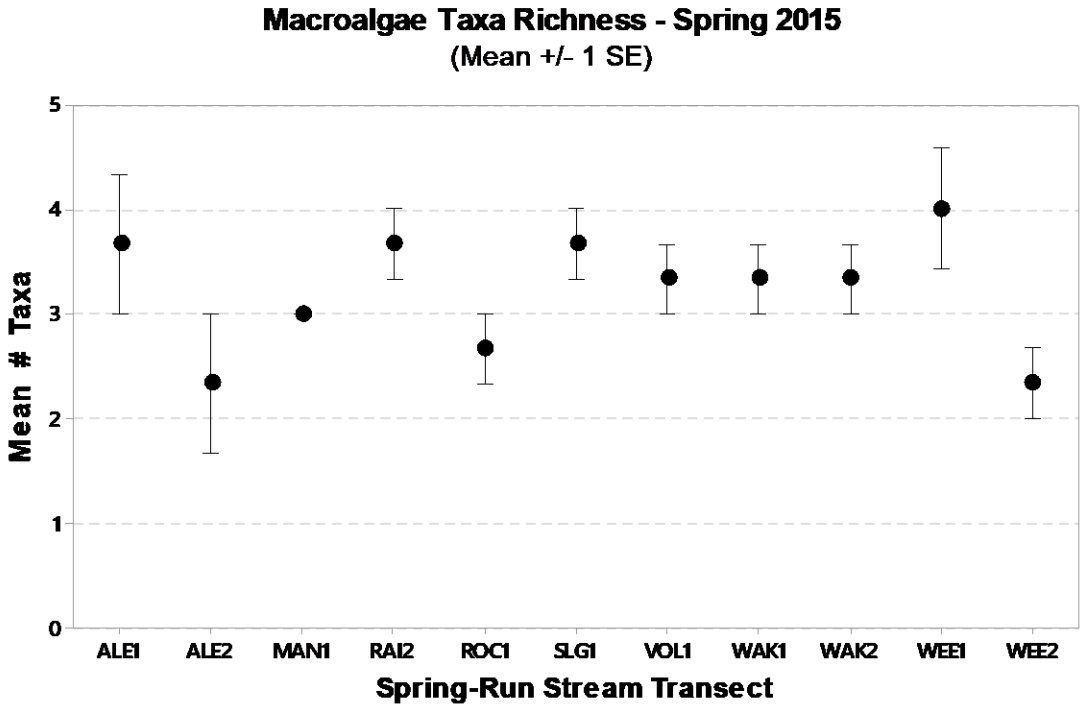
As noted earlier, "Macroalgae" refers to the filamentous, mat forming algal taxa and their attached epiphytes.

Taxa Richness and Composition

Spring 2015. Eleven (11) spring-run stream transects supported macroalgal mats extensive enough to be sampled in the spring season (Appendix D presents taxa lists). A total of 23 algal taxa were collected in the macroalgal mats, with major groups being Cyanophyta (10 taxa), Chlorophyta (6 taxa), Bacillariophyta (4 taxa), Rhodophyta (2 taxa) and Xanthophyta (Yellow-green algae;1 taxon). The major filamentous forms comprising the macroalgal mats included the cyanobacterium *Microseira* (formerly *Lyngbya*) *wollei*, the green algae *Cladophora glomerata* (at WAK2), *Dichotomosiphon tuberosus* (at MAN1 and WEE 1 and 2), *Hydrodictyon reticulatum* (at ALE1) and *Rhizoclonium hieroglyphicum* (at ALE1 and VOL1), an unidentified filamentous red alga (WAK1), and the yellow-green alga *Vaucheria* sp. at ROC1, VOL1, and WAK1. *Microseira wollei* was the most commonly occurring algal taxon, found at most of the stream sites. *Rhizoclonium hieroglyphicum* and the filamentous diatom *Terpsinoe musica* were the second most commonly occurring algal taxa (found at 5 of the 11 sites).

Highest mean macroalgal taxa richness was at WEE1 (Figure 9 – 4 taxa). As for the epiphytic algae, cluster analysis of the macroalgal taxa composition had to be done using the individual samples from the transects. This analysis indicated four (4) significant groups of sites (Figure 10). One small group consisted of only two samples, both from the upstream site located in Alexander Spring and Volusia Blue Spring. SIMPER analysis showed that transects from these two springs were distinguished by high abundances of *R. hierogylphicum*. A group of eight (8) samples from O transects had high abundances of *D. tuberosus*, while a similar sized group of samples from mixed transects had high abundances of *Vaucheria*. The largest grouping comprised of samples from a mix of upstream and downstream SJR and O transects had higher abundances of *M. wollei*. ANOSIM found significant differences in macroalgal composition between samples from SJR versus O sites ($R=0.151$, $P_{perm} = 0.01$) in the Spring. SIMPER analysis revealed that these differences were attributable to increased abundances of *M. wollei* and *Vaucheria* at SJR sites and increased abundances of *D. tuberosus* at O sites.

Fall 2015. Fewer spring-run stream transects (9) supported macroalgal mats in the fall, although they were mostly the same streams as sampled in spring 2015. A total of 16 macroalgal taxa or epiphytes were collected (Appendix D); Cyanophyta (4 taxa), Chlorophyta (5 taxa), Bacillariophyta (4 taxa), Rhodophyta (2 taxa), and Xanthophyta (1 taxon). Generally, the same filamentous macroalgal taxa dominated the sample sites in the fall as in the spring (*M. wollei, D. tuberosus, R. hieroglyphicum*, and *Vaucheria* sp.). *Microseira wollei* again occurred in most of the spring-run streams. The red alga *Compsopogon coeruleus* was the dominant algal taxon at WAK1. The filamentous diatoms *Pleurosira laevis* and *Terpsinoe musica* were also components of the algal mats, as they were in the spring.



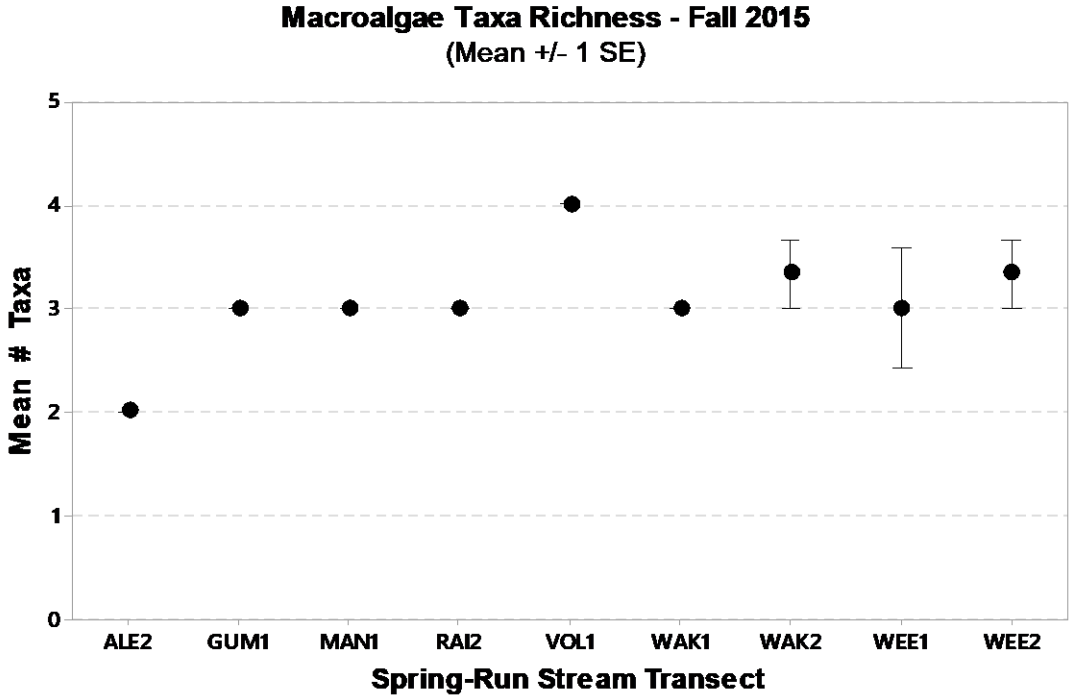


Figure 9. Mean macroalgal (and associated epiphyte) taxa richness in spring and fall 2015 at the sampling transects where macroalgal mats occurred. See Table 2 for definitions of site abbreviations.

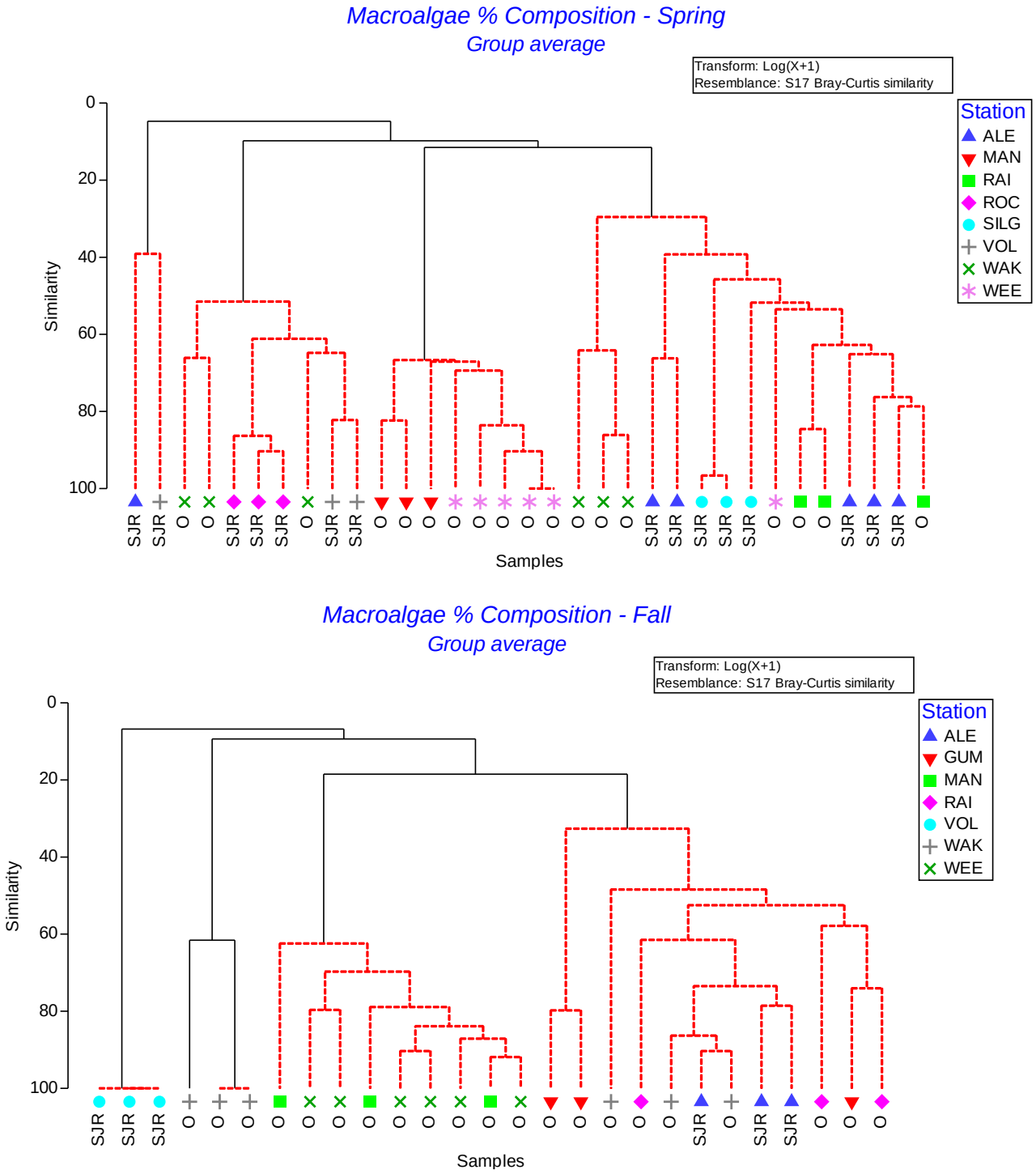


Figure 10. Cluster analyses of the macroalgal/epiphyte taxa composition raw samples. Clusters connected with a solid line are significantly different. See Table 2 for definitions of site abbreviations.

Highest total macroalgal taxa richness (6) was collected at MAN1, RAI2, and WAK2. Volusia Blue Spring Run (VOL1) had the highest mean taxa richness (Figure 9). Again, cluster analysis of the macroalgal data had to be conducted on the individual samples from all transects. This analysis indicated five (5) significant groupings (Figure 10). One upstream Wakulla sample grouped by itself based on the occurrence of *Dichotomosiphon/Vaucheria* and *M. wollei*, with the other two upstream Wakulla samples clustered in a separate group based on the abundance of *Compsopogon coeruleus*. All the Volusia Blue Spring Run samples formed a third group based on the abundance of *Dichotomosiphon*/*Vaucheria*. The grouping of nine samples from Manatee Springs Run and the Weeki Wachee River was based on the abundance of *D. tuberosus*, whereas the large grouping of SJR and O samples was based on the abundance of *M. wollei*. ANOSIM found significant differences in macroalgal composition between samples from SJR versus O sites ($R=0.245$, $P_{perm} = 0.024$). SIMPER analysis revealed that these differences were attributable to increased abundances of *M. wollei* and *Dichomosiphon*/ *Vaucheria* at SJR sites and increased abundances of *D. tuberosus* at O sites, very similar to what was found in the Spring.

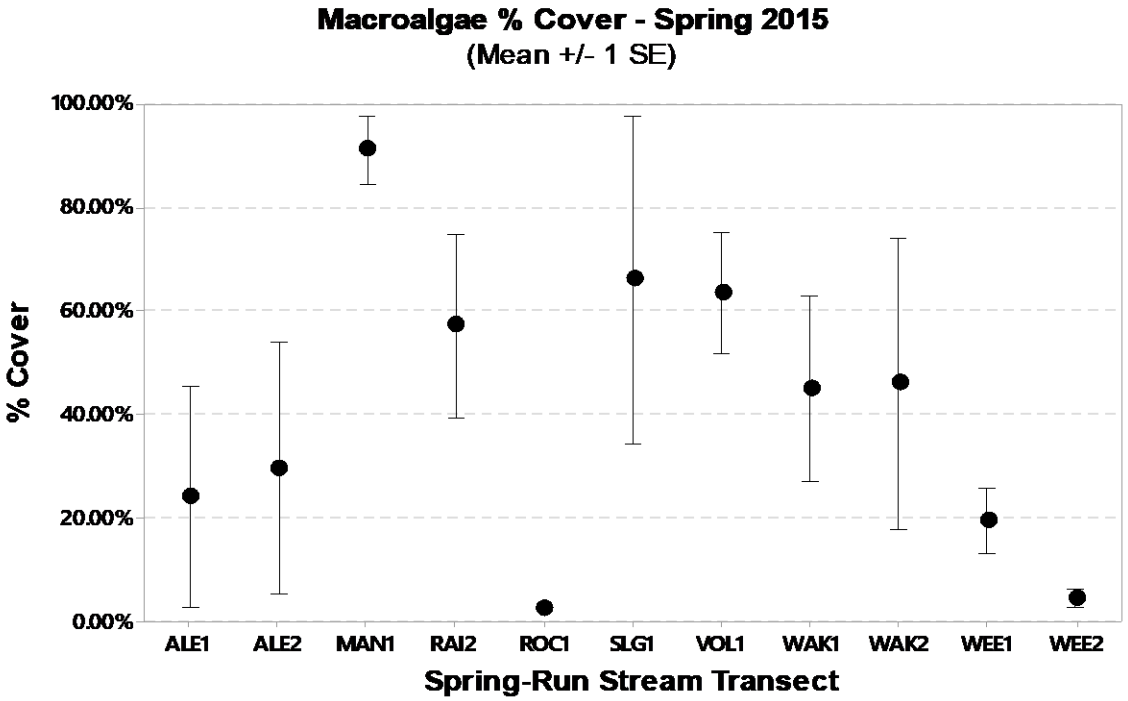
Cover

Spring 2015. Mean macroalgal cover in spring 2015 varied widely, although cover at most sites exceeded 20% (Figure 11). Highest mean cover ($\geq$50%) was seen at MAN1, RAI2, SLG1, and VOL1. ROC1 and WEE2 had lowest mean macroalgal cover. Where multiple sites were sampled in the same spring-run stream, slightly higher mean cover was generally seen at the downstream site (ALE-Alexander Springs Creek and WAK-Wakulla River), although at Weeki Wachee River, the downstream site (WEE2) had lower mean macroalgal cover. Stevenson et al. (2007) generally found highest overall macroalgal cover at upstream sites closest to the headsprings. Cluster analysis of macroalgal cover indicated no significant differences among the transects. ANOSIM analysis showed no significant difference in macroalgal cover between SJR and O sites.

Fall 2015. As seen in the spring, most transects supported mean macroalgal cover >20% in the fall (Figure 11). ALE1, ROC1, and SLG1 did not support algal mats extensive enough to sample in the fall, while GUM1 had algal mats extensive enough to sample in the fall but not spring. Highest mean macroalgal cover in the fall ($\geq$50%) was seen at GUM1, MAN1, VOL1, and WAK1. Mean macroalgal cover at RAI2 was substantially lower in fall (10%) versus spring (>50%). Cluster analysis of macroalgal cover likewise showed no significant differences among sites based on cover and ANOSIM showed no significant difference in macroalgal cover between SJR and O sites.

Chlorophyll a

Spring 2015. Highest Chl *a* densities in spring (>400 mg/m^2) were seen at ALE2, RAI2, and SLG1 (Figure 12). Lowest Chl *a* densities (<100 mg/m^2) were seen at MAN1, VOL1, WAK1 and WAK2 (Figure 12). Cluster analysis showed no significant groupings of samples based on macroalgal Chl *a* densities in the spring. In addition, ANOSIM analysis revealed no significant difference between samples from SJR versus O sites.



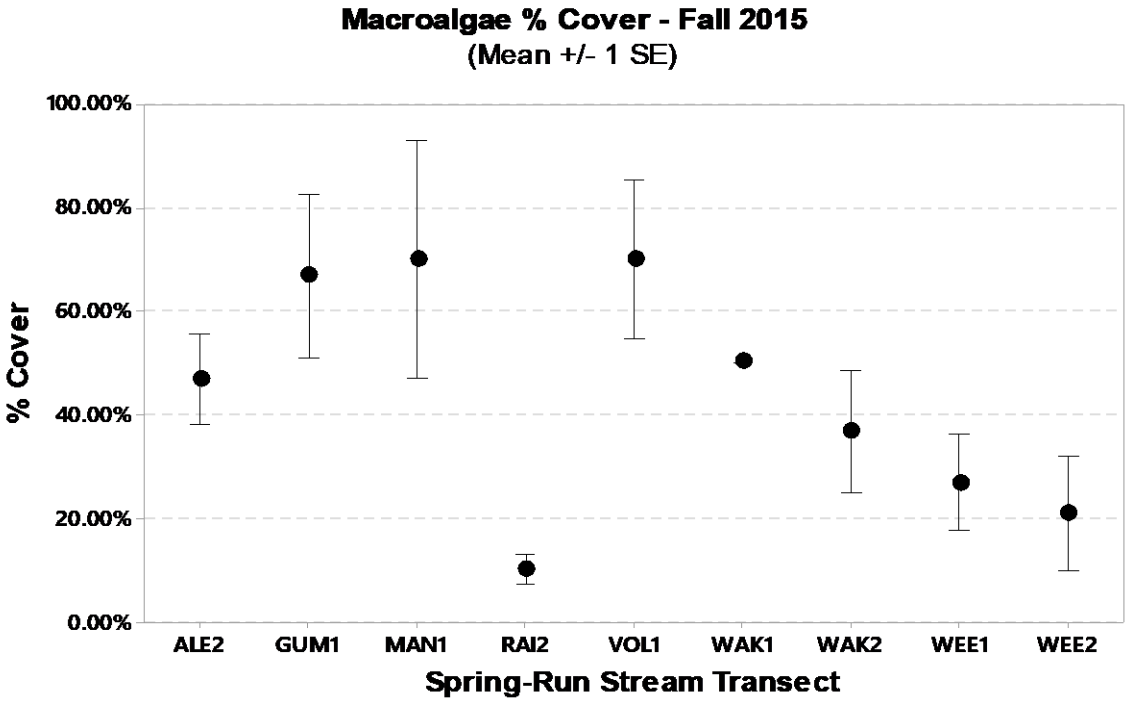


Figure 11. Mean macroalgae (and associated epiphyte) cover (%) in spring and fall 2015. See Table 2 for definitions of site abbreviations.

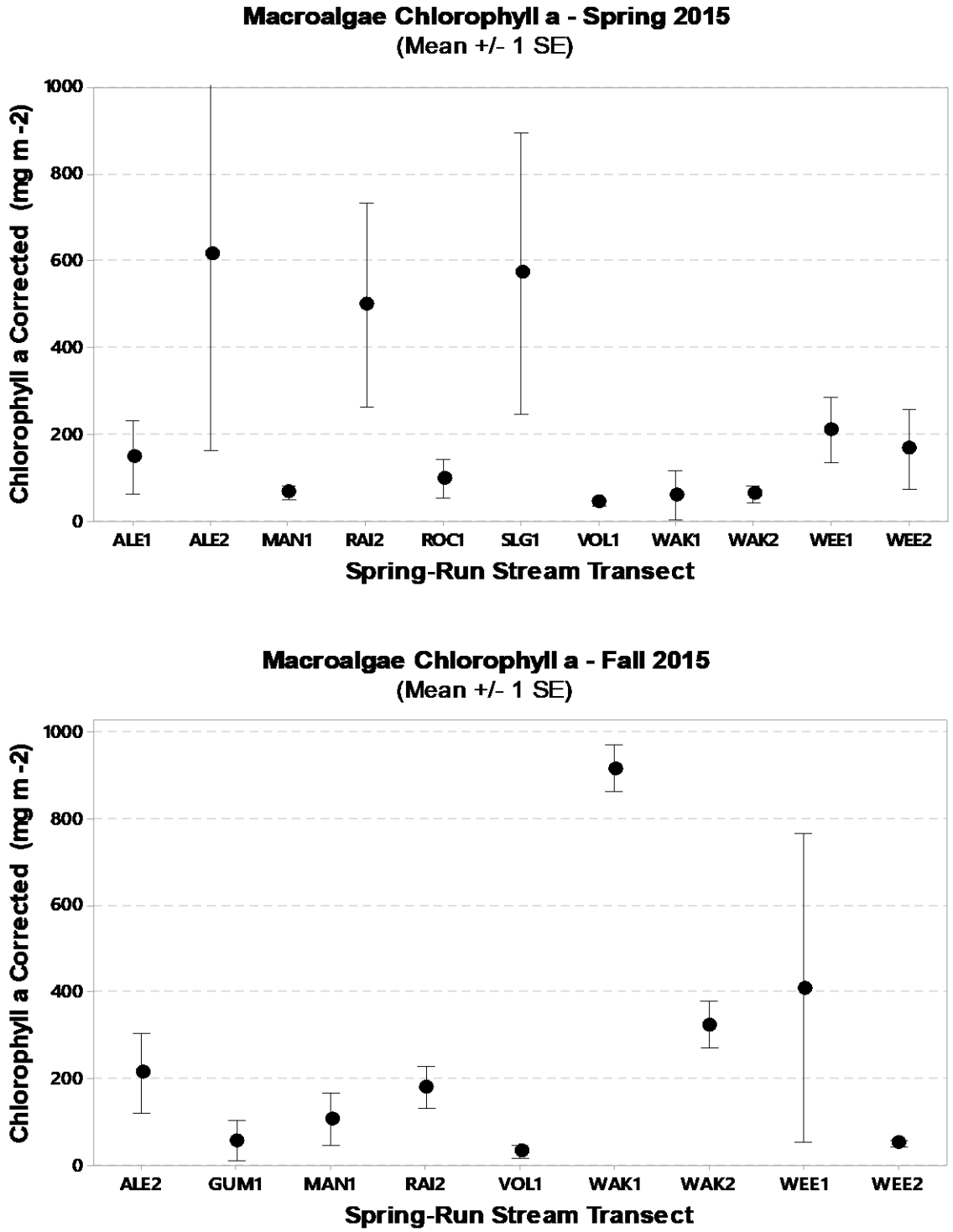


Figure 12. Mean macroalgae (and associated epiphyte) Chlorophyll a density in spring and fall 2015 at the transects where macroalgal mats occurred. See Table 2 for definitions of site abbreviations.

Fall 2015. In fall, macroalgal Chl *a* densities were generally lower compared to spring (Figure 12). WAK1 and WEE1 were the only transects with Chl *a* densities >400 mg/m^2. Lowest Chl *a* densities (<100 mg/m^2) were seen at GUM1 (which did not support macroalgae in the spring), MAN1, VOL1 and WEE2. Transects ALE1, ROC1, and SLG1 did not support macroalgal mats in the fall (Figure 12). Fall Chl *a* densities were lower at ALE2, RAI2, and WEE2, and densities were higher at MAN1, WAK 1 and 2, and WEE1. Similar densities were seen at VOL1 in spring and fall. Cluster analysis showed no significant groupings of samples based on epiphytic Chl *a* densities. In addition, ANOSIM analysis revealed no significant difference between samples from high or low velocity sites or from SJR versus O sites.

