Biological Sciences

Springs Coast long-term fish community assessment: The Rainbow River System

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Abstract Florida has one of the highest concentrations of freshwater springs in the world, including six Outstanding Florida Springs (OFS) systems within the Southwest Florida Water Management District (SWFWMD). This study characterized the fish community of the Rainbow River System, one of the SWFWMD's OFS systems, over a five-year period. Thirty-seven species were collected, with Spotted Sunfish (*Lepomis punctatus*) making up 39% of the total catch. There were no seasonal or yearly differences in species diversity; however, diversity was higher in the Lower River compared to the Upper River. Fish assemblages were most distinguished by location and were largely driven by the abundances of Spotted Sunfish, Coastal Shiner (*Notropis petersoni*), Inland Silverside (*Menidia beryllina*), Warmouth (*Lepomis gulosus*), Eastern Mosquitofish (*Gambusia holbrooki*), Redeye Chub (*Notropis harperi*), Bluegill (*Lepomis macrochirus*), Redbreast Sunfish (*Lepomis auritus*), Redear Sunfish (*Lepomis microlophus*), and Florida Bass (*Micropterus salmoides floridanus*). Species more abundant in the Upper River included Spotted Sunfish, Redeye Chub, and Florida Bass, while species with higher Lower River abundances included Coastal Shiner, Warmouth, Bluegill, Redbreast Sunfish, and Redear Sunfish. This long-term dataset, which serves as a baseline to monitor future fish assemblage changes and ecological health, contributes to improving the understanding and management of the Rainbow River System.

Keywords Rainbow River, Rainbow Springs, Florida Springs Fish Assemblages

Introduction

Florida has one of the largest concentrations of freshwater springs in the world. These springs are one of Florida's most cherished natural resources, providing water for people, wildlife, rivers, and estuaries and offering unique recreational opportunities. A key feature of Florida springs systems is their crystal-clear water with relatively stable year-round temperatures. Unlike typical rivers and streams, springs systems are generally characterized as stable ecosystems with unique faunal and floral assemblages that persist under relatively static physical and chemical conditions (Hubbs 2001, Walsh 2001, Work et al. 2010). Springs are important not only for their ecological value but also for their economic benefits to the surrounding communities (SWFWMD 2015a).

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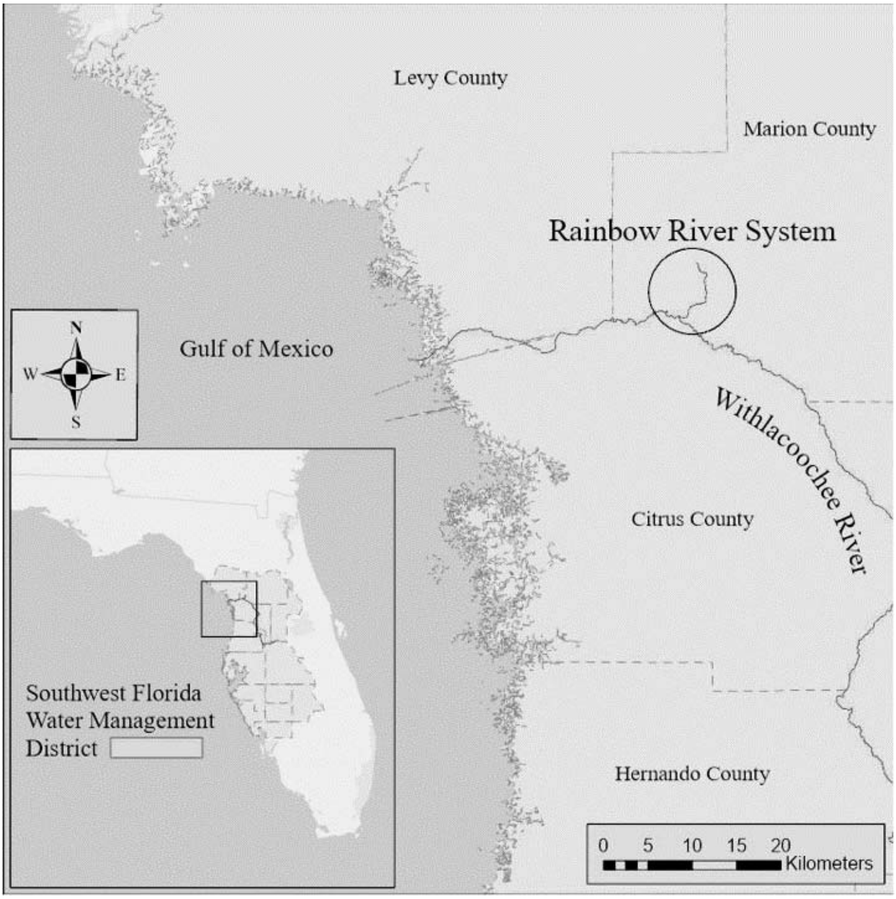

Figure 1. Location of the Rainbow River System, located within Southwest Florida Water Management District boundaries in relation to Florida.