Ash-Free Dry Weight

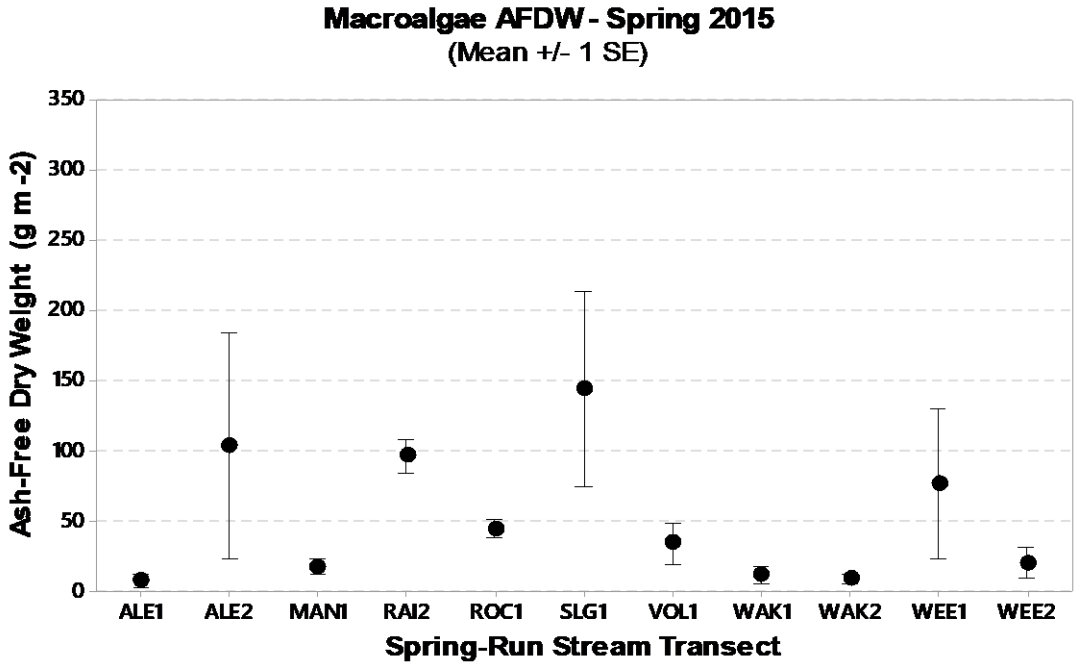
Spring 2015. Highest AFDW (>50 g/m^2) was seen at ALE2, RAI2, SLG1, and WEE1 (Figure 13). ALE2 and SLG1 had AFDW exceeding 100 g/m2. Lowest AFDW (<10 g/m^2) was exhibited at ALE1 and WAK2 (Figure 13). Cluster analysis revealed no significant groupings of samples based on macroalgae AFDW in the Spring. ANOSIM analysis revealed no significant differences in AFDW from samples based on location (SJR versus O).

Fall 2015. Macroalgal abundance as AFDW was generally reduced in the fall (Figure 13). Most sites were <50 g/m^2. Highest AFDW (>50 g/m^2) was seen at WAK1 and WEE1. At the low end of the scale, no transect had <10 g/m^2 AFDW. Lowest AFDW (<20 g/m^2) was seen at MAN1 and WEE2. Higher AFDW in fall was seen at WAK1; most other sites had similar or lower AFDW in fall versus spring (Figure 13). Cluster analysis revealed no significant groupings of samples based on macroalgae AFDW. ANOSIM analysis revealed no significant differences in AFDW from samples based on location (SJR versus O).

Summary of Seasonal Differences Among Transects

Perhaps one of the most basic findings from this study is the high amount of spatial and temporal variability in algal abundance (cover, chlorophyll a, and AFDW) for both epiphytic and macroalgae. This makes it difficult to discern trends over time and to compare the data collected in this study with other (historic) studies of algae in spring-run streams without more extensive sampling.

Epiphytic Algae. Epiphytic algal percent cover did not display consistent seasonal differences between spring and fall (Figure 14). Half of the transects (12/24) had higher cover in spring and half in fall. Chl *a* trends were somewhat more distinct; 17 of 24 transects displayed higher spring Chl *a* (Figure 14). AFDW was similar, with 16 of 24 transects displaying higher spring AFDW (Figure 14). However, different transects exhibited different patterns. ALE2 had higher Chl *a* in spring, but higher AFDW in fall (Figure 14); ICH1 had higher spring AFDW, but higher fall Chl *a*. Chl *a* is a more accurate measure of standing crop of photosynthetic organisms, while AFDW measures standing crop of all biota in the epiphytic community, including photosynthetic and non-photosynthetic organisms and any extracellular organic material.



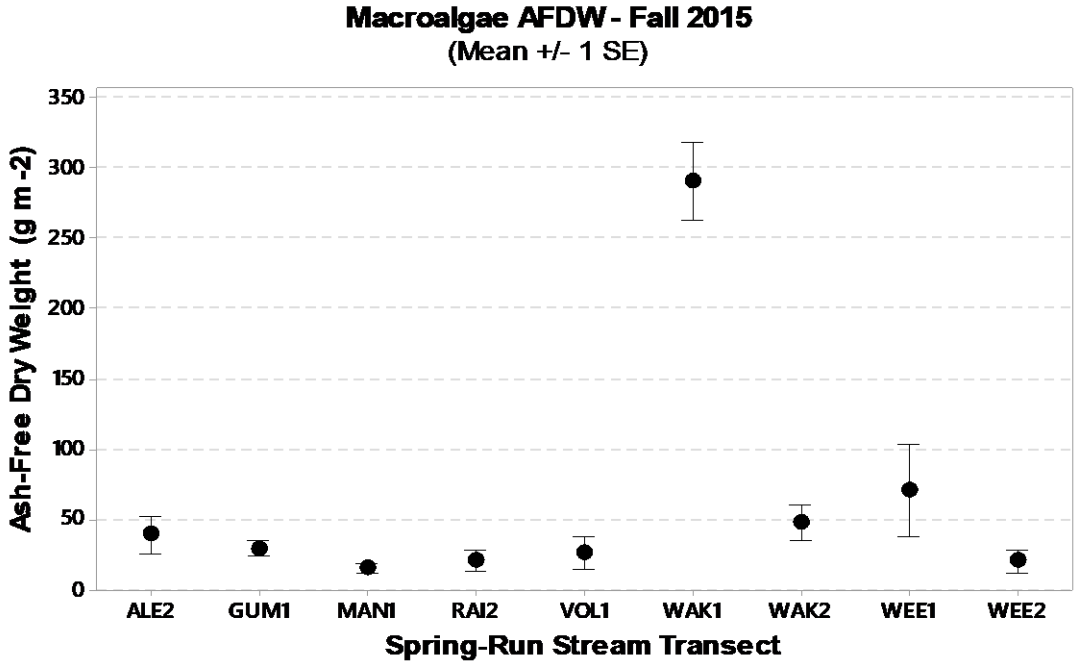


Figure 13. Mean macroalgae (and associated epiphyte) Ash-Free Dry Weight standing crop in spring and fall 2015 at the transects where macroalgal mats occurred. See Table 2 for definitions of site abbreviations.

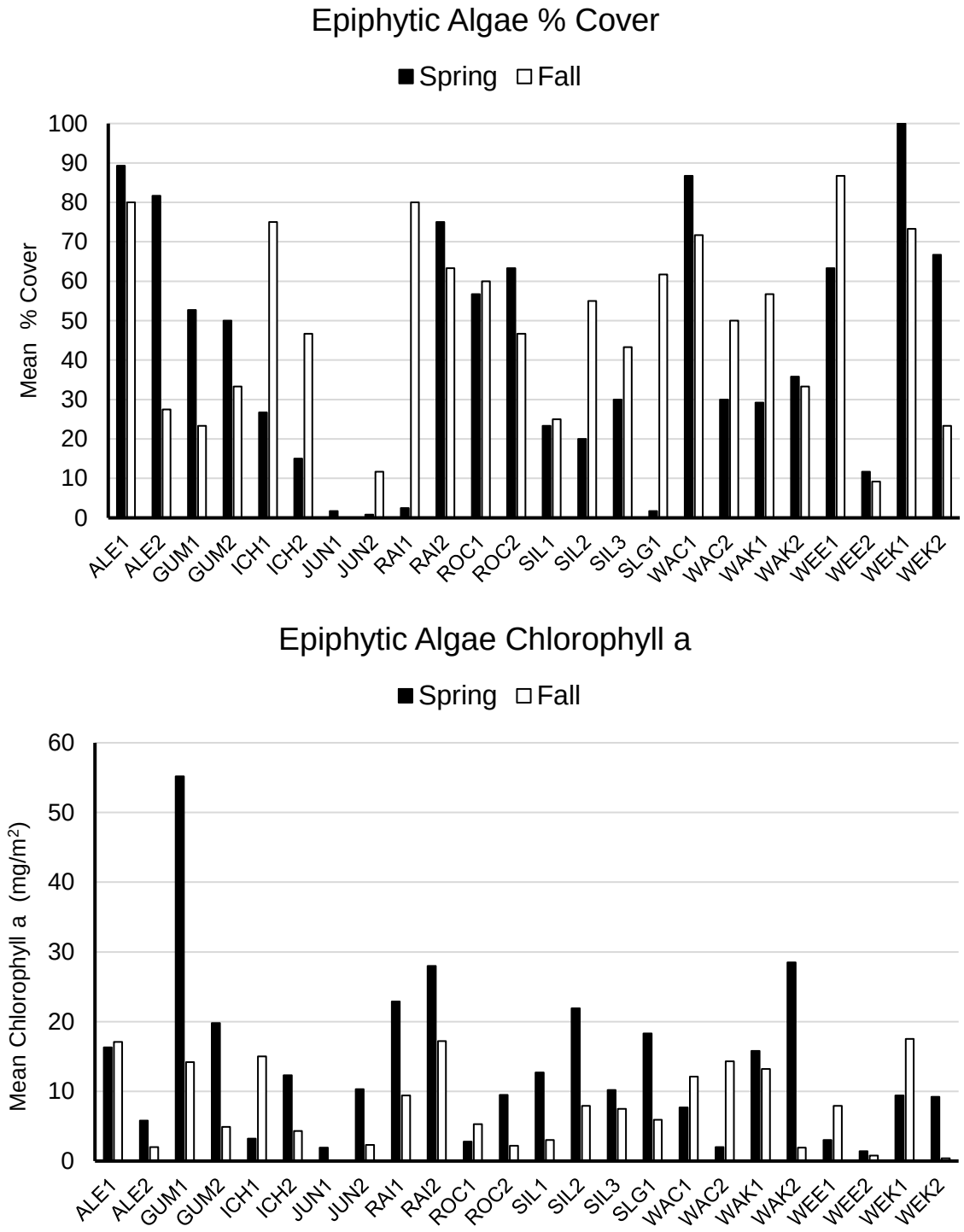


Figure 14. Comparisons of mean epiphytic algal abundance (% Cover, Chlorophyll a, and AFDW) in spring versus fall at the transects supporting macrophytes with epiphytic algae. See Table 2 for definitions of site abbreviations.

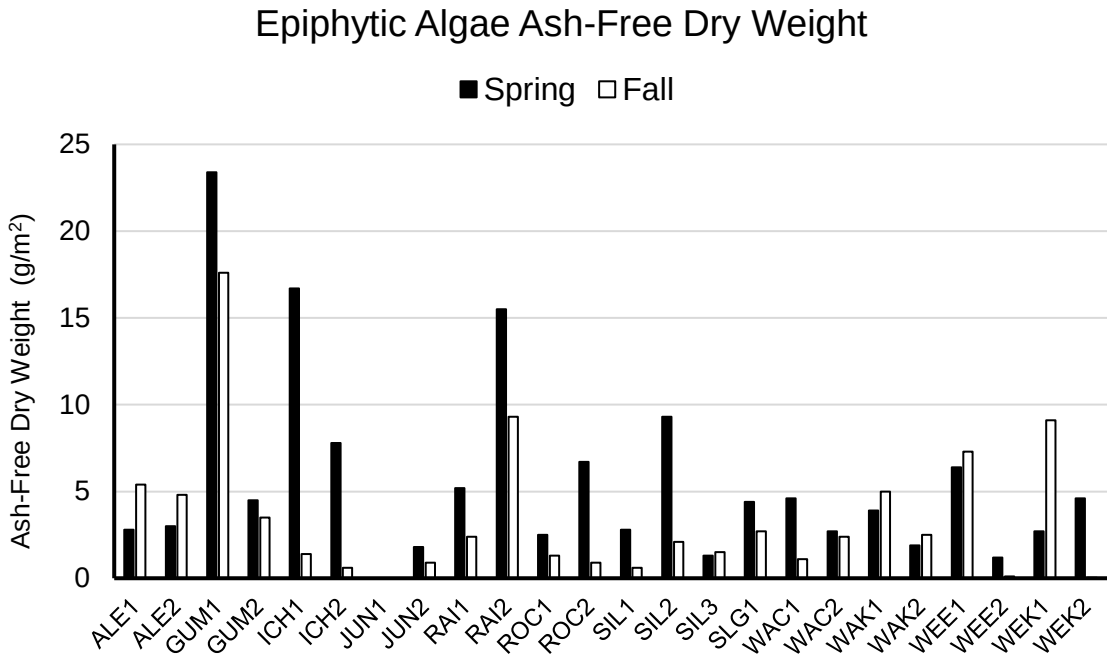


Figure 14. Continued.

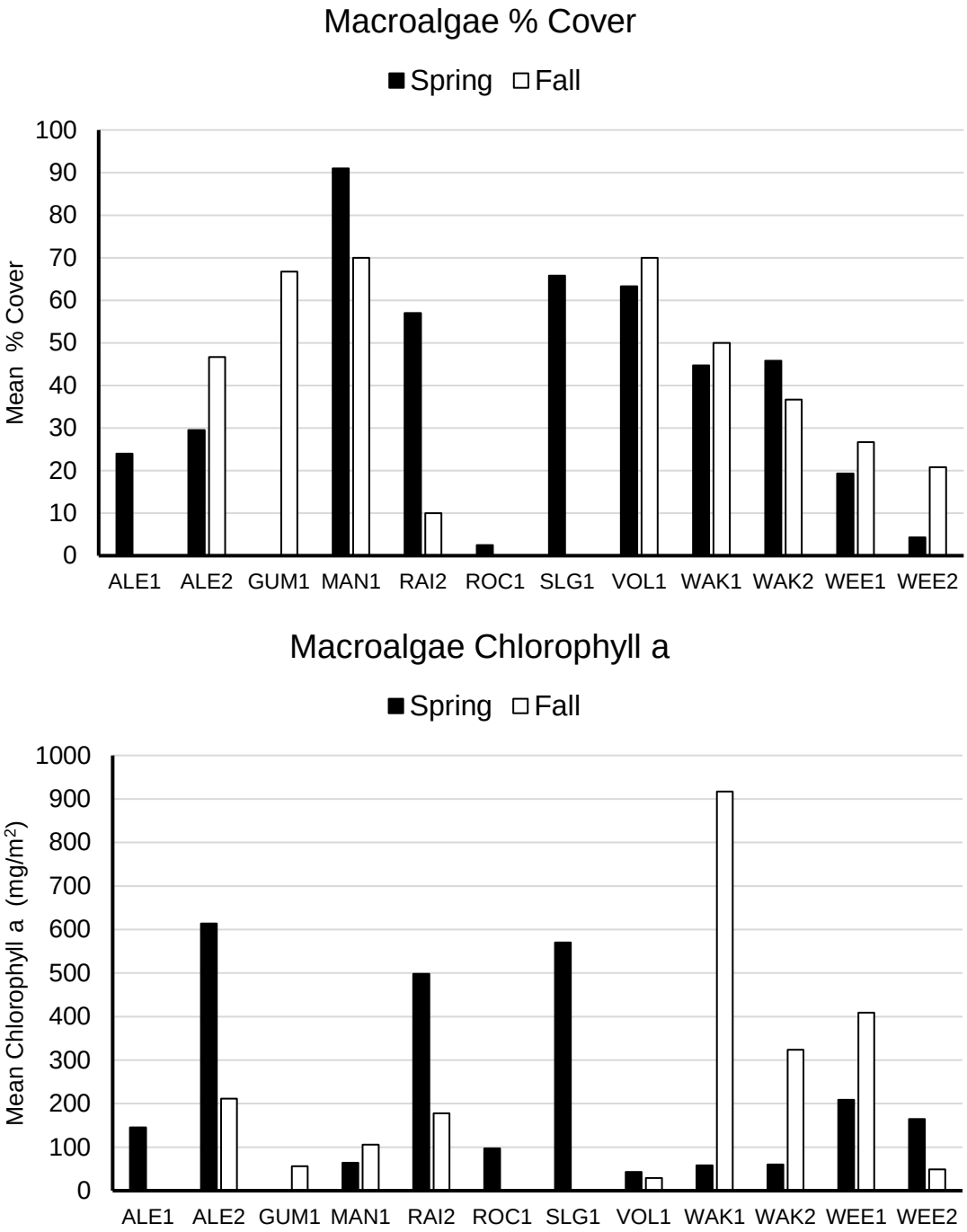


Figure 15. Comparisons of macroalgal abundance (% Cover, Chlorophyll a, and AFDW) in spring versus fall at the transects supporting macroalgal mats. See Table 2 for definitions of site abbreviations.

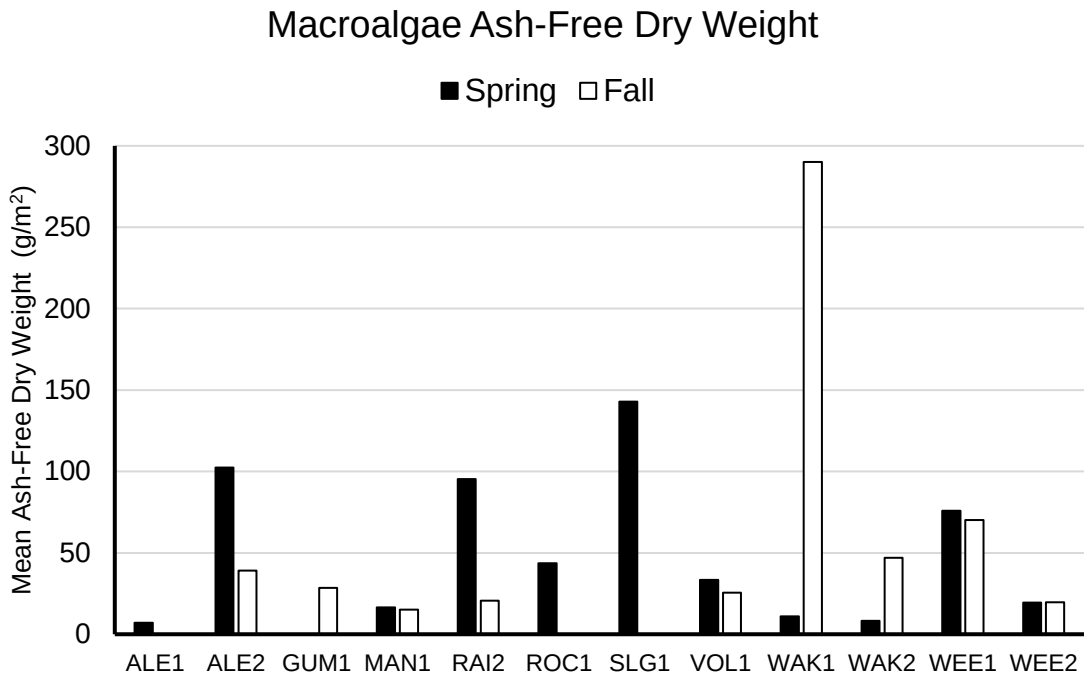


Figure 15. Continued

Macroalgae. Macroalgal percent cover, Chl *a* and AFDW did not display any clear trends between spring and fall for the 12 transects that supported macroalgal mats (Figure 15). For all three measures, about half of the transects were higher in spring and the other half higher in fall. At three of the 12 transects (ALE1, ROC1, and SLG1), macroalgal mats were present in spring, but not in fall; one transect (GUM1) had macroalgal mats present in fall but not spring.

ECOLOGICAL RELATIONSHIPS

Hynes (1970), Whitton (1975) and Stevenson et al. (1996) provided reviews of the environmental factors influencing stream benthic algal communities (which includes epiphytes and macroalgae) including physical (substrate, light, and current velocity) and chemical factors (pH, conductivity, and nutrients; Hynes 1970; Whitton 1975; Stevenson et al. 1996). Additionally, biological factors such as competition with macrophytes (Doyle and Smart 1998; Cohen et al. 2017) and grazing by herbivores can be significant (Steinman 1996). Current velocity is generally regarded as a major factor affecting benthic algal communities, by both physical sloughing/removal of algal biomass and influencing nutrient supply (Hynes 1970; Whitton 1975). Light is important for algae as primary producers, and Odum (1957b) and Cohen et al. (2017) showed that light levels are one of the major drivers of primary production in Florida spring-run streams. Nutrient concentrations are also important determinants of algal abundance (Stevenson et al. 2007) and will be discussed more specifically below.

Epiphytic Algae

Taxa Composition. BEST analysis (Table 4) showed low correlation between epiphyte taxa composition and the suite of environmental variables in spring (conductivity, pH; R=0.236) and in fall (water depth, conductivity, DO, turbidity; R=0.205).

Cover. Reaver et al. (2019) found a significant negative relationship between current velocity and epiphyte cover ("periphyton" in their terminology) using data from multiple Florida spring-run streams and experimental studies in Silver River. They reported a threshold velocity of ~0.22 m/s, below which significantly higher epiphytic algal cover was measured on macrophyte leaf blades. For epiphyte cover, ANOSIM revealed a significant difference (R=0.36, P_{perm} = 0.025) between sites in spring (Table 4), based on current velocity, with lower velocity sites having greater cover of epiphytic algae. Low velocity sites had flow rates ≤ 0.21 m/s and high velocity sites had flow rates ≥ 0.22 m/s. ANOSIM analysis revealed no significant difference in epiphyte percent cover based on current velocity in the fall (Table 4).

Chlorophyll a. ANOSIM analysis revealed no significant difference in epiphyte Chl *a* densities in spring 2015 from sites with high NOx levels (≥ 0.465 mg/L) or low levels (<0.465 mg/L). This threshold was chosen based on the judgement of multiple SJRWMD staff to create two groupings of sites based on NOx. However, ANOSIM did indicate significant differences (R=0.246, P_{perm} = 0.02) in epiphyte Chl *a* densities from sites with high versus low current velocity (Table 4). SIMPER analysis revealed that Chlorophyll densities were higher in

Table 4. Summary of multivariate analyses of algal and physicochemical data. R is the test statistic. Permutation probability ("P_{perm}") shown in parentheses, where P_{perm} <0.05 the test statistic is significant. NS=not significant. Environmental variables: Cond=conductivity, Curr V=current velocity, Dep=water depth, DO=dissolved oxygen, SAV canopy=macrophyte canopy height, Temp=water temperature, Tree canopy=tree canopy cover, Turb=turbidity, Width=stream channel width.

Analysis	Epiphyte Taxa Rich	Epiphyte Cover	Epiphyte Chl a	Epiphyte AFDW	Macroalgal Taxa Rich	Macroalgal Cover	Macroalgal Chl a	Macroalgal AFDW
BEST								
Spring	R=0.236 *Cond*	R=0.301 *Cond, Curr V*	R=0.181 *Temp, DO, Turb*	R=0.122 *Tree canopy, DO, Turb*	R=0.347 *Tree canopy, Cond, pH, Turb, Width*	R=0.718 *Curr V*	R=0.274 *Temp, Width*	R=0.140 *Tree canopy, Temp, Turb*
Fall	R=0.205 *Dep, Cond, DO, Turb*	R=0.256 *Cond, Turb, Curr V*	R=0.122 *Turb*	R=0.175 *Curr V, SAV canopy*	R=0.589 *SAV canopy, Temp, Cond, DO*	R=0.390 *pH, Curr V*	R=0.201 *Tree canopy, Dep, Cond, Width*	R=0.326 *Tree canopy, Temp*
ANOSIM								
Spring	No Pchem effects	R=0.36 (0.025) *Curr V*	R=0.246 (0.02) *Curr V*	No Pchem effects	No Pchem effects	R=0.825 (0.006) *Curr V*	No Pchem effects	R=0.094 (0.035) *NOx*
Fall	No Pchem effects	No Pchem effects	No Pchem effects	No Pchem effects	No Pchem effects	R=0.394 (0.04) *Curr V*	No Pchem effects	R=0.264 (0.009) *NOx*
SIMPER								
Spring	NS	Higher cover low-velocity sites (≤0.21 m/sec)	Higher Chl *a* low-velocity sites (≤0.21 m/sec)	NS	NS	Higher cover low-velocity sites (≤0.21 m/sec)	NS	Higher AFDW at sites with higher NOx
Fall	NS	NS	NS	NS	NS	Higher cover low-velocity sites (≤0.21 m/sec)	NS	Higher AFDW at sites with lower NOx

samples from sites with low current velocity (Table 4). BEST analysis showed low correlation (R=0.181) between spring season epiphyte Chlorophyll a densities and environmental values (water temperature, DO, turbidity). ANOSIM analysis (Table 4) revealed no significant difference between samples from high or low velocity sites and no significant difference in sample densities from sites with high or low NOx in fall. BEST analysis showed low correlation (R=0.221) between Chlorophyll a densities in fall and environmental values (turbidity).

Ash-Free Dry Weight. In spring, ANOSIM analysis revealed no significant differences among sites for epiphyte AFDW (Table 4) based on current velocity (high versus low) or NOx concentration (high versus low). BEST analysis showed a weak correlation between AFDW and the suite of environmental variables (canopy cover, DO, turbidity; R= 0.122). In fall, ANOSIM analysis also revealed no significant differences in AFDW (Table 4) among sites based on current velocity (high versus low) or NOx concentration (high versus low). BEST analysis showed a weak correlation between AFDW and the suite of environmental variables (current velocity, SAV canopy height; R= 0.175).

Macroalgae

Taxa Composition. BEST analysis (Table 4) showed moderate correlation between macroalgal taxa composition in spring and the suite of environmental variables (canopy cover, conductivity, pH, turbidity, stream width; R=0.347) and in fall (SAV canopy height, water temperature, conductivity, DO; R=0.589).

Cover. ANOSIM analysis (Table 4) showed significant differences ($R = 0.825$, $P_{perm} = 0.006$) in macroalgal cover between high and low velocity sites in spring, with low velocity sites having higher macroalgae percent cover based on SIMPER analysis. In addition, BEST analysis showed a high correlation between percent macroalgae cover and current velocity in spring (R=0.718). ANOSIM also revealed significant differences ($R = 0.394$, $P_{perm} = 0.04$) between high and low velocity sites in fall, with low velocity sites having higher macroalgae percent cover based on SIMPER analysis. BEST analysis showed a moderate correlation between percent macroalgae cover and the suite of environmental variables (pH, current velocity; R=0.390) during the fall. Figure 16 shows the relationship between macroalgal cover and current velocity in both seasons. Our results were similar to those of King (2014), who found significant negative relationships between macroalgal cover and current velocity in Gum Slough, with a threshold of ~0.22 m/s, above which macroalgal cover was significantly reduced. Higher macroalgal cover values (>50%) measured in this study were evident below this threshold (Figure 16). In contrast, Reaver et al. (2019) found no relationship between current and macroalgal cover using data from multiple Florida spring-run streams.

Chlorophyll a. ANOSIM analysis (Table 4) of spring samples revealed no significant difference in Chl *a* between sites with regard to velocity or NOx. BEST analysis showed low correlation between macroalgae Chl *a* densities and the suite of environmental values (water

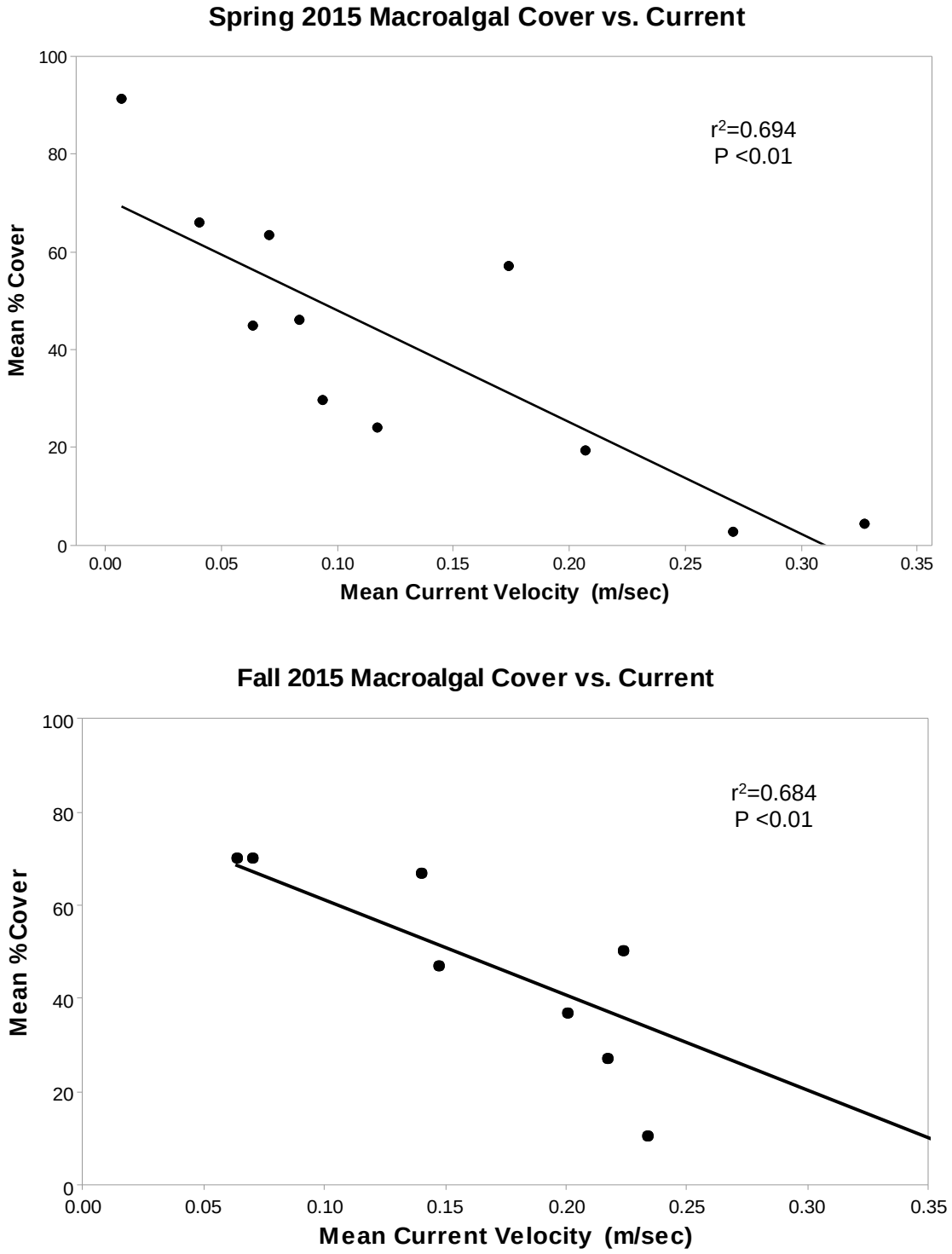


Figure 16. Plots of mean macroalgal cover versus current velocity in spring and fall 2015 at the spring-run stream transects supporting macroalgal mats.

temperature, stream width; R=0.274) in spring. In fall, ANOSIM analysis revealed no significant difference in Chl *a* between high or low velocity sites. However, ANOSIM did reveal differences approaching significance (R=0.14, P_{perm} = 0.065) in Chl *a* densities from sites with high versus low NOx. BEST analysis showed low correlation between Chl *a* densities and the suite of environmental values (canopy cover, water depth, conductivity, stream width; R=0.201) in the fall.

Ash-Free Dry Weight. ANOSIM analysis (Table 4) revealed no significant differences in spring season AFDW from sites based on current velocity (high versus low). However, it did show significant differences (R=0.094, P_{perm} = 0.035) between samples based on NOx level (high versus low) in spring. SIMPER analysis revealed that macroalgae AFDW was higher at sites with high NOx. BEST analysis showed a weak correlation between AFDW and the suite of environmental variables in spring (canopy cover, water temperature, turbidity; R= 0.140). ANOSIM analysis revealed no significant differences in AFDW from samples in fall based on current velocity (high versus low), but it did show significant differences (R=0.264, P_{perm} = 0.009) among sites based on NOx level (high versus low). However, SIMPER analysis revealed that macroalgae AFDW was higher at sites with low NOx. BEST analysis showed a moderate correlation between AFDW and environmental variables in the fall (canopy cover, water temperature; R= 0.326).

Nutrient concentrations (primarily nitrogen as nitrate) and their effects on algal communities in Florida spring-run streams has historically been a management issue of concern. As discussed above, we found weak relationships between NOx and epiphytic and macro- algal abundance measures. Recent work in Silver River (Cohen et al. 2017) and reviews of the literature (Brown et al. 2008; Heffernan et al. 2010) indicated generally poor relationships between algal abundance and nutrient supply in spring-run streams, but other evidence indicates that nutrients do influence algal growth and abundance in spring-run streams (Osborne et al. 2017; Cowell and Dawes 2004; Stevenson et al. 2007; Sickman et al. 2009; Nifong 2017; Jacoby et al. 2008; GreenWater Laboratories 2010). For stream ecosystems in general, more nutrient-enriched streams support higher benthic algal abundance and may be characterized by "nuisance" blooms of macroalgae (Hynes 1970; Biggs 1996; Dodds et al. 1998; Hudon et al. 2014). Evidence for direct links between the documented enrichment of NOx in Florida spring-run streams (Scott et al. 2004) and algal abundance is mixed. It may be that other environmental factors correlated with NOx may influence algal abundance and distribution in spring-run streams, and these may vary among spring ecosystems.

COMPARISON WITH PRIOR STUDIES OF FLORIDA SPRING-RUN STREAMS

The first published study of the algal communities of Florida springs and spring-run streams was Whitford (1956). He conducted sampling in 30 Florida spring-run streams (including the headspring area and down the spring run) over a period of 3.5 years in the early and mid-1950s. Many of the species collected in the present study were also found in this earlier study (Table 5). In terms of taxonomic composition, species from Cyanophyta, Chlorophyta, and Bacillariophyta were the main components of spring algal communities both historically and

Table 5. Comparison of the algal taxa collected in Florida springs and spring-run streams by Whitford (1956), Stevenson et al. (2004), GreenWater Labs (2010) and in this study.

ALGAL TAXON	Whitford 1956	Stevenson 2004	GreenWater 2010	This Study 2015
CYANOPHYTA				
Aphanocapsa sp.	XX		XX	XX
Aphanothece sp.		XX	XX	
Calothrix sp.	XX		XX	XX
Hapalosiphon sp.			XX	XX
Heteroleibleinia sp.			XX	XX
Heteroleibleinia/*Leptolyngbya*				XX
Homoeothrix sp.			XX	XX
cf. *Hydrococcus* sp.			XX	XX
Lyngbya cf.*martensiana*	XX			XX
Lyngbya sp.	XX	XX	XX	XX
Lyngbya/*Phormidium* sp.	XX		XX	XX
Microchaete sp.			XX	XX
Microcoleus amoenus/*autumnalis*				XX
Microcoleus cf. *paludosus*				XX
Microcoleus sp./spp.	XX			XX
Microseira wollei	XX[1]		XX	XX
Oscillatoria ornata v. *crassa*				XX
Oscillatoria cf. *princeps*	XX		XX	XX
Oscillatoria sp.	XX	XX	XX	XX
Oscillatoria/*Phormidium* sp.	XX		XX	XX
Phormidium sp.	XX	XX	XX	XX
Phormidium/*Microcoleus* sp.	XX			XX
Scytonema sp.			XX	XX
Tapinothrix sp.				XX
cf. *Wollea* sp.				XX
CHLOROPHYTA				
Chaetomorpha sp.		XX	XX	
Cladophora glomerata		XX	XX	XX
Cladophora sp./spp.	XX			XX
Dichotomosiphon tuberosus				XX
Dichotomosiphon/*Vaucheria* sp.	XX	XX	XX	XX
Enteromorpha sp.		XX		
Hydrodictyon reticulatum	XX		XX	XX
Hydrodictyon sp.		XX		
Oedogonium sp./spp.	XX	XX	XX	XX
Rhizoclonium hieroglyphicum	XX[2]	XX	XX	XX
Schizomeris leibleinii	XX		XX	XX
Schizomeris sp.		XX		
Spirogyra spp.	XX	XX	XX	XX

Table 5. Continued.

ALGAL TAXON	Whitford 1956	Stevenson 2004	GreenWater 2010	This Study 2015
CHLOROPHYTA				
Stigeoclonium sp.	XX	XX	XX	XX
Ulothrix sp.			XX	XX
Ulva sp.			XX	XX
BACILLARIOPHYTA				
Achnanthes cf. *inflexa*	XX[3]			XX
Bacillaria paradoxa			XX	XX
Cymbella cf. *mexicana*	XX			XX
Epithemia adnata/turgida				XX
Epithemia sp./spp.	XX		XX	XX
Eunotia sp./spp.	XX[4]		XX	XX
Melosira varians	XX		XX	XX
Nitzschia sp./spp.	XX		XX	XX
Pleurosira laevis	XX[5]	XX	XX	XX
Rhopalodia gibba			XX	XX
Terpsinoe musica	XX	XX	XX	XX
Ulnaria cf. *ulna*	XX[6]			XX
Ulnaria spp.	XX[6]		XX	XX
RHODOPHYTA				
Audouionella sp.		XX		
Batrachospermum sp.		XX	XX	XX
Caloglossa sp.		XX		
Compsopogon coeruleus	XX		XX	XX
Compsopogon sp.		XX		
Polysiphonia sp.	XX	XX		XX
XANTHOPHYTA				
Vaucheria spp.	XX	XX	XX	XX

1 – *Plectonema wollei* in Whitford (1956)
2 – Whitford collected *Rhizoclonium* spp.
3 – Whitford collected *Achnanthes* spp.
4 – Whitford collected *E. pectinalis*
5 – *Biddulphia laevis* in Whitford (1956)
6 – formerly *Synedra ulna* or *Synedra* spp. collected by Whitford

at currently. A few species/taxa from the Rhodophyta and Xanthophyta were also common components of the spring algal community. Diatoms in the genus *Ulnaria* (formerly *Synedra*

spp.) are a common component of the algal flora in "hard, freshwater" springs, such as Silver River, Weeki Wachee River, Ichetucknee River, and Wakulla River (Appendices C and D).

Many of the filamentous algal taxa that can form extensive mats were present in Florida springs in the 1950s, and Whitford comments on the occurrence of algal mats composed of *Microseira* (=*Plectonema*) *wollei*, *Cladophora* spp., *Spirogyra* spp., *Rhizoclonium* spp., *Hydrodictyon reticulatum*, and/or *Vaucheria* spp. In terms of species composition, the algal communities growing in Florida springs today are overall very similar to those found nearly 70 years ago (Table 5).

Stevenson et al. (2004, 2007) conducted extensive sampling of the algal communities of 29 Florida springs (mainly focusing on the headspring areas, not downstream in the spring run), primarily in the spring and fall of 2003. The same Divisions collected in this study were also collected by Stevenson and coworkers; Cyanophyta (5 taxa), Chlorophyta (10 taxa), Bacillariophyta (3 filamentous taxa), Rhodophyta (5 taxa), and Xanthophyta (1 taxon). The two most frequently occurring filamentous macroalgal taxa across the 29 springs they sampled were the cyanobacterium *Lyngbya* (=*Microseira*/ *Plectonema*) *wollei* and the xanthophyte *Vaucheria* spp. Other common mat-forming macroalgal taxa were *Dichotomosiphon* sp. (Chlorophyta) and *Compsopogon* sp. (Rhodophyta). In our study *Vaucheria* spp. did not seem as prevalent compared to what Stevenson reported, but *M. wollei* was present at most sites in the macroalgal mats (Appendix D).

GreenWater Laboratories (2010) conducted quarterly quantitative sampling of algal communities in five spring-run systems between 2007-2009: Alexander Springs Creek, Juniper Creek, Rock Springs Run, Silver Glen Run, and Wekiva River. They sampled algae on the dominant natural substrata (woody debris, emergent and submergent vegetation, sediment and macroalgal mat) at upstream and downstream transects on most of these spring-run streams (one transect at Silver Glen). They analyzed their samples for taxa richness, algal cell density and biovolume (by lowest practical taxon; down to species if possible), Chl *a* and AFDW. As in the above prior studies, the four major algal taxa (Divisions) present on all substrata were Cyanophyta, Chlorophyta, Bacillariophyta, and Rhodophyta. Diatoms dominated the taxa richness at all their sites. Diatoms and/or cyanobacteria dominated the cell density, while diatoms generally dominated the biovolume estimates at all their sites.

Stevenson et al. (2004) collected a total of 24 taxa (identified down to genus/species) of macroalgae in the 29 springs they sampled in 2003. In this study we collected 23 taxa of macroalgae (and attached epiphytes) in the spring and 16 taxa in fall of 2015, generally similar to Stevenson's results. Somewhat higher taxa richness was seen in the epiphytic algal community in this study: 39 taxa in the spring and 31 in fall. GreenWater Laboratories (2010) collected more epiphytic algal taxa at similar locations on submerged macrophytes in Alexander Springs Creek, Juniper Creek, Silver Glen Springs Run, and Wekiva River, probably due to a more extended sampling period and greater frequency, as well as a broader "reach" at their sampling locations, whereas this study sampled at a single transect.

Trends in Algal Abundance

A major question that has drawn attention over the past 30 years is, "Is there more algae in Florida springs now than occurred historically?" Worldwide, the occurrence of nuisance algal blooms (both phytoplankton and benthic macroalgae) in freshwater ecosystems appears to be an increasing phenomenon (Stevenson et al. 2007; Hudon et al 2014). For benthic macroalgae in freshwater, the primary "nuisance algae" are filamentous taxa in the Cyanophyta and Chlorophyta (e.g., *Lyngbya*/*Microseira* spp., *Cladophora* spp.). Hudon et al. (2014) indicated that the occurrence of blooms of the nuisance cyanobacterium *Lyngbya*/*Microseira wollei*, appears to be due to this species' wide environmental tolerance and ability to proliferate under a variety of conditions.

Unfortunately, there are few historical quantitative data with which to compare current levels of algal abundance in springs and assess trends over time. Anecdotal observations indicate that changes have occurred in some springs (Figure 17), primarily a loss of submerged macrophytes and replacement by or substantial increases in filamentous algal mats. One of us (RAM) has observed the loss of macrophytes and replacement with filamentous algal mats in Manatee Spring and Run on the lower Suwannee River over the period 1988-2005. Frazer et al. (2006) documented the loss of submerged macrophytes and replacement by macroalgal mats in the Homosassa River between 1998-2005. While mats of filamentous macroalgae have always been present in Florida springs and spring-run streams (Whitford 1956; Odum 1957b), they did not appear to be as extensive historically compared to what is observed today.

Odum (1957b) conducted extensive aquatic ecological studies in the headspring area of Silver Springs in the 1950s. A follow-up study was conducted in 2003-2005 using similar methods in the same area surveyed by Odum to assess changes during the intervening 50-year period (Munch et al. 2006). Submerged macrophyte abundance in the Munch study was similar to what was measured by Odum, however, algal abundance (both epiphytic algae and macroalgae) appeared to be substantially higher than what Odum measured (Munch et al. 2006; Quinlan et al. 2008; Mattson et al. 2019). Odum did not provide any data on macroalgal abundance, as he noted that the occurrence of macroalgal mats was so sparse that they were not a major component of the primary producer community in Silver Springs.

Standing crop of epiphytic algae (growing on the blades of *Sagittaria kurziana*, the dominant macrophyte in his sampling area) was estimated by Odum (1957b) to be 188 g Dry Weight (DW)/m^2. Munch et al. (2006) measured epiphyte standing crops of 397 g DW/m^2, double the 1950s standing crop. In the present study, epiphyte standing crop was estimated to be 249 g DW/m^2. Both Odum (1957b) and Munch et al. (2006) did scrapings of epiphytic algae from the macrophyte blades (just as we did in this study), but in both cases they somehow "scaled up" these smaller measurements to reflect the total algal standing crop on the macrophytes covering a 1 m^2 area of bottom habitat. Neither study described the methodology to do this. Odum (1957b) indicated that the average amount of macrophyte (*Sagittaria*) blade surface area covering 1 m^2 of bottom in his study area was 24.3 m^2. We multiplied the average DW

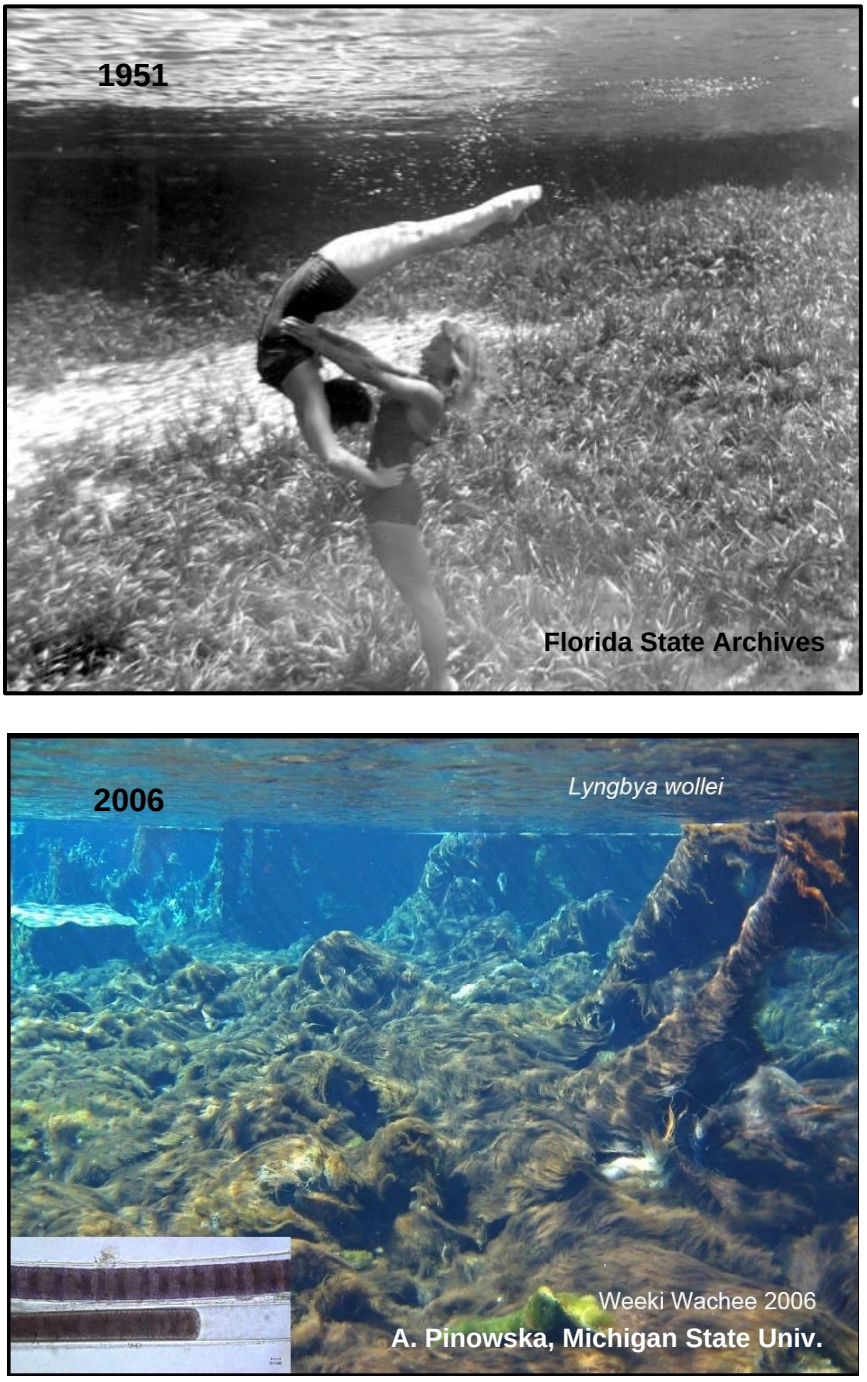


Figure 17. Photographs of Weeki Wachee Spring in 1951 (top) and 2006 (bottom), showing general changes in submerged aquatic plant communities and algal proliferation.

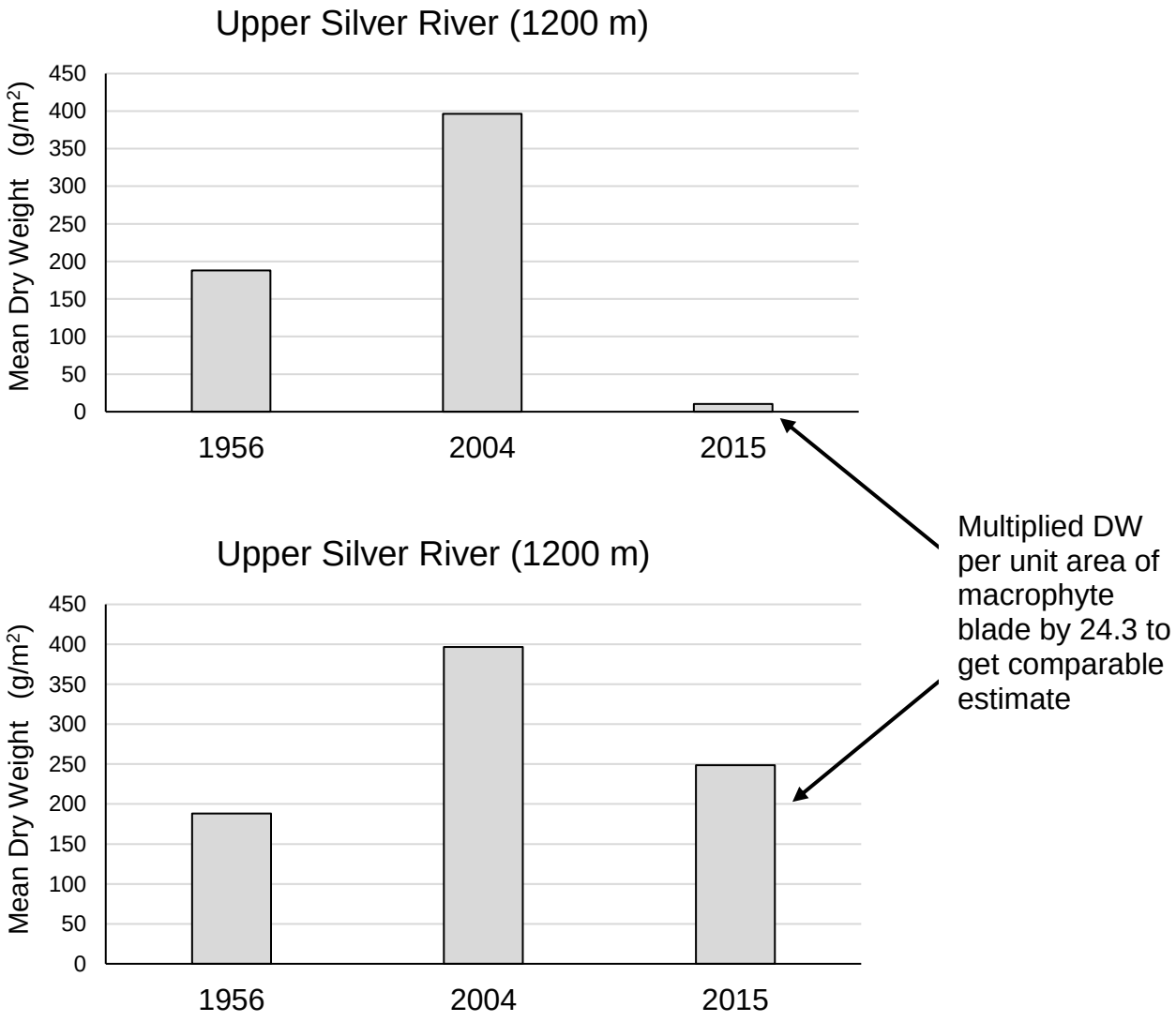


Figure 18. Comparison of epiphytic algal standing crop (g Dry Weight [DW]/m^2) in the upper reach of Silver River. Data from 1956 (Odum 1957b); 2004 (Munch et al. 2006); and 2015 (this study). Data from this study (2015) have been "scaled up" as described in the text.

epiphyte standing crop on individual macrophyte blades at the two stations we sampled (in the same reach as the prior two studies) by 24.3 to obtain a roughly comparable figure (Figure 18). The estimated 2015 standing crop was about 33% greater than that estimated in the 1950s by Odum. This suggests that epiphytic algal standing crop is higher currently than historically measured in Silver Springs, corroborating the findings of Munch et al. (2006) and Quinlan et al. (2008).

The data from Silver River appear to represent the only "long-term" (multi-decadal) quantitative algal data available to assess trends in algal abundance in Florida springs. Stevenson et al. (2007) and GreenWater Laboratories (2010) both quantitatively sampled epiphytic algae in Florida springs in 2003 and 2007-2009 (respectively). We compared their data with the data collected in this study to assess trends over the past 15+ years in the same springs. In all cases, we used the data collected from the upstream reach in our study (near the headspring) to compare with similar locations sampled by these two prior studies. Our study and GreenWater Laboratories sampled at similar locations using similar methods (blade scrapings off macrophytes). Stevenson et al. sampled over a more extensive area (multiple collection locations near the headspring) but used similar lab analytical methods.

Epiphytic algal AFDW and Chl *a* from Alexander Spring, Juniper Spring, Silver Glen Spring and Wekiwa Spring are shown in Figures 19-22. Considerable seasonal variation is seen among all three studies. Stevenson routinely measured higher standing crops than either GreenWater Laboratories or this study (particularly for Chlorophyll a), so the data may not be entirely comparable. No consistent temporal trends were evident over the period 2003-2015, other than periods of higher and lower standing crop. Looking at just the data from this study and GreenWater Laboratories, both measures of algal abundance exhibit considerable variability, on the order of 3- to 7-fold changes over the course of these two studies. The differences between the Stevenson et al. data and the other two studies in Wekiwa Spring is not as great for AFDW, but one value from spring 2003 is substantially higher (Figure 22). All three studies saw overall lowest epiphyte abundance in Juniper Spring. Stevenson generally measured higher epiphyte abundance in spring, while GreenWater Laboratories and this study saw highest abundance in the spring in some streams (Alexander and Juniper; Figures 19 and 20) but in summer and fall/winter in others (Silver Glen and Wekiwa; Figures 21 and 22). In Silver River Munch et al. (2006) also concluded that epiphyte quantitative data exhibit high variation and that this indicates that consistent, long-term data are needed to better define temporal trends in epiphytic algal abundance.

As with epiphytic algae, historical data on macroalgal abundance in springs is lacking. We compared our upstream stations (near the headspring) with one or more similar Stevenson sites (selected by comparing their site descriptions and geographic coordinates). As with the epiphyte data, Stevenson's measurements tended to be considerably higher than measured in this study (Figure 23), possibly indicating a different sampling method (lab methods were similar), so unfortunately the data may not be directly comparable in terms of assessing changes between 2003-2015. In terms of relative differences, results were mixed. Stevenson et al. tended to measure higher Chl *a* density in the fall versus spring while this study tended

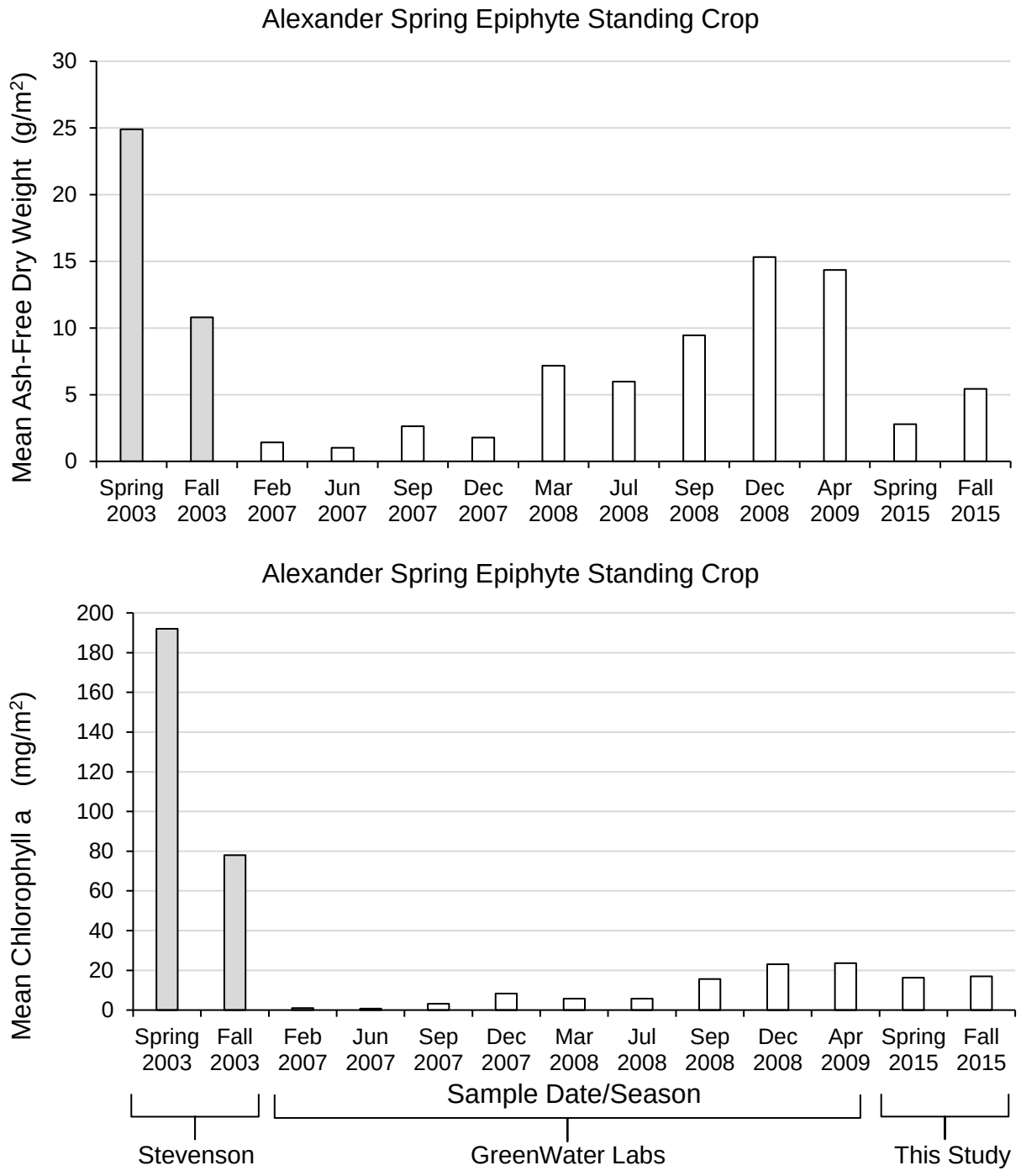


Figure 19. Comparison of epiphytic algal standing crop (as AFDW and Chlorophyll a per unit blade area) at Alexander Spring from Stevenson et al. (2007), GreenWater Laboratories (2010) and this study. Gray shading indicates Stevenson data may not be completely comparable to the other two studies.

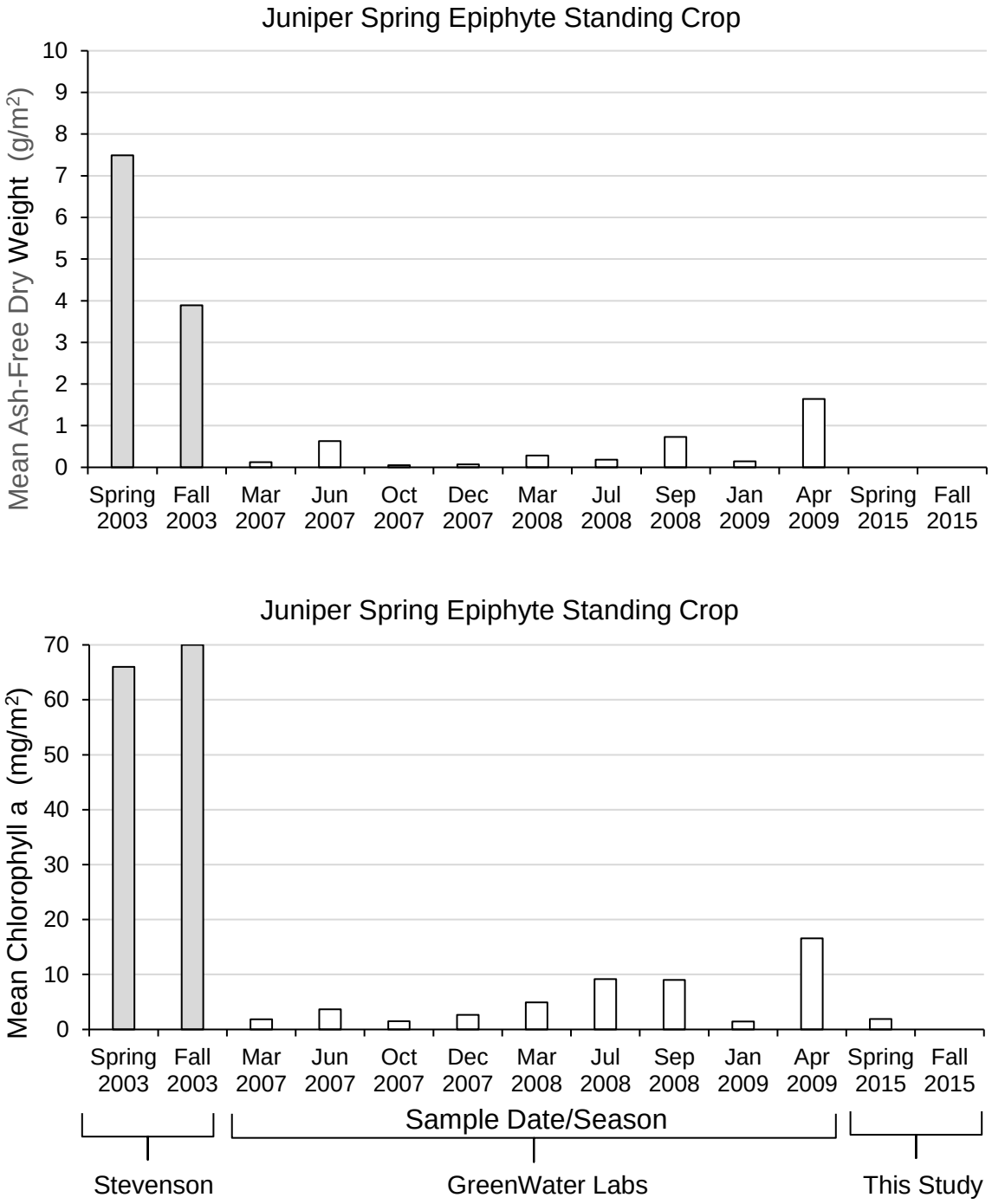


Figure 20. Comparison of epiphytic algal standing crop (as AFDW and Chlorophyll a per unit blade area) at Juniper Spring from Stevenson et al. (2007), GreenWater Laboratories (2010) and this study. Gray shading indicates Stevenson data may not be completely comparable to the other two studies.

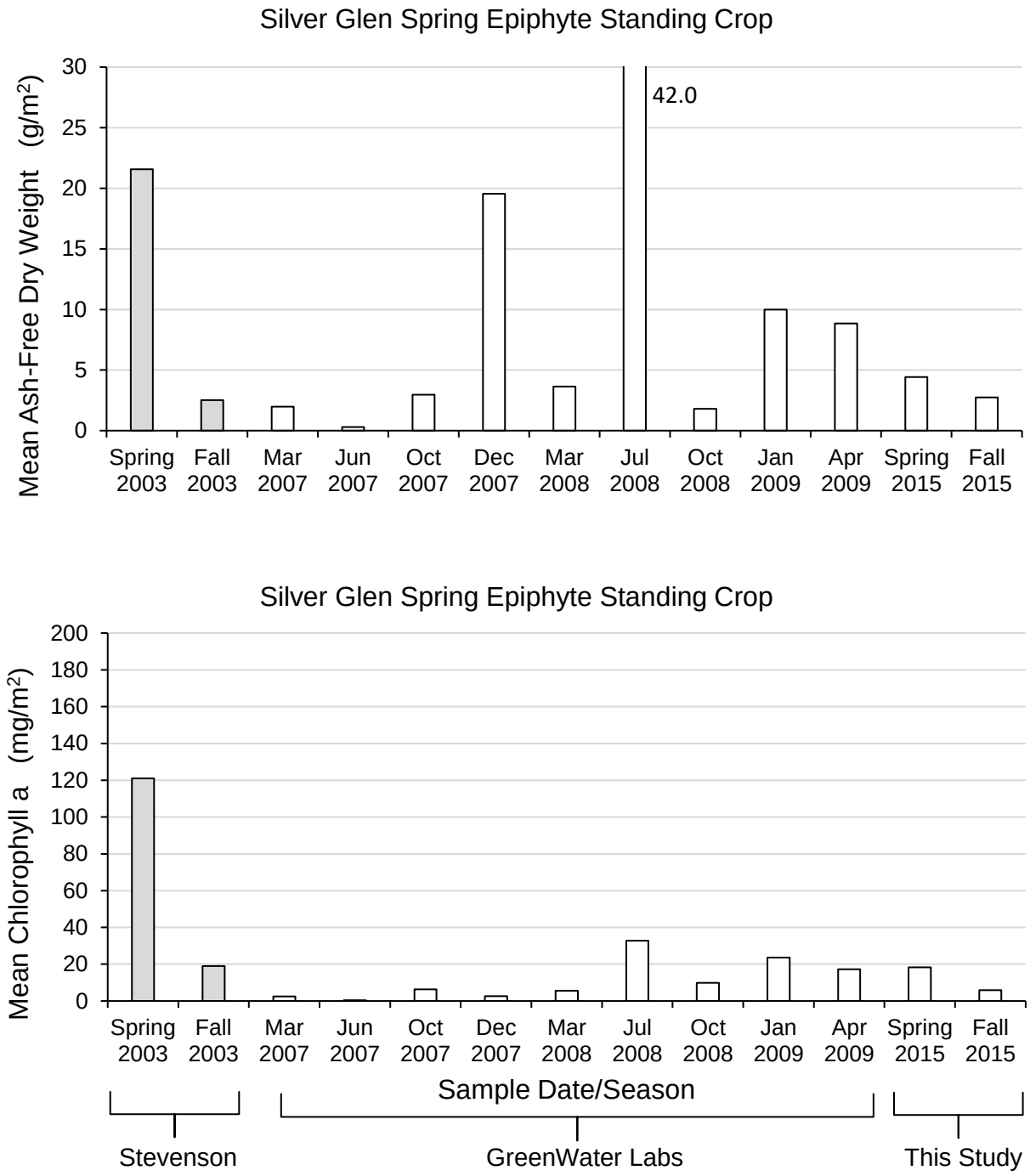


Figure 21. Comparison of epiphytic algal standing crop (as AFDW and Chlorophyll a per unit blade area) at Silver Glen Spring from Stevenson et al. (2007), GreenWater Laboratories (2010) and this study. Gray shading indicates Stevenson data may not be completely comparable to the other two studies.

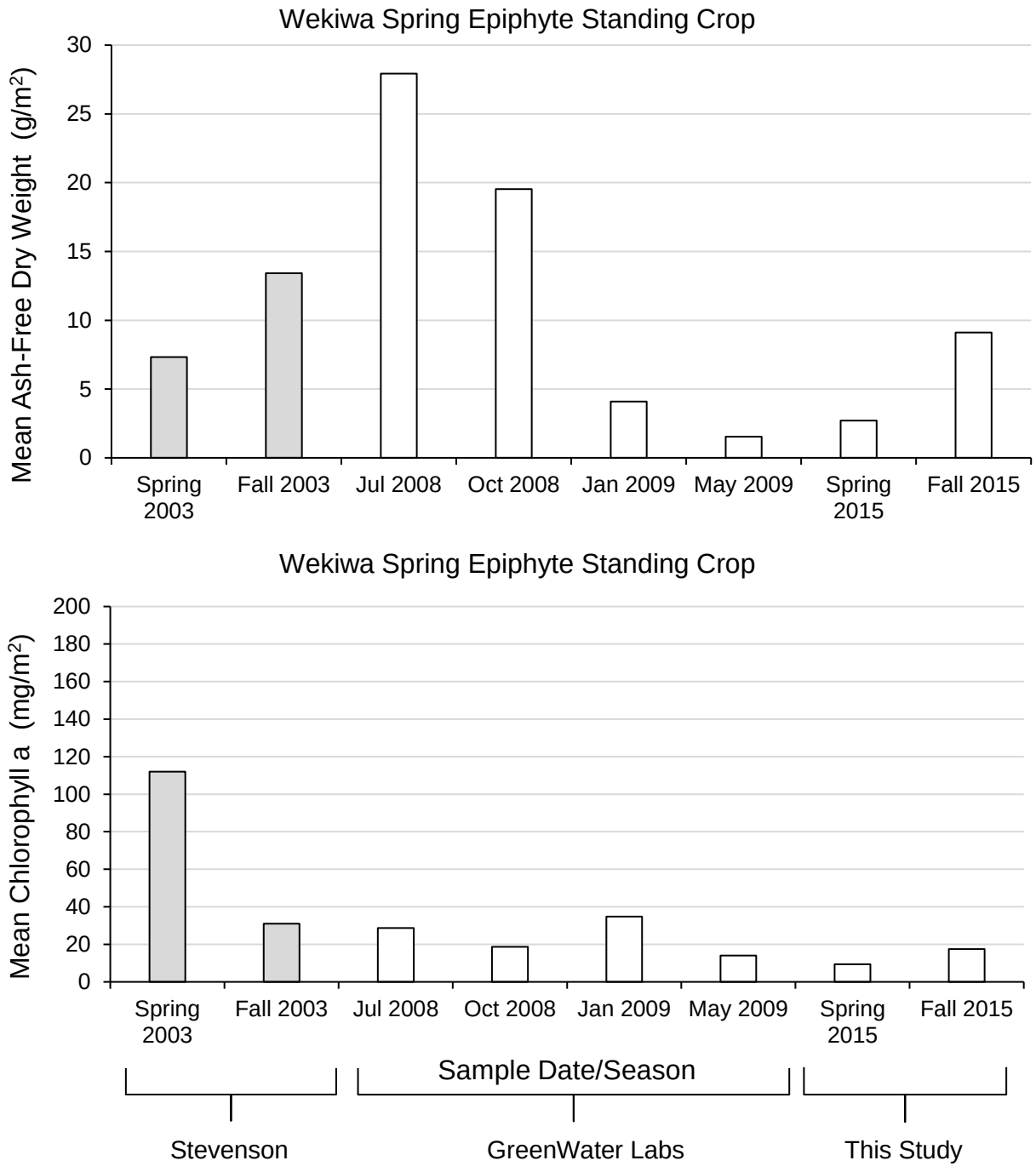


Figure 22. Comparison of epiphytic algal standing crop (as AFDW and Chlorophyll a per unit blade area) at Wekiwa Spring from Stevenson et al. (2007), GreenWater Laboratories (2010) and this study. Gray shading indicates Stevenson data may not be completely comparable to the other two studies.

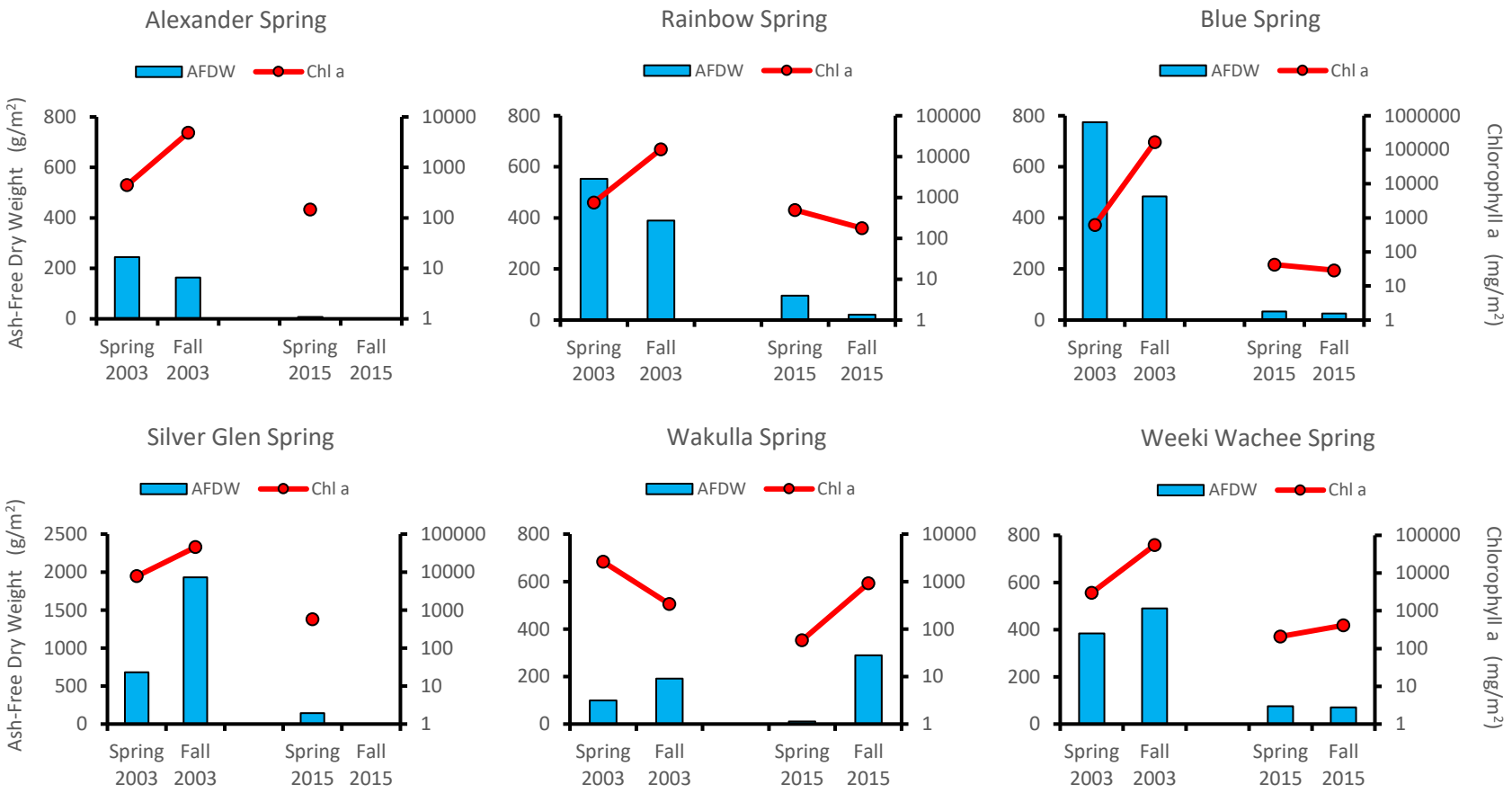


Figure 23. Comparison of macroalgal Ash-Free Dry Weight and Chlorophyll a (corrected) at similar sites sampled in 2003 (Stevenson et al. 2007) and in this study.

to measure the opposite. AFDW standing crop differences were mixed; both Stevenson et al. and this study measured higher fall AFDW at some springs and lower at others. Stevenson et al. measured highest macroalgal AFDW at Silver Glen Spring, whereas in this study it was considerably lower (no macroalgal mats were present at the Silver Glen transect in fall in this study). Generally lowest macroalgal AFDW was measured at Alexander Spring by both Stevenson et al. and this study.

Algal Abundance Thresholds

Related to the question of historical changes in algal abundance in springs is the question "How much algae is too much algae?", or alternatively, "What constitutes a 'natural background' level of algae in a Florida spring?" Use of algal abundance thresholds that constitute "nuisance" or undesirable conditions in lakes is well-established in lake management but has rarely been done for streams (Dodds et al. 1998). The only study providing some type of background or "reference" condition for a Florida spring-run stream is that of Odum (1957b) in Silver Springs/Silver River. Based on this work, a "reference abundance" of epiphytic algae would be <200 g/m^2 dry weight (Figure 18).

Studies conducted in rocky northwestern US streams, concluded that a threshold of 150 mg/m^2 Chl *a* constituted an aesthetic nuisance level of macroalgal growth, mainly by interfering with recreational fishing (Welch et al. 1988). This equated to a macroalgal coverage of about 20%. Horner et al. (1983) also suggested a nuisance Chl *a* value of 150 mg/m^2, based on experiments in artificial stream channels and a broader review of maximum periphytic algal biomass levels reported in the stream literature. Biggs (1996), citing his own and others work in streams worldwide, suggested thresholds of 40% maximum cover of benthic filamentous algae, and/or maximum biomass of 100 mg/m^2 Chl *a* or40 g/m^2 AFDW. He noted that choice of a particular threshold is dependent upon the specific resource value to be protected (e.g., water abstraction, recreation, aquatic life protection). About half of the spring-run streams that supported macroalgal mats in this study (5/12 sites) had mean Chl *a* levels $\geq$ 150 mg/m^2 threshold (Figure 12) in both spring and fall 2015, and up to 7 of these streams had maximum Chl *a* levels exceeding this level in one or both seasons (Appendix F). Mean macroalgal coverage exceeding 20% in one or both seasons was seen in most of the streams (Figure 11) and mean macroalgal cover in up to 6 streams exceeded 40% cover in spring or fall (Figure 11 and Appendix F). Five of the 11 springs sampled in spring 2015 and 6 of 11 streams sampled in fall had mean macroalgal AFDW $\geq$40 g/m^2 (Figure 13). Odum (1957b) did not collect macroalgal data to provide what might be considered a background or reference abundance of macroalgae in Silver Springs/River.

Excessive growth of epiphytic algae on macrophytes can reduce the amount of light energy reaching the blades of the "host plant", with consequent negative effects. Szafraniec (2014) found that epiphyte growth on the leaves of *Sagittaria kurziana* could intercept 20-75% of the incident light reaching the blades in the Rainbow and Weeki Wachee Rivers. This reduction is in addition to any water column attenuation of light, potentially resulting in substantial cumulative reductions of light energy available for macrophyte growth and proliferation. Additionally, Szafraniec's work found that the epiphytes were particularly

efficient at intercepting light in the blue range of the spectrum, a wavelength that also appeared important for the growth and persistence of *Sagittaria*. Guan et al. (2020) proposed an epiphyte burden of 4-5 mg Dry Weight/cm^2 of leaf surface to be an unacceptable level of epiphytic growth on *Vallisneria* in the Chassahowitzka River, based on the epiphyte load reducing growth rate of the macrophyte and other changes in the plants' physiology. Dry weight (DW) is not reported in this report but was collected. Using the spring and fall DW and AFDW data, the average conversion from DW to AFDW is 0.35. This would equate to an epiphyte abundance of 1.4-1.75 mg AFDW/cm^2 of leaf surface, or 14-17.5 g/m^2 AFDW. The majority of the transects sampled in this study fell below these thresholds in both spring and fall (Figure 8)

In general, filamentous macroalgal taxa in the Cyanobacteria and Chlorophyta are regarded as the principal "nuisance" taxa (Welch et al. 1988; Biggs 1996; Hudon et al. 2014). Mattson (2009) found that more sensitive benthic invertebrate groups, such as the "EPT" taxa (Ephemeroptera, Plecoptera, and Trichoptera; mayflies, stoneflies, and caddisflies, respectively), generally dropped out of the invertebrate community with increasing relative abundance of Cyanobacteria and Chlorophyta in the benthic algal community in Florida springs (as indicated by the EPT Score). Thresholds in the EPT Score were seen at ~20% and 40% relative abundance, with highest EPT scores seen at <20% and the EPT taxa essentially disappearing at >40% relative abundance of Cyanobacteria and Chlorophyta. In contrast, the EPT score increased with increasing relative abundance of diatoms in the benthic algal community in springs (Mattson 2009). Macroalgal mats at most of the springs sampled in this study were dominated by filamentous Cyanobacteria and Chlorophyta (Appendix D).

Ecosystem Effects of Algal Proliferation

Proliferation of macroalgal mats and their replacement of submerged macrophyte beds can affect aquatic animal community structure. Hudon et al. (2014) reviewed work done in aquatic ecosystems worldwide and found that mats of *Lyngbya* (now *Microseira*) *wollei* were used as habitat by species of amphipods, chironomid larvae, and copepods, but that gastropods (abundant in macrophyte beds) were not found in these mats. Power (1990) also found a dominance of chironomids in mats of *Cladophora* sp. and believed that these aquatic insect larvae were more tolerant of the widely fluctuating environmental conditions in the algal mats. More sensitive benthic invertebrate taxa such as mayflies and stoneflies were absent from mats (Power 1990). Both Power (1990) and Hudon et al. (2014) reported that the mats provided the resident invertebrate taxa with predation refuges, making them less available to higher trophic levels, with potential effects "up the food web".

A review of the literature and existing data relating benthic macroinvertebrate community characteristics to macroalgal proliferation in Florida springs and concluded that while some invertebrate community characteristics (taxa richness and abundance) were increased by more abundant algae, others (diversity, evenness, and the % dominance index) declined, reflecting that macroalgal mats may only be preferred as habitat by a few, more tolerant invertebrate groups (Mattson 2009). Camp et al. (2014) saw similar trends in comparison of

macrophyte beds versus macroalgal mats in the Homosassa and Chassahowitzka Rivers (higher density but reduced diversity). Macroinvertebrate data collected in this study, in both macrophyte and macroalgal habitats, will be presented in a subsequent report.

Macroalgal proliferation and its effects on food bases can have cascading effects on higher trophic levels in aquatic ecosystems. Camp et al. (2014) found that proliferation of macroalgal mats in the Homosassa River (a spring-run stream on the Florida Gulf coast) resulted in higher densities of invertebrates and small bodied fishes in the algal habitat versus macrophyte beds, but reduced diversity. The lack of larger predators, such as Spotted sunfish (*Lepomis punctatus*), that feed on these species in the Homosassa River, versus the adjacent Chassahowitzka River (also a spring-run stream, with more abundant submerged macrophyte beds) suggested that lack of access to these food resources due to better predation refuges may "cascade" up trophic levels. Other studies have shown that replacement of submerged macrophytes by filamentous macroalgal mats was associated with changes in trophic relationships (Lauretta et al. 2019; Hudon et al. 2014).

Lack of grazing has been proposed as a mechanism to explain the proliferation of macroalgae in Florida springs (Dormsjo 2008; Heffernan et al. 2010; Liebowitz et al. 2014). While these studies showed that algal grazing in spring-run streams can be significant, other lines of evidence indicate that grazing may not be a widespread mechanism influencing the abundance of macroalgal mats in springs. Grazing/herbivorous invertebrates living in algal mats, particularly mats of the cyanobacterium *M. wollei*, appear to feed on the epiphytic algae and detritus in the mats rather than on the macroalgal filaments (Hudon et al. 2014) and other work has shown that *Lyngbya/Microseira* is not consumed by the amphipod *Hyalella azteca* (Camacho and Thacker 2006). Recent work done on the food web in the Silver River using stable isotopes of C and N likewise found that the "nuisance" filamentous macroalgae (*Lyngbya*/*Microseira* and *Vaucheria*) were generally not consumed by herbivorous invertebrates and fish (Frazer et al. 2017).

Liebowitz et al. (2014) found that above a threshold of 20-25 g/m^2 algal biomass, small grazers in Florida springs (primarily the gastropod *Elimia floridensis*) did not reduce or limit algal abundance by their grazing activity. Mullet (*Mugil* spp.) have been observed foraging in filamentous macroalgal mats in Silver Glen Spring, but the stable isotope work in Silver River indicates they are feeding on the epiphytes and not the macroalgae (Frazer et al. 2017). Mullet were historically much more abundant at Silver Springs (Munch et al. 2006), but based on this evidence, they were probably not a major controlling factor on macroalgal abundance. Lab mesocosm experiments found that growth rates of most of the common taxa of filamentous macroalgae in Silver Springs (*Lyngbya/Microseira*, *Vaucheria*, *Spirogyra*, *Cladophora*, and *Rhizoclonium*) were generally equivalent to the grazing rates of most of the common small grazers (gastropods and crustaceans; Frazer et al. 2017), indicating that their grazing activity could not constrain algal abundance. Only herbivory of grass shrimp (*Palaemonetes paludosus*) on the xanthophyte *Vaucheria* sp. appeared to have the potential to limit the abundance of this alga (Frazer et al. 2017). These algal and grazer taxa are common in most Florida springs. Finally, field experiments with exclosures to restrict predation on small grazers (potentially allowing them to proliferate and reduce algal

abundance) had no effect on small grazer or algal abundance in Silver River (Frazer et al. 2017).

CONCLUSIONS AND RECOMMENDATIONS

Fourteen springs and their associated spring-run streams in north and central Florida were intensively sampled in 2015 for selected physicochemical characteristics and quantitative measurement of submerged aquatic vegetation (SAV — macrophytes and algae) and associated macroinvertebrates. This report focused on the algal community data.

Florida springs and their associated spring-run stream exhibit a wide range of flow and water chemistry characteristics (dissolved solids, nutrient concentrations, etc.). Springs along the mainstem of the St. Johns River system generally exhibited higher dissolved salts and minerals.

A total of 39 taxa of epiphytic algae (on macrophytes; primarily *Vallisneria americana* and *Sagittaria kurziana*) and 23 taxa of macroalgae were identified. Most taxa were members of the Cyanophyta ((blue-green algae), Chlorophyta (green algae) and Bacillariophyta (diatoms), with a few taxa of Rhodophyta (red algae) and Xanthophyta (yellow-green algae). Mean epiphyte taxa richness ranged from 2.7-7.3 over both seasons among the 12 streams that supported macrophytes. Mean macroalgal taxa richness ranged from 2.3-4.0 among the 12 streams that had macroalgal mats at the sampling transects.

Epiphytic and macro- algal abundance were measured as % Cover, Chlorophyll a "density" as mg/m^2, and Ash-Free Dry Weight (AFDW) as g/m^2. Mean epiphyte % Cover ranged from <1-100% over both spring and fall; mean epiphyte Chl *a* ranged from <1-55.2 mg/m^2; mean epiphyte AFDW ranged from 0.05-23.42 g/m^2. Generally higher epiphyte Chl *a* and AFDW occurred in the spring sampling period while % Cover was mixed (not clearly higher in spring or fall). Mean macroalgal % Cover ranged from 2.5-91% over both spring and fall (both extreme values occurred in spring). Mean macroalgal Chl *a* ranged from 29.1-917 mg/m2 over both sampling periods (both extremes occurring in the fall sampling). Mean macroalgal AFDW ranged from 7.0-290.1 g/m^2 during this study. Macroalgal abundance measures displayed no clear trend towards being higher in spring versus fall.

Multivariate permutation analyses of the algal data indicated that transects in springs associated with the St. Johns River tended to cluster together as a group based on similarities in epiphytic algal and macroalgal species composition. However, no distinct groupings or clusters of sites were detected based on any of the measures of algal abundance (% Cover, Chlorophyll a, and AFDW).

Comparison of the algal abundance data with selected physical and chemical characteristics (stream canopy cover, channel width, conductivity, turbidity, pH, DO, water temperature, current velocity and long-term nutrient concentrations) generally detected weak relationships. Stronger relationships were identified between epiphyte and macro- algal abundance and current velocity as "low velocity" (< 0.22 m/second) and "high velocity" ($\geq$ 0.22 m/ second) systems. No differences in epiphytic algal abundance measures was seen in comparing sites with "low" (<0.465 mg/L NOx) versus "high" (> 0.465 mg/L NOx) nitrate concentrations.

Higher macroalgal abundance (as AFDW) was seen at sites with "high nitrate" concentrations.

Many of the taxa/species of algae found in Florida springs in the 1950s are still found in Florida springs and their spring-run streams today. Overall, the species composition of the algal communities of Florida spring-run streams are similar to those reported historically.

Trends in algal abundance in Florida springs and spring-run streams over the past several decades cannot be determined due to lack of quantitative data. Anecdotal observations indicate an increase in algal abundance in some spring-run stream systems, and replacement of submerged macrophyte beds by algal mats. Our data, along with other quantitative algal data from recent studies indicate considerable temporal variation in epiphytic algal abundance. Macroalgal abundance data are very sparse.

The lack of historical quantitative algal data precludes a determination of a "background" or "reference" condition for algal abundance. Macroalgae mats were not a major component of the primary producer community in Silver Springs in the 1950s, but macroalgal mats have been historically observed in many Florida springs and spring-run streams. The literature on benthic algae suggests potential "nuisance" or undesirable thresholds of 100–150 mg/m^2 Chl *a* or 20–40% macroalgal cover. Relative abundance of Cyanobacteria and Chlorophyta >20% may also be a possible threshold for an undesirable abundance of macroalgae due to effects on sensitive macroinvertebrate taxa. Half or more of the spring-run streams sampled in this study had macroalgal abundance exceeding one or more of these thresholds.

Our results corroborated those of prior studies showing that current velocity is an important environmental variable that can limit algal populations in springs and spring-run streams. Results of this study also indicated generally weak relationships between water quality characteristics and algal abundance in springs and spring-run streams.

Biological monitoring of SAV should be incorporated as a component of springs monitoring programs (along with discharge and water quality monitoring) to help further understand the causes of variability in springs algal communities and document long-term trends.

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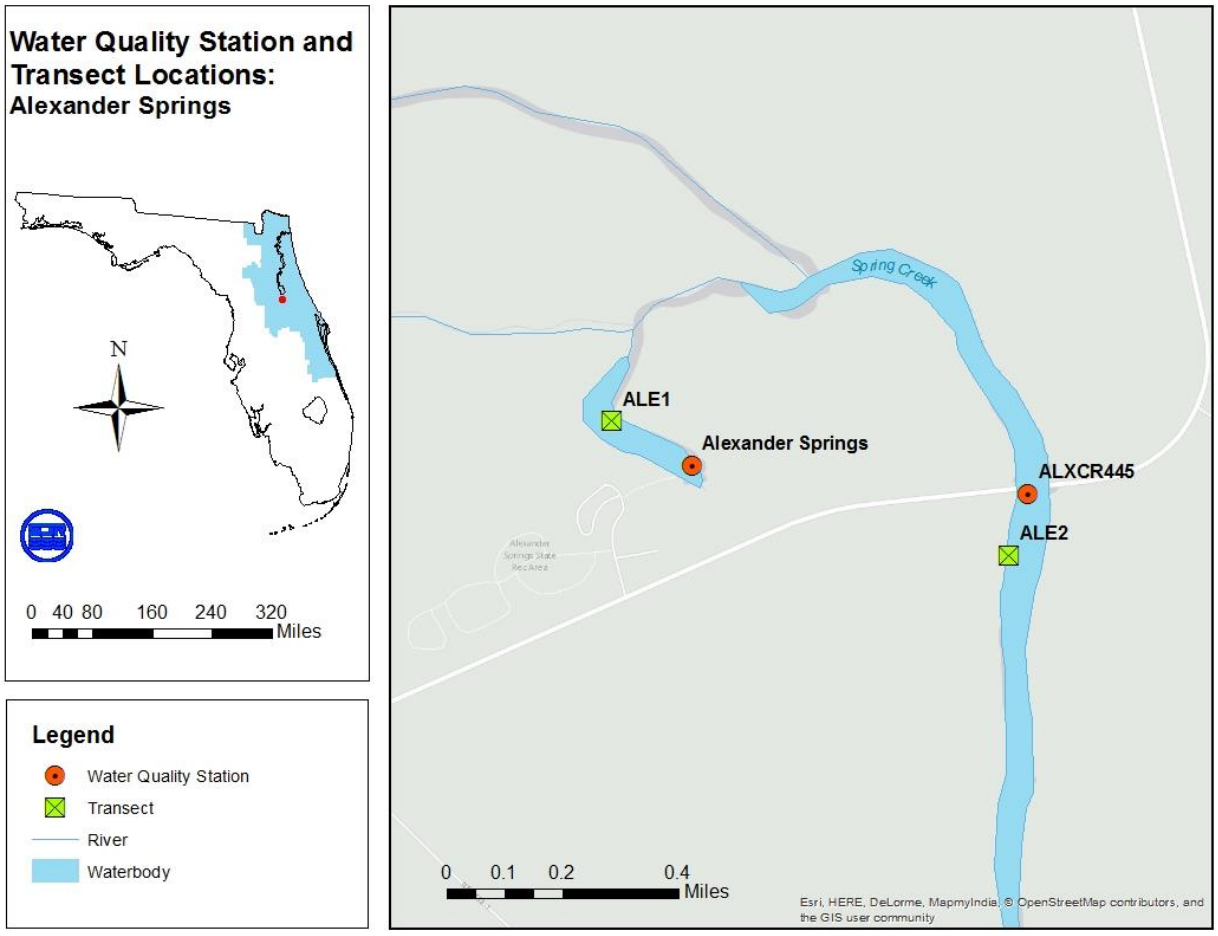
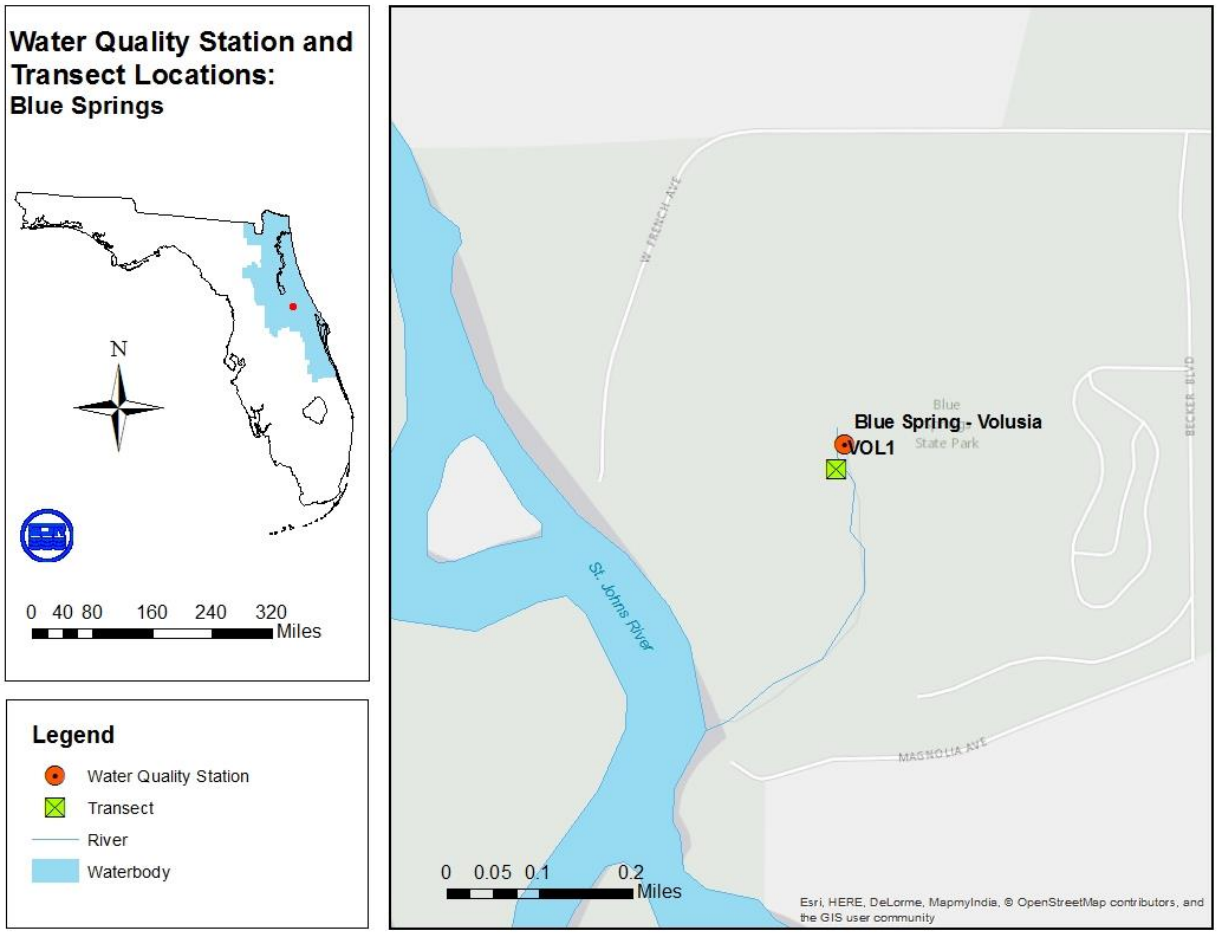
APPENDIX A—TABLE OF ST. JOHNS RIVER SPRINGS DISCHARGE DATA

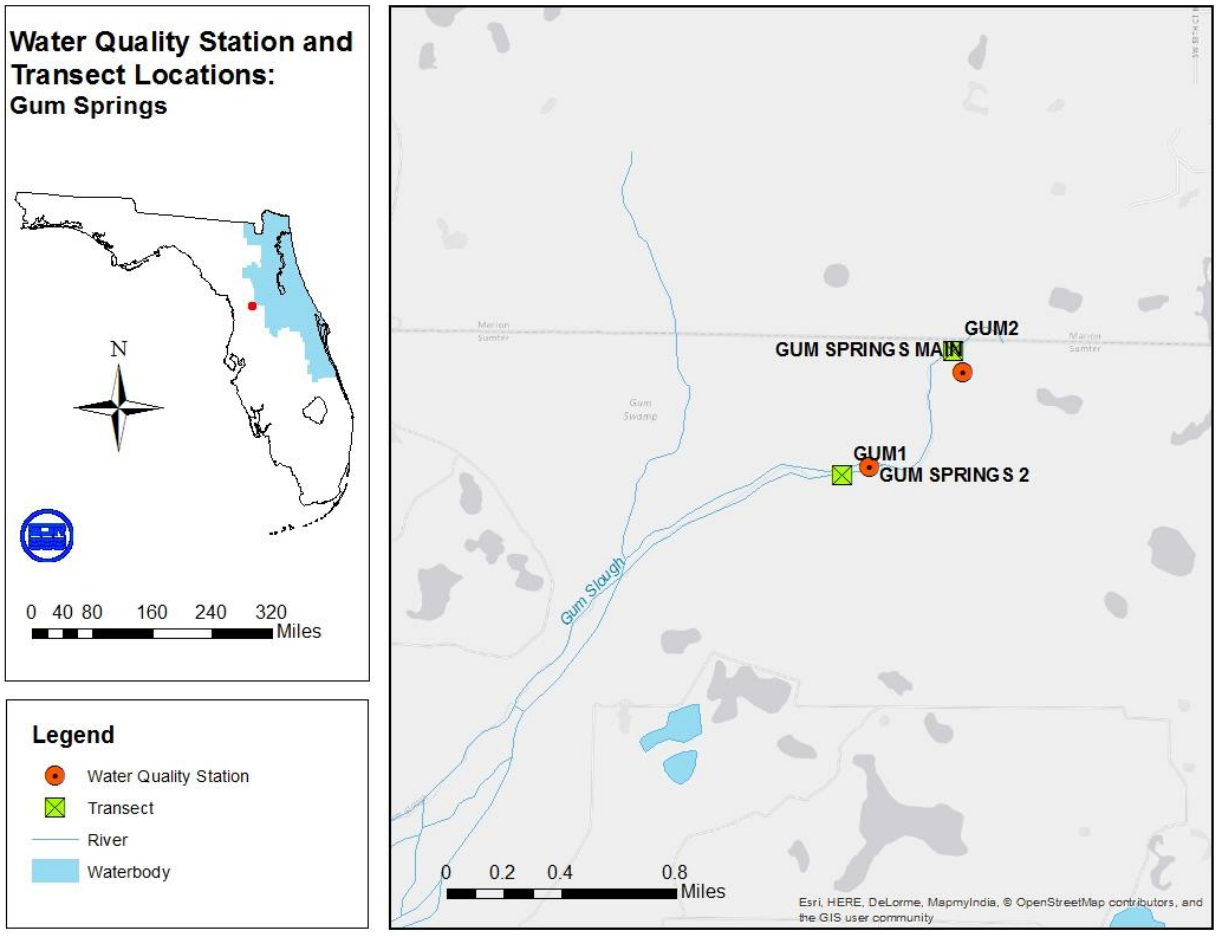
Appendix A Table1. Average discharge rate, magnitude, and data period of record of 25 springs in SJRWMD. Shading indicates first-, second-, and third-magnitude. Data from SJRWMD databases and table from Di and Mattson (unpublished report).

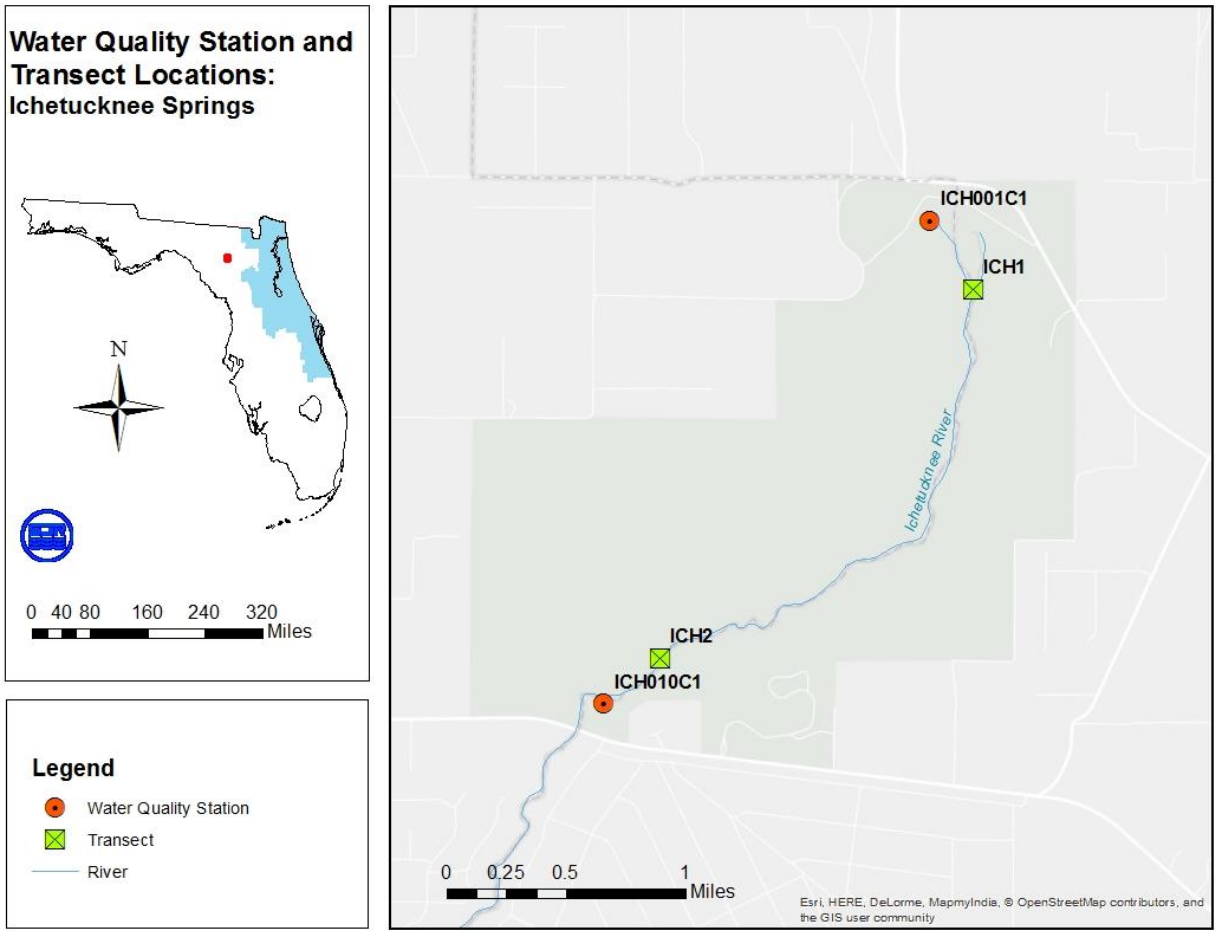
Spring	Mean Discharge (cfs)	Magnitude	Start	End*
Silver Springs	714	First	10/1932	04/2014
Blue Spring - Volusia	144	First	03/1932	09/2013
Alexander Springs	102	First	02/1931	04/2014
Silver Glen Springs	101	First	03/1931	09/2011
Salt Springs	79	Second	02/1929	06/2014
Croaker Hole Spring	69	Second	07/1998	03/2014
Wekiwa Springs	62	Second	03/1932	03/2014
Rock Springs	54	Second	02/1931	05/2014
Apopka Spring	25	Second	05/1971	03/2014
Ponce De Leon Springs	23	Second	02/1983	06/2014
Sanlando Springs	19	Second	11/1941	05/2014
Sweetwater Springs	13	Second	11/1980	06/2014
Starbuck Spring	12	Second	07/1944	05/2014
Bugg Spring Run	11	Second	03/1990	10/2013
Fern Hammock Springs	11	Second	12/1935	04/2014
Juniper Springs	11	Second	04/1935	04/2014
Gemini Springs	10	Second	04/1972	05/2014
Palm Springs - Seminole	6	Third	11/1941	05/2014
Miami Springs	5	Third	08/1945	05/2014
Orange Spring	3	Third	09/1972	06/2014
Holiday Springs Dstm	3	Third	04/1946	10/2011
Green Cove Spring	3	Third	02/1929	06/2014
Blue Spring Yal Run	3	Third	01/2002	10/2011
Double Run Spring	2	Third	10/1991	10/2011
Green Springs	1	Third	04/1972	05/2014

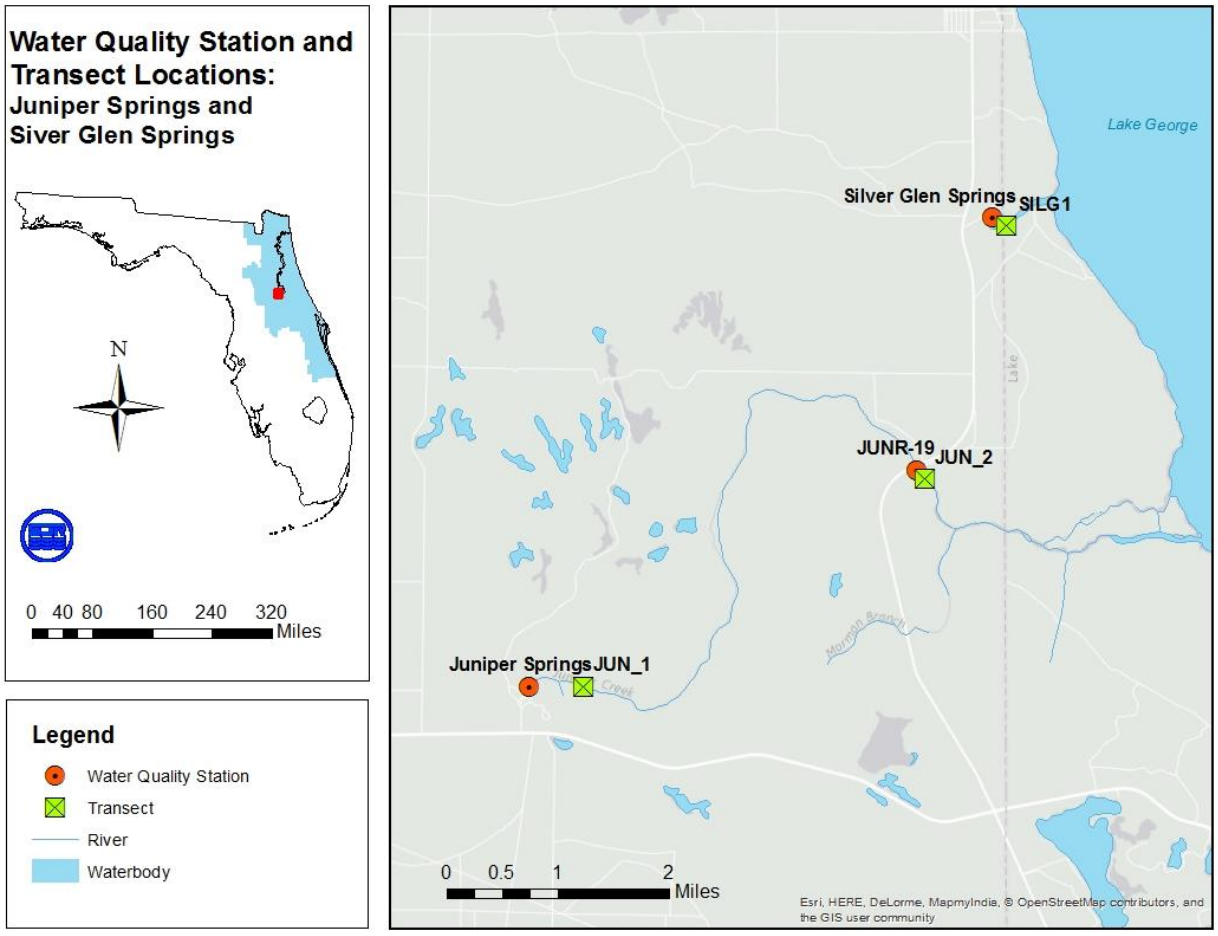
APPENDIX B—MAPS OF SAMPLING SITES


Water Quality Station and Transect Locations:
Alexander Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
Spring Creek
ALE1
Alexander Springs
ALXCR445
ALE2
0 0.1 0.2 0.4
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Blue Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
Blue Spring - Volusia
VOL1
Blue Spring State Park
St. Johns River
W FRENCH AVE
MAGNOLIA AVE
BECKER BLVD
0 0.05 0.1 0.2
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Gum Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
GUM2
GUM SPRINGS MAIN
GUM1
GUM SPRINGS 2
Gum Slough
Gum Swamp
0 0.2 0.4 0.8
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Ichetucknee Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
ICH001C1
ICH1
Ichetucknee River
ICH2
ICH010C1
0 0.25 0.5 1
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Juniper Springs and Siver Glen Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
Lake George
Silver Glen Springs
SILG1
JUNR-19
JUN_2
Juniper Springs
JUN_1
0 0.5 1 2
Miles
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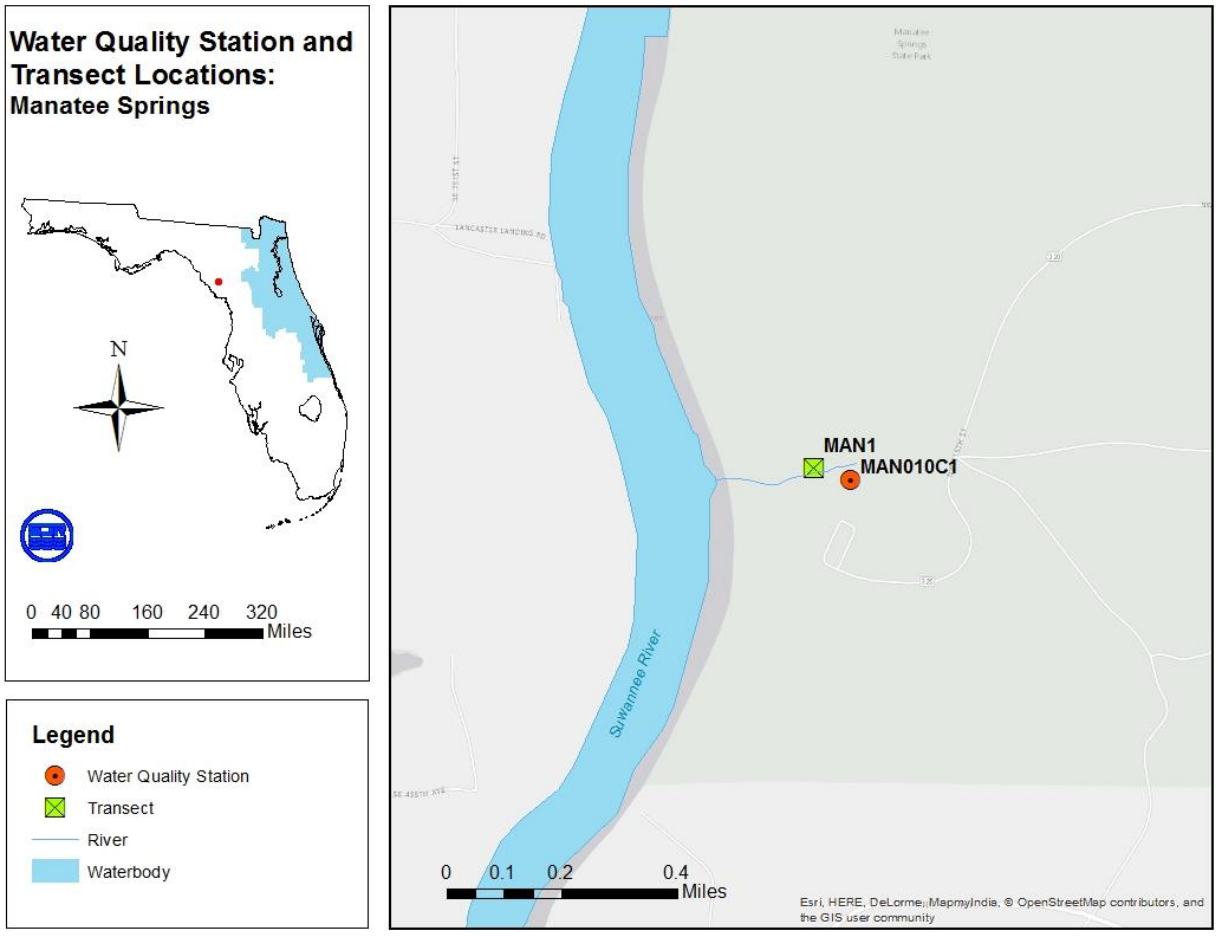
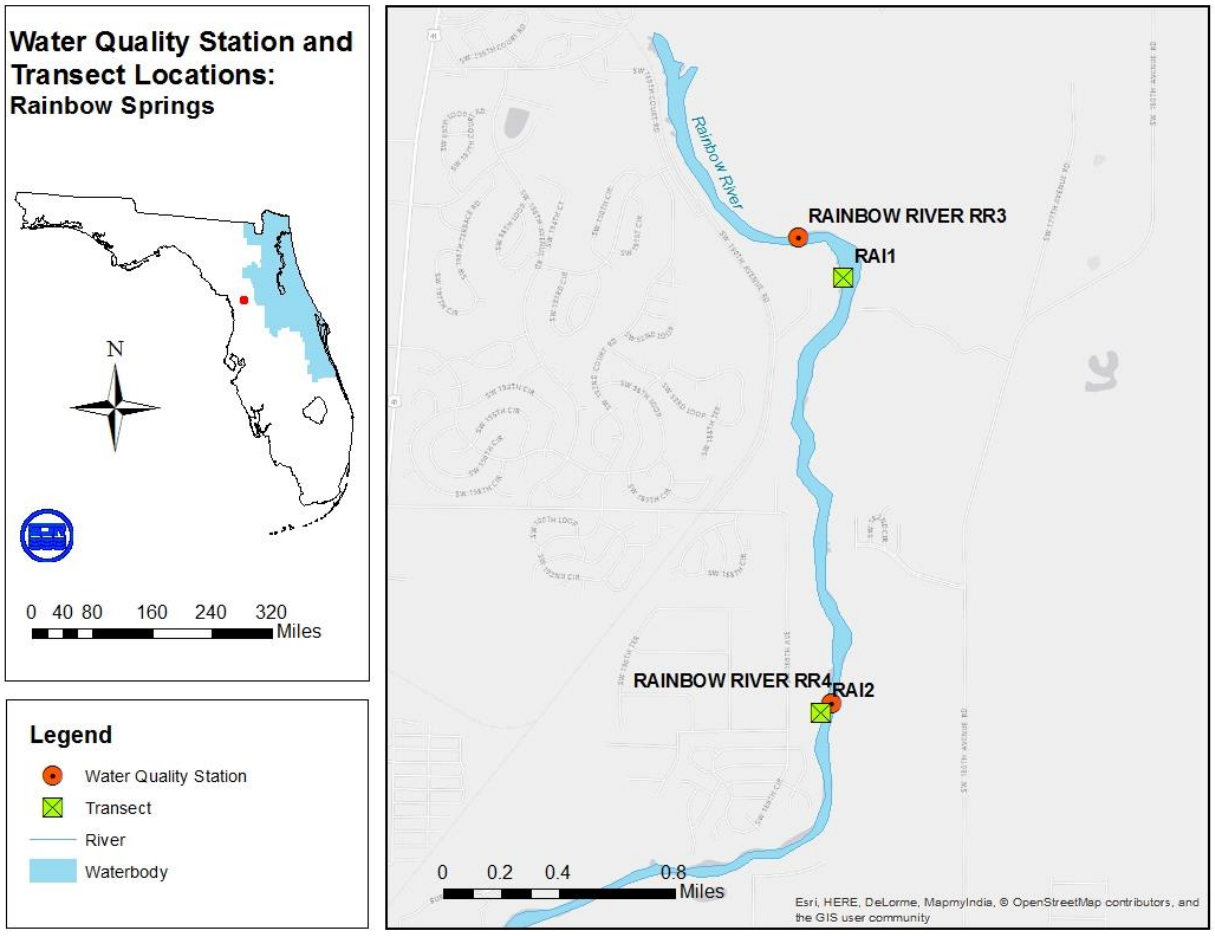
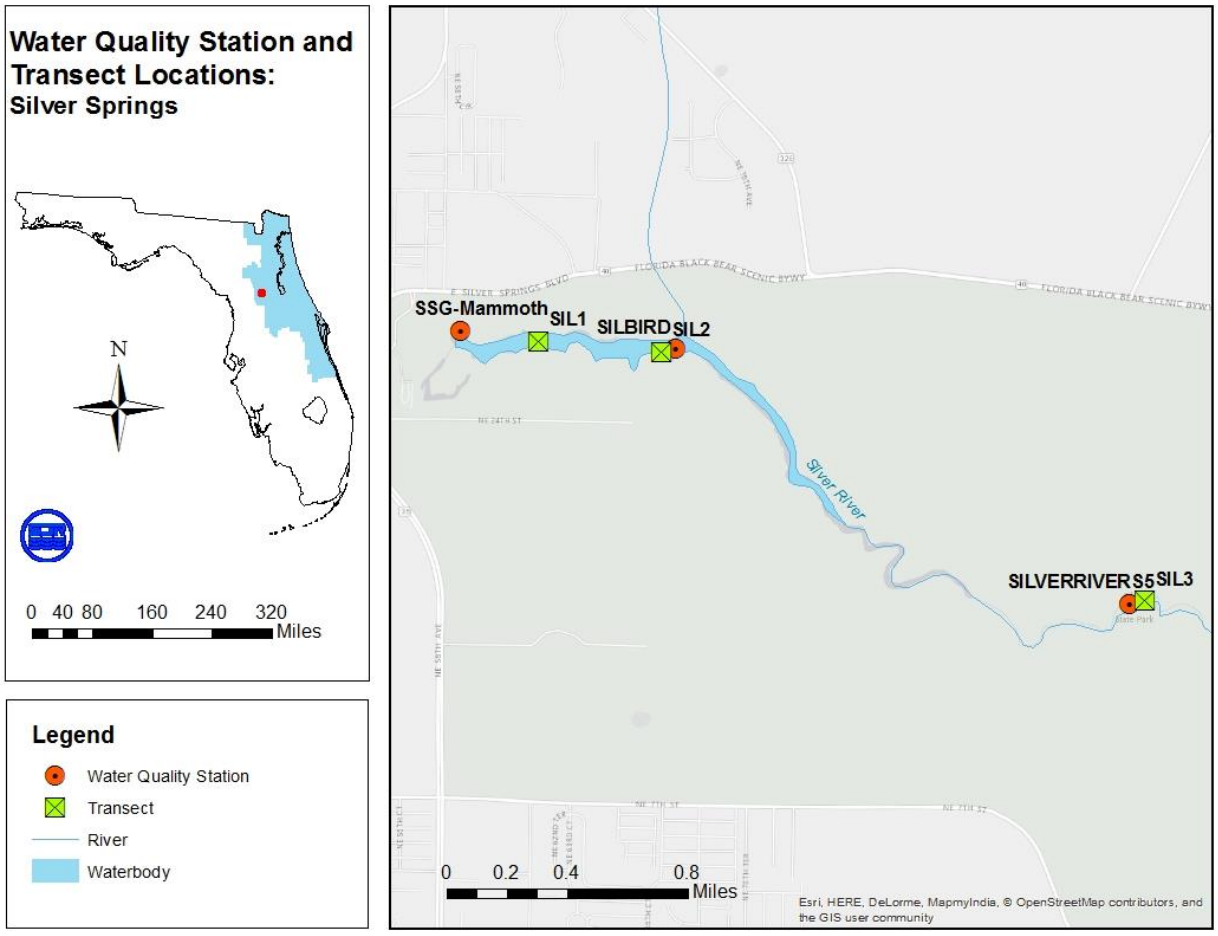
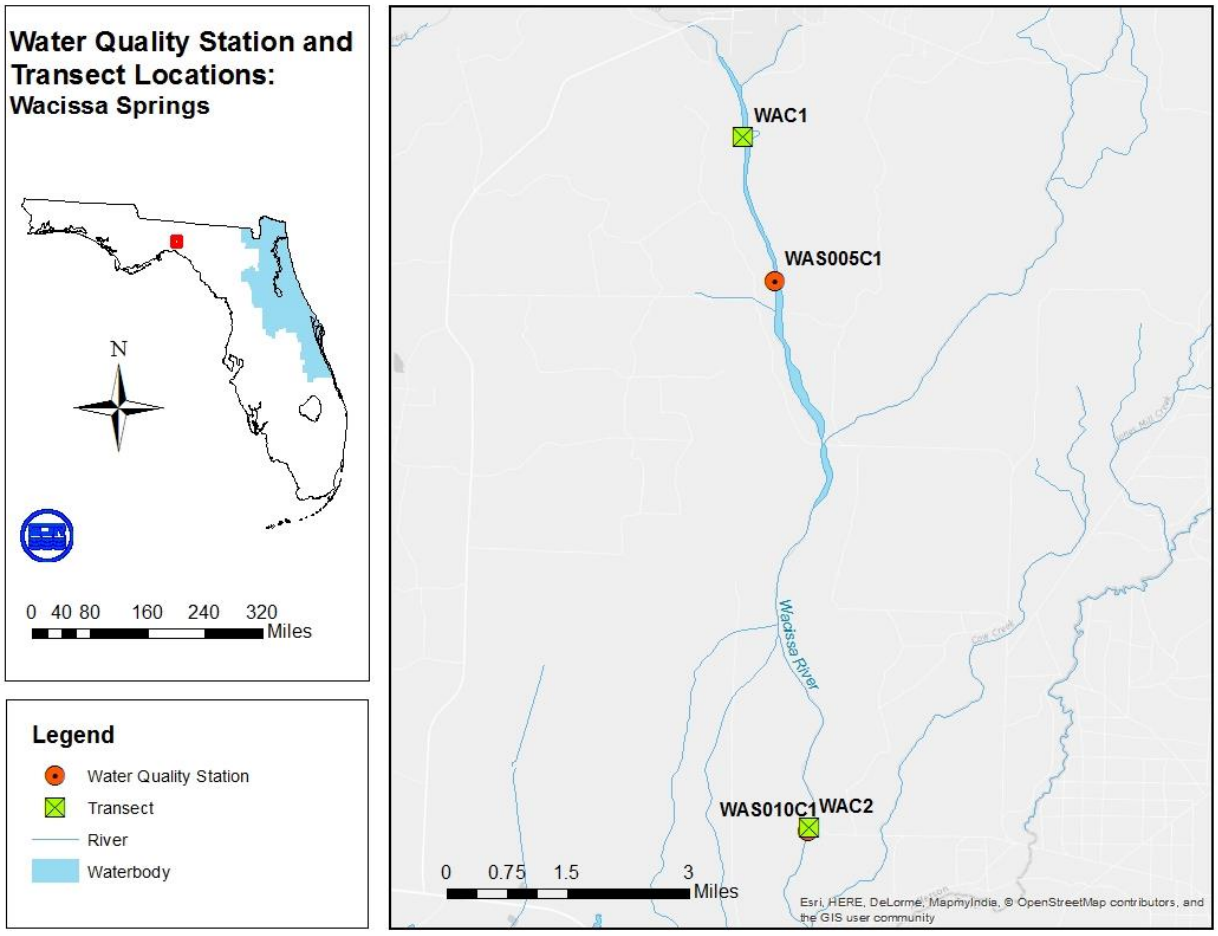
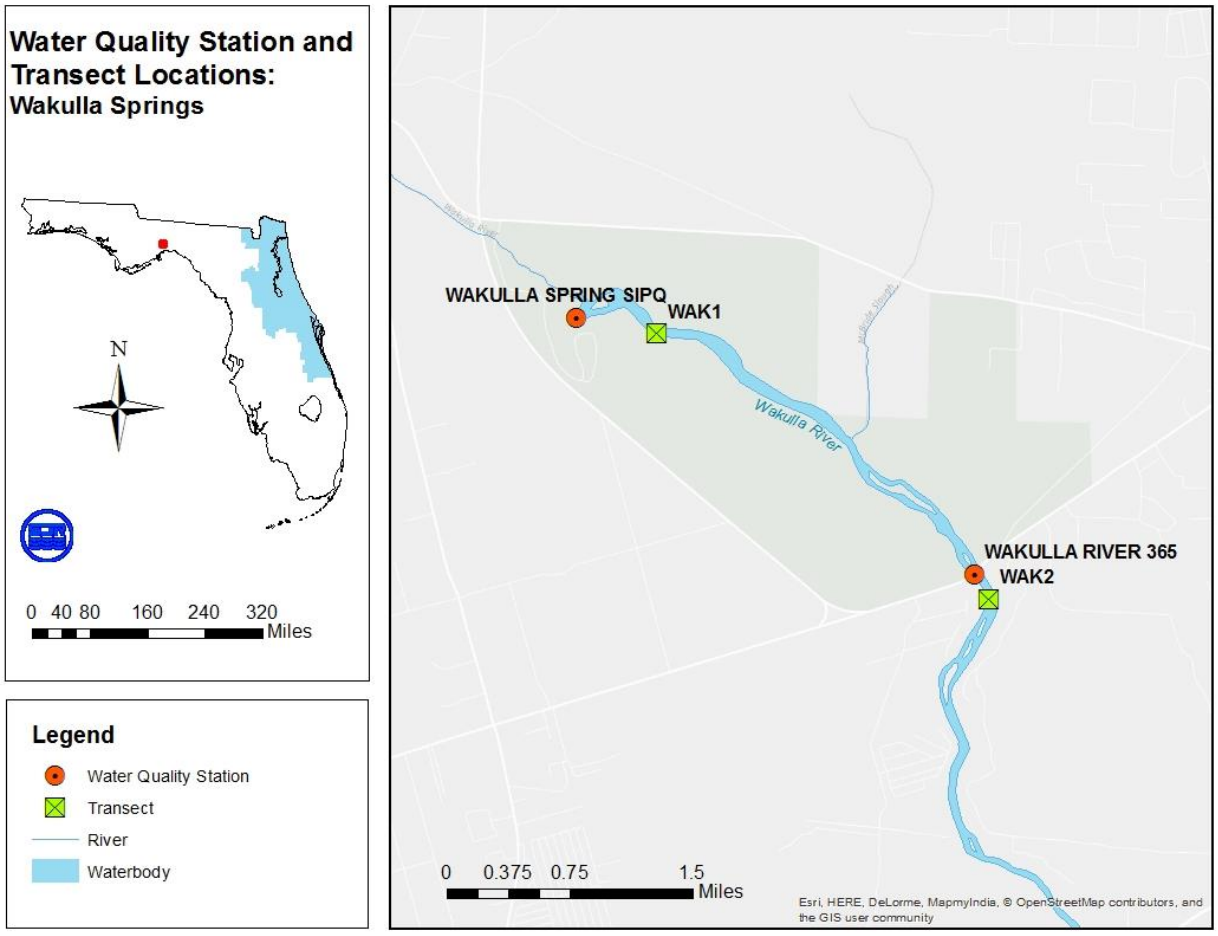

Water Quality Station and Transect Locations:
Manatee Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
MAN1
MAN010C1
Suwannee River
0 0.1 0.2 0.4
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Rainbow Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
Rainbow River
RAINBOW RIVER RR3
RAI1
RAINBOW RIVER RR4
RAI2
0 0.2 0.4 0.8
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Silver Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
SSG-Mammoth
SIL1
SILBIRD
SIL2
Silver River
SILVERRIVER
S5
SIL3
0 0.2 0.4 0.8
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Wacissa Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
WAC1
WAS005C1
WAS010C1
WAC2
Wacissa River
0 0.75 1.5 3
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Wakulla Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
WAKULLA SPRING SIPQ
WAK1
Wakulla River
WAKULLA RIVER 365
WAK2
0 0.375 0.75 1.5
Miles
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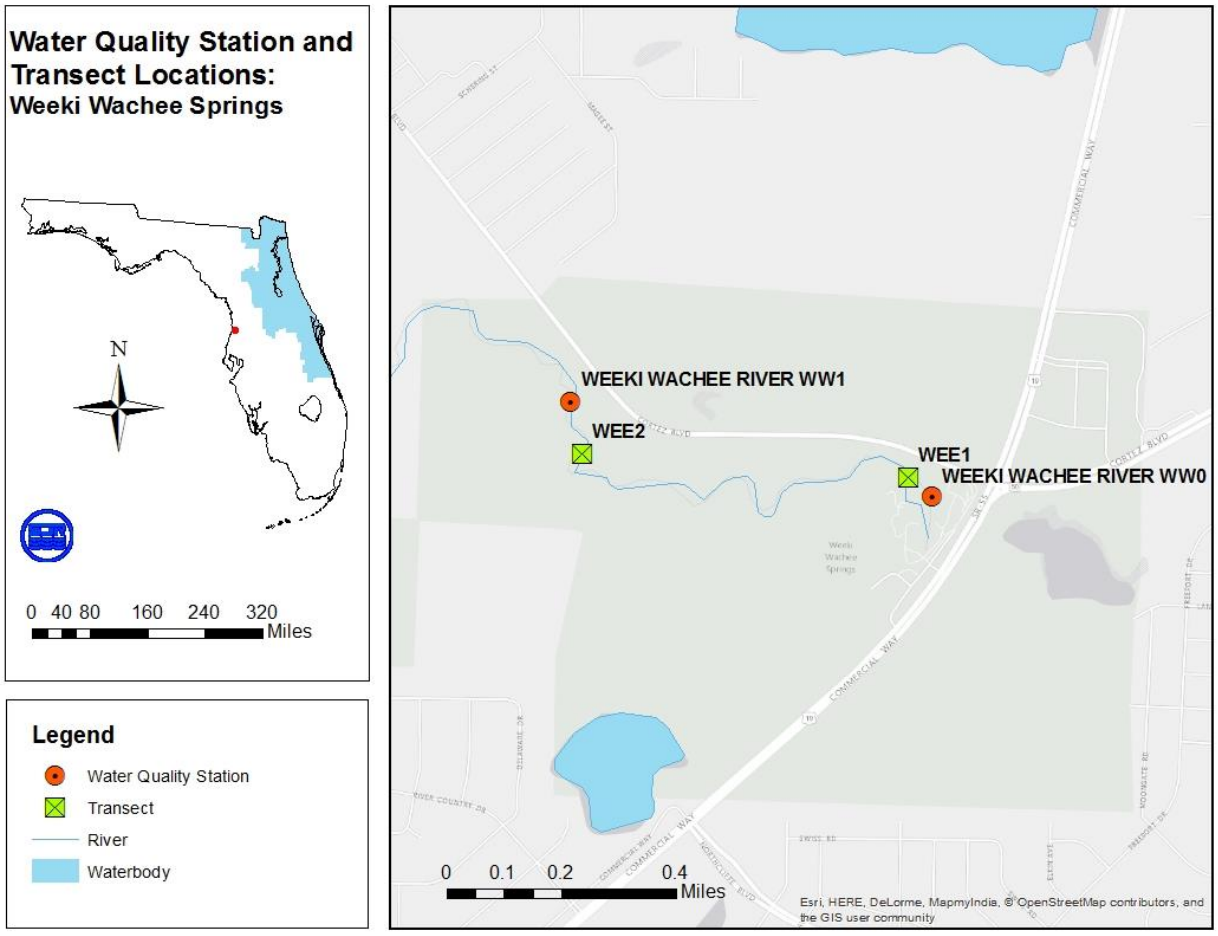
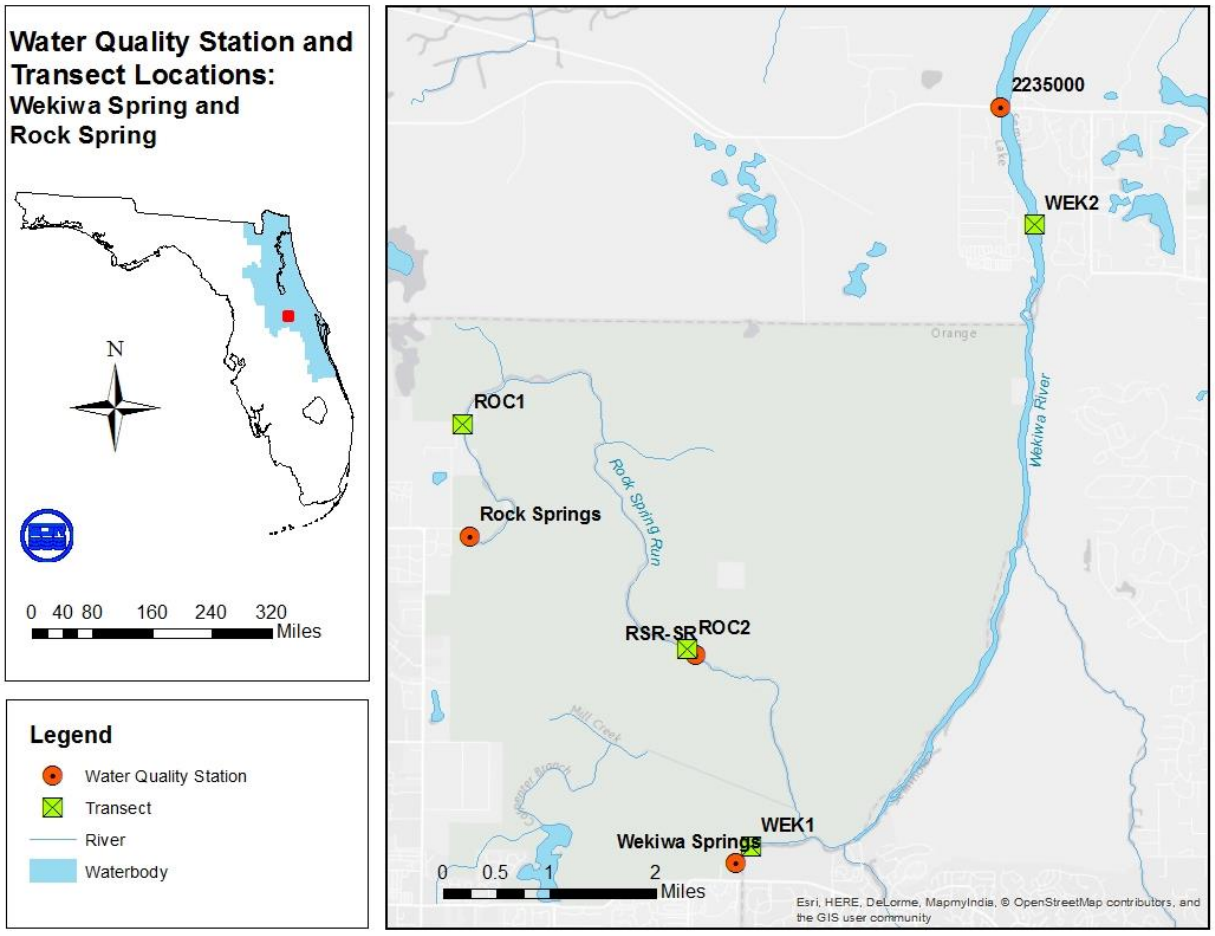

Water Quality Station and Transect Locations:
Weeki Wachee Springs
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
WEEKI WACHEE RIVER WW1
WEE2
WEE1
WEEKI WACHEE RIVER WW0
0 0.1 0.2 0.4
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community


Water Quality Station and Transect Locations:
Wekiwa Spring and Rock Spring
N
0 40 80 160 240 320
Miles
Legend
Water Quality Station
Transect
River
Waterbody
2235000
WEK2
ROC1
Rock Springs
Rock Spring Run
RSR-SR
ROC2
Wekiwa River
Mill Creek
WEK1
Wekiwa Springs
0 0.5 1 2
Miles
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

APPENDIX C—SUMMARIES OF EPIPHYTIC ALGAL TAXA COLLECTED AT THE SAMPLING TRANSECTS

Epiphyte Species Spring 2015

	ALE1	ALE2	GUM1	GUM2	ICH1	ICH2	JUN1	JUN2	RAI1	RAI2
CYANOPHYTA										
Calothrix sp.										
Hapalosiphon sp.		X								
Heteroleibleinia sp.				X						X
Heteroleibleinia/*Leptolyngbya*										
Homoeothrix sp.					X	X		X	X	
cf. *Hydrococcus* sp.							X			
Lyngbya sp.	X								X	
Lyngbya/*Phormidium* sp.										
Microchaete sp.		X								
Microcoleus amoenus/*autumnalis*				X						
Microcoleus sp.			X							
Microseira wollei	X		X						X	X
Oscillatoria ornata v. *crassa*	X									
Oscillatoria sp.										
Oscillatoria/*Phormidium* sp.										
Phormidium sp.	X	X						X		
Phormidium/*Microcoleus* sp.					X					
Scytonema sp.			X						X	
Tapinothrix sp.								X		
cf. *Wollea* sp.	X									
Nostocalean filament sp.	X	X	X							
Oscillatorialean filament sp.				X						
CHLOROPHYTA										
Cladophora glomerata	X					X			X	X
Oedogonium sp./spp.	X	X								X
Rhizoclonium hieroglyphicum									X	X
Schizomeris leibleinii						X				
Spirogyra sp.										
Stigeoclonium sp.	X	X			X	X				X
Ulothrix sp.										
Ulva sp.										
BACILLARIOPHYTA										
Bacillaria paradoxa								X		
Cymbella cf. *mexicana*		X			X		X	X		
Epithemia adnata/*turgida*		X								
Eunotia sp.					X				X	
Melosira varians										
Rhopalodia gibba		X								
Terpsinoe musica			X	X				X		
Ulnaria cf. *ulna*	X	X		X	X	X	X	X		X
Ulnaria spp.										
Centric diatom chain sp.									X	
Pennate diatom spp.			X	X		X		X	X	X

	ALE1	ALE2	GUM1	GUM2	ICH1	ICH2	JUN1	JUN2	RAI1	RAI2
RHODOPHYTA										
Batrachospermum sp.			X	X	X	X	X		X	
Compsopogon coeruleus										
Rhodophyte filament sp.										
TOTAL TAXA RICHNESS	**10**	**10**	**7**	**7**	**7**	**7**	**4**	**8**	**10**	**8**

	ROC1	ROC2	SIL1	SIL2	SIL3	SLG1	WAC1	WAC2	WAK1	WAK2
CYANOPHYTA										
Calothrix sp.						X				
Hapalosiphon sp.										
Heteroleibleinia sp.					X					
Heteroleibleinia/*Leptolyngbya*						X				
Homoeothrix sp.			X							X
cf. *Hydrococcus* sp.										
Lyngbya sp.						X				
Lyngbya/*Phormidium* sp.						X				
Microchaete sp.						X				
Microcoleus amoenus/ *autumnalis*										
Microcoleus sp.										
Microseira wollei							X	X		X
Oscillatoria ornata v. *crassa*										
Oscillatoria sp.										
Oscillatoria/*Phormidium* sp.		X								
Phormidium sp.						X				
Phormidium/*Microcoleus* sp.										
Scytonema sp.										
Tapinothrix sp.										
cf. *Wollea* sp.										
Nostocalean filament sp.										
Oscillatorialean filament sp.										
CHLOROPHYTA										
Cladophora glomerata	X	X	X	X		X	X	X	X	X
Oedogonium sp./spp.	X					X				X
Rhizoclonium hieroglyphicum							X			
Schizomeris leibleinii					X					
Spirogyra sp.						X			X	X
Stigeoclonium sp.	X	X				X			X	X
Ulothrix sp.										
Ulva sp.						X				
BACILLARIOPHYTA										
Bacillaria paradoxa										
Cymbella cf. *mexicana*			X	X						
Epithemia adnata/*turgida*										
Eunotia sp.			X	X						
Melosira varians							X			

Rhopalodia gibba										
	ROC1	ROC2	SIL1	SIL2	SIL3	SLG1	WAC1	WAC2	WAK1	WAK2
Terpsinoe musica			X							
Ulnaria cf. *ulna*	X	X	X	X	X		X	X		X
Ulnaria sp./spp.	X							X	X	X
Centric diatom chain sp.										
Pennate diatom spp.		X	X		X		X	X	X	X
RHODOPHYTA										
Batrachospermum sp.				X	X		X	X	X	X
Compsopogon coeruleus					X					
Rhodophyte filament sp.									X	
TOTAL TAXA RICHNESS	**5**	**5**	**7**	**5**	**6**	**11**	**7**	**6**	**7**	**10**

	WEE1	WEE2	WEK1	WEK2
CYANOPHYTA				
Calothrix sp.				
Hapalosiphon sp.				
Heteroleibleinia sp.		X		X
Heteroleibleinia/*Leptolyngbya*				
Homoeothrix sp.	X	X		
cf. *Hydrococcus* sp.				
Lyngbya sp.				
Lyngbya/*Phormidium* sp.				
Microchaete sp.				
Microcoleus amoenus/*autumnalis*				
Microcoleus sp.				
Microseira wollei	X	X		
Oscillatoria ornata v. *crassa*				
Oscillatoria sp.		X		
Oscillatoria/*Phormidium* sp.				
Phormidium sp.				
Phormidium/*Microcoleus* sp.				
Scytonema sp.				
Tapinothrix sp.				
cf. *Wollea* sp.				
Nostocalean filament sp.				
Oscillatorialean filament sp.			X	
CHLOROPHYTA				
Cladophora glomerata	X		X	X
Oedogonium sp./spp.			X	X
Rhizoclonium hieroglyphicum				
Schizomeris leibleinii				
Spirogyra sp.			X	X
Stigeoclonium sp.		X		X
Ulothrix sp.			X	
Ulva sp.				
BACILLARIOPHYTA				

Bacillaria paradoxa				
Cymbella cf. *mexicana*				
	WEE1	WEE2	WEK1	WEK2
Epithemia adnata/turgida				
Eunotia sp.	X			
Melosira varians				
Rhopalodia gibba				
Terpsinoe musica	X		X	
Ulnaria cf. *ulna*	X	X	X	X
Ulnaria spp.				
Centric diatom chain sp.				
Pennate diatom spp.	X	X		X
RHODOPHYTA				
Batrachospermum sp.	X	X	X	X
Compsopogon coeruleus				X
Rhodophyte filament sp.				
TOTAL TAXA RICHNESS	**8**	**8**	**8**	**9**

Epiphyte Species Fall 2015

	ALE1	ALE2	GUM1	GUM2	ICH1	ICH2	JUN1	JUN2	RAI1	RAI2
CYANOPHYTA										
Aphanocapsa sp.		X								
Heteroleibleinia sp.						X		X		
Homoeothrix sp.						X				
Lyngbya sp.	X								X	
Microcoleus cf. *amoenus*										
Microcoleus cf. *paludosus*	X									
Microcoleus/*Phormidium* sp.			X							
Microseira wollei	X	X	X	X					X	X
Oscillatoria sp.								X		
Phormidium sp.	X									
Scytonema sp.									X	
Tapinothrix sp.										
CHLOROPHYTA										
Cladophora glomerata				X		X		X		X
Cladophora sp.										
Oedogonium sp./spp.	X								X	X
Rhizoclonium hieroglyphicum			X						X	
Spirogyra sp.	X									
Stigeoclonium sp.										
BACILLARIOPHYTA										
Achnanthes cf. *inflexa*	X									
Cymbella cf. *mexicana*			X		X	X			X	
Epithemia sp.	X	X						X		

Eunotia sp.					X				X	
Nitzschia sp.										
Pleurosira laevis	X									
	ALE1	ALE2	GUM1	GUM2	ICH1	ICH2	JUN1	JUN2	RAI1	RAI2
Rhopalodia gibba		X								
Terpsinoe musica	X		X	X				X		
Ulnaria cf. *ulna*		X	X	X	X				X	X
Ulnaria sp.		X								
Centric diatom chain sp.										X
Centric diatom sp.										X
Pennate diatom spp.	X	X	X		X	X		X	X	X
RHODOPHYTA										
Batrachospermum sp.				X	X	X		X		
Compsopogon coeruleus										
Polysiphonia sp.								X		
TOTAL TAXA RICHNESS	**11**	**7**	**7**	**5**	**5**	**6**	**0**	**8**	**9**	**7**

	ROC1	ROC2	SIL1	SIL2	SIL3	SLG1	WAC1	WAC2	WAK1	WAK2
CYANOPHYTA										
Aphanocapsa sp.										
Heteroleibleinia sp.		X	X					X		
Homoeothrix sp.			X	X			X	X		
Lyngbya sp.										
Microcoleus cf. *amoenus*			X							
Microcoleus cf. *paludosus*										
Microcoleus/*Phormidium* sp.										
Microseira wollei						X	X	X		
Oscillatoria sp.										
Phormidium sp.			X			X				
Scytonema sp.										
Tapinothrix sp.			X					X		
CHLOROPHYTA										
Cladophora glomerata					X	X	X	X	X	
Cladophora sp.										
Oedogonium sp./spp.	X				X	X		X		X
Rhizoclonium hieroglyphicum		X								
Spirogyra sp.							X			
Stigeoclonium sp.	X	X								X
BACILLARIOPHYTA										
Achnanthes cf. *inflexa*										
Cymbella cf. *mexicana*			X	X			X			
Epithemia sp.			X	X						
Eunotia sp.			X	X						

Nitzschia sp.		X								
Pleurosira laevis										
Rhopalodia gibba										
Terpsinoe musica				X		X				
	ROC1	ROC2	SIL1	SIL2	SIL3	SLG1	WAC1	WAC2	WAK1	WAK2
Ulnaria cf. *ulna*	X	X	X	X	X		X	X	X	X
Ulnaria sp.									X	X
Centric diatom chain sp.										
Centric diatom sp.										
Pennate diatom spp.	X	X	X	X		X	X	X	X	X
RHODOPHYTA										
Batrachospermum sp.			X		X				X	
Compsopogon coeruleus					X				X	
Polysiphonia sp.										
TOTAL TAXA RICHNESS	**4**	**6**	**11**	**7**	**5**	**6**	**7**	**8**	**6**	**5**

	WEE1	WEE2	WEK1	WEK2
CYANOPHYTA				
Aphanocapsa sp.				
Heteroleibleinia sp.				X
Homoeothrix sp.	X	X		
Lyngbya sp.				
Microcoleus cf. *amoenus*				
Microcoleus cf. *paludosus*				
Microcoleus/*Phormidium* sp.				
Microseira wollei		X		
Oscillatoria sp.				
Phormidium sp.				X
Scytonema sp.				
Tapinothrix sp.				
CHLOROPHYTA				
Cladophora glomerata	X	X	X	
Cladophora sp.				
Oedogonium sp./spp.			X	
Rhizoclonium hieroglyphicum				
Spirogyra sp.			X	
Stigeoclonium sp.	X	X		X
BACILLARIOPHYTA				
Achnanthes cf. *inflexa*				
Cymbella cf. *mexicana*				
Epithemia sp.				
Eunotia sp.				X
Nitzschia sp.				

Pleurosira laevis				
Rhopalodia gibba				
Terpsinoe musica	X		X	X
Ulnaria cf. *ulna*	X	X	X	
Ulnaria sp.	X			
	WEE1	WEE2	WEK1	WEK2
Centric diatom chain sp.				
Centric diatom sp.				
Pennate diatom spp.		X		X
RHODOPHYTA				
Batrachospermum sp.	X		X	X
Compsopogon coeruleus			X	
Polysiphonia sp.				
TOTAL TAXA RICHNESS	**7**	**6**	**7**	**7**

APPENDIX D—SUMMARIES OF MACROALGAL (AND ASSOCIATED EPIPHYTE) TAXA COLLECTED AT THE SAMPLING TRANSECTS

Macroalgae and Epiphytes Spring 2015 (** - Abundant to Dominant taxa present in the samples, as defined in Methods Section)

	ALE1	ALE2	MAN1	RAI2	ROC1	SLG1	VOL1	WAK1	WAK2	WEE1	WEE2
CYANOPHYTA											
Heteroleibleinia sp.						X					
Homoeothrix sp.										X	
Lyngbya cf.*rtensiana*	X										
Lyngbya sp.		X				X					X
Lyngbya/*Phormidium* sp.						X					
Microchaete sp.						X					
Microcoleus cf. *paludosus*	X										
Microseira wollei	X**	X**	X	X**	X	X**		X**	X**	X**	
Oscillatoria ornata v. *crassa*	X										
Phormidium sp.			X			X					
CHLOROPHYTA											
Cladophora glomerata							X		X**	X	
Dichotomosiphon tuberosus			X**							X**	X**
Hydrodictyon reticulatum	X**										
Oedogonium sp.		X	X								
Rhizoclonium hieroglyphicum	X**	X	X	X			X**				
Spirogyra sp.								X	X	X	
BACILLARIOPHYTA											
Eunotia sp.							X			X	
Pleurosira laevis		X					X	X			
Terpsinoe musica			X	X	X		X	X		X	
Ulnaria cf. *ulna*				X	X					X	X
Centric diatom chain sp.				X							
Centric diatom sp.				X							
RHODOPHYTA											
Batrachospermum sp.							X		X		

	ALE1	ALE2	MAN1	RAI2	ROC1	SLG1	VOL1	WAK1	WAK2	WEE1	WEE2
Compsopogon coeruleus								X			
Rhodophyte filament sp.								X**			
XANTHOPHYTA											
Vaucheria sp.					X**		X**	X**	X		
TOTAL TAXA RICHNESS	**6**	**5**	**6**	**6**	**4**	**6**	**7**	**7**	**5**	**8**	**3**

Macroalgae and Epiphytes Fall 2015 (** - Abundant to Dominant taxa present in the samples, as defined in Methods Section)

	ALE2	GUM1	MAN1	RAI2	VOL1	WAK1	WAK2	WEE1	WEE2
CYANOPHYTA									
Microseira wollei	X**	X**	X	X**		X	X**	X	X
Oscillatoria cf. *princeps*			X						
Oscillatoria sp.			X						
Scytonema sp.				X					
Oscillatorialean filament sp.	X								
CHLOROPHYTA									
Cladophora glomerata			X	X					
Dichotomosiphon tuberosus			X**					X**	X**
Dichotomosiphon/*Vaucheria* sp.					X**	X	X		
Rhizoclonium hieroglyphicum		X**	X	X					X
Spirogyra sp.							X		
Chlorophyte filament sp.	X								
BACILLARIOPHYTA									
Eunotia sp.					X				
Pleurosira laevis					X		X		
Terpsinoe musica	X	X			X		X	X	
Ulnaria cf. *ulna*				X		X		X	X
Centric diatom chain sp.				X					

	ALE2	GUM1	MAN1	RAI2	VOL1	WAK1	WAK2	WEE1	WEE2
RHODOPHYTA									
Batrachospermum sp.									X
Compsopogon coeruleus						X**	X		
Rhodophyte filament sp.						X			
XANTHOPHYTA									
Vaucheria sp.		X**							
TOTAL TAXA RICHNESS	**4**	**4**	**6**	**6**	**4**	**5**	**6**	**4**	**5**

APPENDIX E—SUMMARY STATISTICS FOR EPIPHYTIC ALGAL TAXA RICHNESS AND ABUNDANCE MEASURES

Column Headings in Tables

MEAN – mean value
SE – Standard error
SD – Standard deviation
MIN – Minimum value
25 %ile – 25th Percentile value
MEDIAN – Median value
75 %ile – 75th Percentile value
MAX – Maximum value

Appendix E. Table 1. Summary statistics for epiphytic algal taxa richness (# taxa) at the sampling transects supporting macrophytes in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	5.7	0.9	1.5	4.0	4.0	6.0	7.0	7.0
ALE2	5.0	0.6	1.0	4.0	4.0	5.0	6.0	6.0
GUM1	4.3	0.7	1.2	3.0	3.0	5.0	5.0	5.0
GUM2	4.0	0.0	0.0	4.0	4.0	4.0	4.0	4.0
ICH1	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
ICH2	5.0	1.2	2.0	3.0	3.0	5.0	7.0	7.0
JUN1	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
JUN2	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
RAI1	5.3	0.9	1.5	4.0	4.0	5.0	7.0	7.0
RAI2	4.7	1.2	2.1	3.0	3.0	4.0	7.0	7.0
ROC1	2.7	0.3	0.6	2.0	2.0	3.0	3.0	3.0
ROC2	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
SIL1	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
SIL2	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
SIL3	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
SLG1	5.7	1.5	2.5	3.0	3.0	6.0	8.0	8.0
WAC1	5.7	0.3	0.6	5.0	5.0	6.0	6.0	6.0
WAC2	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
WAK1	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
WAK2	5.3	0.7	1.2	4.0	4.0	6.0	6.0	6.0
WEE1	4.3	0.7	1.2	3.0	3.0	5.0	5.0	5.0
WEE2	3.7	0.7	1.2	3.0	3.0	3.0	5.0	5.0
WEK1	4.7	0.3	0.6	4.0	4.0	5.0	5.0	5.0
WEK2	5.0	1.0	1.7	3.0	3.0	6.0	6.0	6.0

Appendix E. Table 2. Summary statistics for epiphytic algal cover (% cover) at the sampling transects supporting macrophytes in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	89.33%	5.67%	9.81%	78.00%	78.00%	95.00%	95.00%	95.00%
ALE2	81.67%	3.33%	5.77%	75.00%	75.00%	85.00%	85.00%	85.00%
GUM1	52.70%	21.10%	36.60%	15.00%	15.00%	55.00%	88.00%	88.00%
GUM2	50.00%	14.40%	25.00%	25.00%	25.00%	50.00%	75.00%	75.00%
ICH1	26.67%	3.33%	5.77%	20.00%	20.00%	30.00%	30.00%	30.00%
ICH2	15.00%	0.00%	0.00%	15.00%	15.00%	15.00%	15.00%	15.00%
JUN1	1.67%	0.83%	1.44%	0.00%	0.00%	2.50%	2.50%	2.50%
JUN2	0.83%	0.83%	1.44%	0.00%	0.00%	0.00%	2.50%	2.50%
RAI1	2.50%	0.00%	0.00%	2.50%	2.50%	2.50%	2.50%	2.50%
RAI2	75.00%	0.00%	0.00%	75.00%	75.00%	75.00%	75.00%	75.00%
ROC1	56.70%	15.90%	27.50%	25.00%	25.00%	70.00%	75.00%	75.00%
ROC2	63.30%	12.00%	20.80%	40.00%	40.00%	70.00%	80.00%	80.00%
SIL1	23.33%	3.33%	5.77%	20.00%	20.00%	20.00%	30.00%	30.00%
SIL2	20.00%	5.00%	8.66%	15.00%	15.00%	15.00%	30.00%	30.00%
SIL3	30.00%	15.00%	26.00%	15.00%	15.00%	15.00%	60.00%	60.00%
SLG1	1.67%	0.83%	1.44%	0.00%	0.00%	2.50%	2.50%	2.50%
WAC1	86.67%	3.33%	5.77%	80.00%	80.00%	90.00%	90.00%	90.00%
WAC2	30.00%	5.00%	8.66%	25.00%	25.00%	25.00%	40.00%	40.00%
WAK1	29.20%	23.00%	39.90%	2.50%	2.50%	10.00%	75.00%	75.00%
WAK2	35.80%	19.50%	33.80%	2.50%	2.50%	35.00%	70.00%	70.00%
WEE1	63.30%	11.70%	20.20%	45.00%	45.00%	60.00%	85.00%	85.00%
WEE2	11.67%	1.67%	2.89%	10.00%	10.00%	10.00%	15.00%	15.00%
WEK1	100.00%	0.00%	0.00%	100.00%	100.00%	100.00%	100.00%	100.00%
WEK2	66.67%	6.67%	11.55%	60.00%	60.00%	60.00%	80.00%	80.00%

Appendix E. Table 3. Summary statistics for epiphytic algal Chlorophyll a (mg/m^2) at the sampling transects supporting macrophytes in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	16.34	6.39	11.06	5.87	5.87	15.25	27.91	27.91
ALE2	5.77	0.45	0.77	5.25	5.25	5.41	6.66	6.66
GUM1	55.20	16.90	29.30	31.10	31.10	46.70	87.70	87.70
GUM2	19.78	1.37	2.38	17.08	17.08	20.73	21.54	21.54
ICH1	3.17	0.87	1.51	1.78	1.78	2.94	4.78	4.78
ICH2	12.33	5.60	9.70	4.84	4.84	8.86	23.28	23.28
JUN1	1.90	0.23	0.40	1.50	1.50	1.90	2.30	2.30
JUN2	10.27	1.39	2.41	8.07	8.07	9.91	12.84	12.84
RAI1	22.90	10.20	17.70	8.70	8.70	17.40	42.70	42.70
RAI2	28.00	16.10	27.90	10.10	10.10	13.70	60.10	60.10
ROC1	2.77	2.21	3.83	0.46	0.46	0.65	7.19	7.19
ROC2	9.51	2.94	5.10	5.38	5.38	7.94	15.21	15.21
SIL1	12.73	6.76	11.70	4.23	4.23	7.88	26.08	26.08
SIL2	21.89	3.01	5.21	17.37	17.37	20.71	27.59	27.59
SIL3	10.23	1.60	2.78	7.96	7.96	9.40	13.33	13.33
SLG1	18.32	5.32	9.21	10.61	10.61	15.82	28.52	28.52
WAC1	7.73	3.56	6.16	2.38	2.38	6.35	14.47	14.47
WAC2	1.95	0.59	1.02	1.11	1.11	1.66	3.09	3.09
WAK1	15.77	0.30	0.52	15.31	15.31	15.67	16.33	16.33
WAK2	28.50	24.50	42.40	3.20	3.20	4.90	77.50	77.50
WEE1	3.04	1.41	2.44	0.82	0.82	2.65	5.66	5.66
WEE2	1.36	0.68	1.17	0.46	0.46	0.93	2.68	2.68
WEK1	9.40	1.59	2.76	6.40	6.40	9.95	11.84	11.84
WEK2	9.16	2.59	4.49	3.98	3.98	11.60	11.90	11.90

Appendix E. Table 4. Summary statistics for epiphytic algal AFDW (g/m^2) at the sampling transects supporting macrophytes in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	2.79	1.89	3.27	0.85	0.85	0.95	6.56	6.56
ALE2	2.95	1.31	2.27	0.99	0.99	2.42	5.43	5.43
GUM1	23.42	9.29	16.09	11.03	11.03	17.63	41.60	41.60
GUM2	4.49	0.94	1.62	3.32	3.32	3.80	6.34	6.34
ICH1	16.70	11.20	19.40	3.50	3.50	7.70	39.00	39.00
ICH2	7.83	3.25	5.63	3.67	3.67	5.58	14.23	14.23
JUN1	*	*	*	*	*	*	*	*
JUN2	1.77	0.23	0.40	1.42	1.42	1.68	2.20	2.20
RAI1	5.24	1.85	3.21	3.10	3.10	3.70	8.93	8.93
RAI2	15.45	2.39	4.14	10.79	10.79	16.83	18.72	18.72
ROC1	2.45	0.81	1.40	0.83	0.83	3.23	3.29	3.29
ROC2	6.71	1.54	2.66	4.73	4.73	5.66	9.74	9.74
SIL1	2.78	0.48	0.84	2.08	2.08	2.56	3.71	3.71
SIL2	9.31	2.22	3.85	5.23	5.23	9.80	12.89	12.89
SIL3	1.31	0.10	0.17	1.15	1.15	1.28	1.49	1.49
SLG1	4.42	1.33	2.30	2.38	2.38	3.96	6.91	6.91
WAC1	4.64	0.74	1.29	3.28	3.28	4.79	5.84	5.84
WAC2	2.70	0.99	1.71	1.66	1.66	1.77	4.68	4.68
WAK1	3.90	1.90	3.30	1.46	1.46	2.59	7.65	7.65
WAK2	1.94	1.04	1.79	0.50	0.50	1.37	3.95	3.95
WEE1	6.38	2.53	4.38	1.82	1.82	6.75	10.56	10.56
WEE2	1.18	0.49	0.84	0.58	0.58	0.81	2.14	2.14
WEK1	2.71	0.96	1.67	1.73	1.73	1.76	4.63	4.63
WEK2	4.61	1.89	3.28	1.34	1.34	4.59	7.90	7.90

Appendix E. Table 5. Summary statistics for epiphytic algal taxa richness (# taxa) at the sampling transects supporting macrophytes in fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	7.3	0.7	1.2	6.0	6.0	8.0	8.0	8.0
ALE2	4.3	0.7	1.2	3.0	3.0	5.0	5.0	5.0
GUM1	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
GUM2	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
ICH1	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
ICH2	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
JUN1	*	*	*	*	*	*	*	*
JUN2	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
RAI1	4.7	0.3	0.6	4.0	4.0	5.0	5.0	5.0
RAI2	4.7	0.7	1.2	4.0	4.0	4.0	6.0	6.0
ROC1	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
ROC2	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
SIL1	5.7	0.3	0.6	5.0	5.0	6.0	6.0	6.0
SIL2	3.7	0.7	1.2	3.0	3.0	3.0	5.0	5.0
SIL3	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
SLG1	3.7	0.7	1.2	3.0	3.0	3.0	5.0	5.0
WAC1	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
WAC2	4.3	0.3	0.6	4.0	4.0	4.0	5.0	5.0
WAK1	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
WAK2	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
WEE1	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
WEE2	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
WEK1	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
WEK2	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0

Appendix E. Table 6. Summary statistics for epiphytic algal cover (% cover) at the sampling transects supporting macrophytes in fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	80.00%	5.77%	10.00%	70.00%	70.00%	80.00%	90.00%	90.00%
ALE2	27.50%	21.40%	37.00%	2.50%	2.50%	10.00%	70.00%	70.00%
GUM1	23.30%	13.60%	23.60%	5.00%	5.00%	15.00%	50.00%	50.00%
GUM2	33.30%	18.30%	31.80%	15.00%	15.00%	15.00%	70.00%	70.00%
ICH1	75.00%	8.66%	15.00%	60.00%	60.00%	75.00%	90.00%	90.00%
ICH2	46.70%	14.80%	25.70%	25.00%	25.00%	40.00%	75.00%	75.00%
JUN1	*	*	*	*	*	*	*	*
JUN2	11.67%	6.67%	11.55%	5.00%	5.00%	5.00%	25.00%	25.00%
RAI1	80.00%	2.89%	5.00%	75.00%	75.00%	80.00%	85.00%	85.00%
RAI2	63.33%	3.33%	5.77%	60.00%	60.00%	60.00%	70.00%	70.00%
ROC1	60.00%	11.50%	20.00%	40.00%	40.00%	60.00%	80.00%	80.00%
ROC2	46.67%	4.41%	7.64%	40.00%	40.00%	45.00%	55.00%	55.00%
SIL1	25.00%	5.00%	8.66%	15.00%	15.00%	30.00%	30.00%	30.00%
SIL2	55.00%	16.10%	27.80%	25.00%	25.00%	60.00%	80.00%	80.00%
SIL3	43.33%	6.67%	11.55%	30.00%	30.00%	50.00%	50.00%	50.00%
SLG1	61.70%	11.70%	20.20%	40.00%	40.00%	65.00%	80.00%	80.00%
WAC1	71.67%	1.67%	2.89%	70.00%	70.00%	70.00%	75.00%	75.00%
WAC2	50.00%	10.40%	18.00%	30.00%	30.00%	55.00%	65.00%	65.00%
WAK1	56.70%	15.90%	27.50%	25.00%	25.00%	70.00%	75.00%	75.00%
WAK2	33.33%	3.33%	5.77%	30.00%	30.00%	30.00%	40.00%	40.00%
WEE1	86.67%	4.41%	7.64%	80.00%	80.00%	85.00%	95.00%	95.00%
WEE2	9.17%	3.63%	6.29%	2.50%	2.50%	10.00%	15.00%	15.00%
WEK1	73.33%	1.67%	2.89%	70.00%	70.00%	75.00%	75.00%	75.00%
WEK2	23.33%	1.67%	2.89%	20.00%	20.00%	25.00%	25.00%	25.00%

Appendix E. Table 7. Summary statistics for epiphytic algal Chlorophyll a (mg/m^2) at the sampling transects supporting macrophytes in fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	17.06	4.31	7.47	10.18	10.18	15.99	25.01	25.01
ALE2	1.95	0.66	1.14	0.96	0.96	1.70	3.19	3.19
GUM1	14.21	3.21	5.56	7.80	7.80	17.06	17.77	17.77
GUM2	4.91	0.95	1.65	3.03	3.03	5.56	6.13	6.13
ICH1	15.04	3.13	5.43	9.27	9.27	15.80	20.04	20.04
ICH2	4.31	1.75	3.02	0.83	0.83	5.85	6.26	6.26
JUN1	*	*	*	*	*	*	*	*
JUN2	2.33	0.63	1.08	1.57	1.57	1.85	3.57	3.57
RAI1	9.41	3.55	6.15	2.46	2.46	11.61	14.15	14.15
RAI2	17.16	0.78	1.36	15.65	15.65	17.57	18.27	18.27
ROC1	5.34	3.70	6.41	1.37	1.37	1.91	12.73	12.73
ROC2	2.17	0.81	1.41	0.57	0.57	2.73	3.21	3.21
SIL1	3.00	0.52	0.90	1.98	1.98	3.37	3.66	3.66
SIL2	7.88	1.30	2.26	5.32	5.32	8.75	9.58	9.58
SIL3	7.46	0.86	1.49	6.12	6.12	7.20	9.07	9.07
SLG1	5.91	1.59	2.75	2.83	2.83	6.79	8.12	8.12
WAC1	12.14	0.91	1.57	10.77	10.77	11.81	13.85	13.85
WAC2	14.30	11.30	19.50	1.60	1.60	4.40	36.80	36.80
WAK1	13.15	1.39	2.41	10.37	10.37	14.38	14.69	14.69
WAK2	1.93	0.74	1.28	1.11	1.11	1.27	3.40	3.40
WEE1	7.86	3.14	5.43	2.49	2.49	7.73	13.35	13.35
WEE2	0.81	0.42	0.73	0.14	0.14	0.69	1.59	1.59
WEK1	17.47	6.24	10.81	7.40	7.40	16.13	28.89	28.89
WEK2	0.35	0.12	0.21	0.15	0.15	0.35	0.56	0.56

Appendix E. Table 8. Summary statistics for epiphytic algal AFDW (g/m^2) at the sampling transects supporting macrophytes in fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	5.43	0.54	0.94	4.42	4.42	5.61	6.27	6.27
ALE2	4.80	1.78	3.08	2.77	2.77	3.29	8.35	8.35
GUM1	17.59	5.08	8.80	9.59	9.59	16.18	27.01	27.01
GUM2	3.47	0.77	1.33	1.96	1.96	4.01	4.45	4.45
ICH1	1.44	0.33	0.56	1.06	1.06	1.18	2.09	2.09
ICH2	0.58	0.03	0.06	0.52	0.52	0.58	0.63	0.63
JUN1	*	*	*	*	*	*	*	*
JUN2	0.89	0.24	0.42	0.58	0.58	0.73	1.37	1.37
RAI1	2.38	0.68	1.18	1.13	1.13	2.54	3.48	3.48
RAI2	9.27	5.29	9.16	2.99	2.99	5.05	19.78	19.78
ROC1	1.34	0.57	0.98	0.41	0.41	1.24	2.36	2.36
ROC2	0.85	0.25	0.43	0.43	0.43	0.84	1.29	1.29
SIL1	0.56	0.13	0.22	0.31	0.31	0.68	0.69	0.69
SIL2	2.07	0.55	0.95	1.24	1.24	1.85	3.11	3.11
SIL3	1.46	0.08	0.14	1.32	1.32	1.46	1.60	1.60
SLG1	2.74	0.40	0.69	1.99	1.99	2.88	3.35	3.35
WAC1	1.12	0.23	0.39	0.67	0.67	1.29	1.39	1.39
WAC2	2.40	1.08	1.87	0.85	0.85	1.88	4.48	4.48
WAK1	5.00	1.85	3.20	1.93	1.93	4.76	8.31	8.31
WAK2	2.51	1.42	2.46	0.97	0.97	1.21	5.34	5.34
WEE1	7.27	4.06	7.04	2.78	2.78	3.65	15.38	15.38
WEE2	0.05	0.02	0.04	0.02	0.02	0.03	0.09	0.09
WEK1	9.11	3.16	5.47	5.38	5.38	6.56	15.39	15.39
WEK2	0.04	0.03	0.05	0.01	0.01	0.01	0.09	0.09

APPENDIX F—SUMMARY STATISTICS FOR MACROALGAL (AND ASSOCIATED EPIPHYTE) TAXA RICHNESS AND ABUNDANCE MEASURES

Column Headings in Tables

MEAN – mean value
SE – Standard error
SD – Standard deviation
MIN – Minimum value
25 %ile – 25th Percentile value
MEDIAN – Median value
75 %ile – 75th Percentile value
MAX – Maximum value

Appendix F. Table 1. Summary statistics for macroalgal taxa richness (# taxa) at the sampling transects supporting algal mats in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	3.7	0.7	1.2	3.0	3.0	3.0	5.0	5.0
ALE2	2.3	0.7	1.2	1.0	1.0	3.0	3.0	3.0
MAN1	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
RAI2	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
ROC1	2.7	0.3	0.6	2.0	2.0	3.0	3.0	3.0
SLG1	3.7	0.3	0.6	3.0	3.0	4.0	4.0	4.0
VOL1	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
WAK1	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
WAK2	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
WEE1	4.0	0.6	1.0	3.0	3.0	4.0	5.0	5.0
WEE2	2.3	0.3	0.6	2.0	2.0	2.0	3.0	3.0

Appendix F. Table 2. Summary statistics for macroalgal cover (% cover) at the sampling transects supporting algal mats in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	24.00%	21.50%	37.20%	2.50%	2.50%	2.50%	67.00%	67.00%
ALE2	29.50%	24.30%	42.10%	2.50%	2.50%	8.00%	78.00%	78.00%
MAN1	91.00%	6.66%	11.53%	78.00%	78.00%	95.00%	100.00%	100.00%
RAI2	57.00%	17.70%	30.60%	28.00%	28.00%	54.00%	89.00%	89.00%
ROC1	2.50%	0.00%	0.00%	2.50%	2.50%	2.50%	2.50%	2.50%
SLG1	65.80%	31.70%	54.90%	2.50%	2.50%	97.00%	98.00%	98.00%
VOL1	63.30%	11.70%	20.20%	45.00%	45.00%	60.00%	85.00%	85.00%
WAK1	44.70%	18.00%	31.10%	12.00%	12.00%	48.00%	74.00%	74.00%
WAK2	45.80%	28.30%	49.00%	2.50%	2.50%	36.00%	99.00%	99.00%
WEE1	19.33%	6.36%	11.02%	8.00%	8.00%	20.00%	30.00%	30.00%
WEE2	4.33%	1.83%	3.18%	2.50%	2.50%	2.50%	8.00%	8.00%

Appendix F. Table 3. Summary statistics for macroalgal Chlorophyll a (mg/m^2) at the sampling transects supporting algal mats in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	145.40	85.70	148.50	34.60	34.60	87.30	314.10	314.10
ALE2	614.00	454.00	787.00	30.00	30.00	304.00	1509.00	1509.00
MAN1	64.40	15.10	26.10	46.80	46.80	52.00	94.40	94.40
RAI2	498.00	235.00	407.00	77.00	77.00	526.00	890.00	890.00
ROC1	96.90	45.30	78.40	6.40	6.40	139.30	145.00	145.00
SLG1	570.00	323.00	559.00	9.00	9.00	574.00	1127.00	1127.00
VOL1	42.80	10.20	17.70	23.50	23.50	46.60	58.20	58.20
WAK1	58.00	55.10	95.40	1.30	1.30	4.60	168.20	168.20
WAK2	60.10	18.10	31.30	25.50	25.50	68.30	86.50	86.50
WEE1	208.80	76.40	132.30	64.20	64.20	238.70	323.70	323.70
WEE2	164.80	91.50	158.50	6.30	6.30	165.00	323.10	323.10

Appendix F. Table 4. Summary statistics for macroalgal AFDW (g/m^2) at the sampling transects supporting algal mats in spring 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE1	7.00	5.15	8.92	0.62	0.62	3.20	17.19	17.19
ALE2	102.30	80.70	139.70	5.50	5.50	39.10	262.50	262.50
MAN1	16.44	5.62	9.73	9.29	9.29	12.52	27.52	27.52
RAI2	95.30	12.40	21.50	76.60	76.60	90.60	118.80	118.80
ROC1	43.62	6.52	11.30	34.62	34.62	39.93	56.30	56.30
SLG1	142.80	69.40	120.10	22.30	22.30	143.80	262.50	262.50
VOL1	33.30	15.00	25.90	12.90	12.90	24.60	62.50	62.50
WAK1	10.86	6.27	10.86	1.01	1.01	9.06	22.51	22.51
WAK2	8.22	3.13	5.42	2.29	2.29	9.45	12.92	12.92
WEE1	75.90	53.30	92.30	8.90	8.90	37.60	181.30	181.30
WEE2	19.50	10.80	18.70	0.80	0.80	19.60	38.20	38.20

Appendix F. Table 5. Summary statistics for macroalgal taxa richness (# taxa) at the sampling transects supporting algal mats in fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE2	2.0	0.0	0.0	2.0	2.0	2.0	2.0	2.0
GUM1	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
MAN1	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
RAI2	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
VOL1	4.0	0.0	0.0	4.0	4.0	4.0	4.0	4.0
WAK1	3.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0
WAK2	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0
WEE1	3.0	0.6	1.0	2.0	2.0	3.0	4.0	4.0
WEE2	3.3	0.3	0.6	3.0	3.0	3.0	4.0	4.0

Appendix F. Table 6. Summary statistics for macroalgal cover (% cover) at the sampling transects supporting algal mats fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE2	46.67%	8.82%	15.28%	30.00%	30.00%	50.00%	60.00%	60.00%
GUM1	66.70%	15.90%	27.50%	35.00%	35.00%	80.00%	85.00%	85.00%
MAN1	70.00%	22.90%	39.70%	25.00%	25.00%	85.00%	100.00%	100.00%
RAI2	10.00%	2.89%	5.00%	5.00%	5.00%	10.00%	15.00%	15.00%
VOL1	70.00%	15.30%	26.50%	40.00%	40.00%	80.00%	90.00%	90.00%
WAK1	50.00%	0.00%	0.00%	50.00%	50.00%	50.00%	50.00%	50.00%
WAK2	36.70%	11.70%	20.20%	15.00%	15.00%	40.00%	55.00%	55.00%
WEE1	26.67%	9.28%	16.07%	15.00%	15.00%	20.00%	45.00%	45.00%
WEE2	20.80%	10.80%	18.80%	2.50%	2.50%	20.00%	40.00%	40.00%

Appendix F. Table 7. Summary statistics for macroalgal Chlorophyll a (mg/m^2) at the sampling transects supporting algal mats fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE2	211.80	94.10	163.00	116.70	116.70	118.60	400.00	400.00
GUM1	56.00	44.90	77.70	2.90	2.90	19.80	145.20	145.20
MAN1	105.70	61.00	105.70	29.50	29.50	61.30	226.40	226.40
RAI2	177.60	48.30	83.60	86.20	86.20	196.40	250.20	250.20
VOL1	29.10	15.20	26.40	2.50	2.50	29.60	55.20	55.20
WAK1	917.00	52.60	91.10	826.00	826.00	916.80	1008.10	1008.10
WAK2	323.60	53.30	92.40	232.30	232.30	321.60	417.00	417.00
WEE1	409.00	358.00	620.00	3.00	3.00	101.00	1123.00	1123.00
WEE2	48.73	7.73	13.39	33.72	33.72	53.00	59.46	59.46

Appendix F. Table 8. Summary statistics for macroalgal AFDW (g/m^2) at the sampling transects supporting algal mats fall 2015.

TRANSECT	MEAN	SE	SD	MIN	25 %ile	MEDIAN	75 %ile	MAX
ALE2	39.00	13.60	23.50	21.20	21.20	30.10	65.60	65.60
GUM1	28.45	5.43	9.40	21.33	21.33	24.91	39.10	39.10
MAN1	15.05	3.30	5.72	10.30	10.30	13.44	21.40	21.40
RAI2	20.60	7.44	12.89	11.07	11.07	15.47	35.27	35.27
VOL1	25.60	11.00	19.00	10.20	10.20	19.60	46.90	46.90
WAK1	290.10	27.40	47.40	239.10	239.10	298.40	332.80	332.80
WAK2	46.90	12.60	21.90	25.00	25.00	46.90	68.80	68.80
WEE1	70.10	33.30	57.70	16.50	16.50	62.50	131.30	131.30
WEE2	19.73	7.76	13.44	10.91	10.91	13.09	35.20	35.20