There are more than 200 documented springs within the Southwest Florida Water Management District (SWFWMD) (SWFWMD 2015a), located on the West-Central Florida Coast from Charlotte County north to Levy County (Figure 1). Within the SWFWMD, there are five first-magnitude Outstanding Florida Springs (OFS) systems that collectively discharge more than one billion gallons of water per day. They include the Rainbow River, Kings Bay/Crystal River, Homosassa River, Chassahowitzka River, and Weeki Wachee River Systems. These OFS systems are located in a region known as the Springs Coast, and all discharge water to the Gulf of Mexico that contains one of the largest seagrass habitats in the world. These unique and important systems face various complex threats as a result of human activities, climate change, and other factors, and efforts to better understand and protect them have increased in recent years.

Long-term monitoring of the SWFWMD's OFS systems is crucial for their management and can provide valuable insights into ecology and environmental change. Ecologists and managers of natural resources readily acknowledge the importance of long-term monitoring for the improved understanding and management of complicated ecological systems (Lindenmayer and Liken 2009). Long-term data are important for many reasons, including evaluating responses to disturbances, such as impacts associated with human activities or climate change; providing baselines to evaluate change; and detecting and evaluating changes in ecosystem structure and function in response to management activities. As a subset of environmental monitoring, fish sampling programs have been an important part of assessing the potential impacts on fish populations for many years (Lohner and Dixon 2013). Without long-term monitoring programs, population evaluations may incorrectly indicate adverse impacts where none exist or no impact where one is likely to occur. In addition, it is often necessary to conduct long-term studies in order to separate subtle changes in population patterns from the year-to-year variability or "noise," which is often large compared to the magnitude of the trend (Lohner and Dixon 2013)

To date, long-term monitoring of fish populations in Florida springs systems is rare (Work et al. 2010, 2017). Single surveys with varying goals and using different methodologies have occasionally been conducted to characterize the fish communities of some of Florida's springs systems (Hubbs and Allen 1943, FWC 1992, Walsh and Williams 2003, WSI 2010), including the SWFWMD's five first-magnitude OFS systems (Herald and Strickland 1949, Frazer et al. 2011, Pine et al. 2011). Fish have been also collected from the OFS systems within the SWFWMD over the years for the Florida Museum of Natural History's (FLMNH's) Ichthyology collection (www.floridamuseum.ufl.edu/fish/ Accessed: July 23, 2020).

The purpose of this study was to conduct long-term monitoring of the fish community of the Rainbow River System to improve the understanding and management of this important natural resource. The fish assemblage was characterized over a five-year period using a standardized protocol. Temporal, seasonal, and spatial differences in the fish assemblages and in notable fish species were evaluated.

Located in Marion County near the town of Dunnellon and approximately 20 miles southwest of Ocala, the Rainbow Springs Group forms the headwaters of the Rainbow River (Figure 1), one of the largest spring runs in the world that flows 9.2 km south into the tannic Withlacoochee River. The Withlacoochee River flows into the Gulf of Mexico near Yankeetown (Holzwart et al. 2017). Phosphate mining occurred in the lower portion of the Rainbow River in the late 1800s and early 1900s (SWFWMD 2015b). In 1909, the Withlacoochee River was dammed nearly 20 km from the Gulf of Mexico, severing any direct connection between the Gulf of Mexico and the Rainbow River. The area around the springs was used as a tourist attraction that included glass-bottom boats and mermaid shows from 1934 to 1973. With exceptional water clarity and unique ecological attributes, the Rainbow River was designated as an Aquatic Preserve in 1986 and an Outstanding Florida Waterway in 1987 by the State of Florida (SWFWMD 2015b). In addition to being an OFS, Rainbow Springs is a National Natural Landmark. The SWFWMD adopted the Rainbow River as a surface water improvement and management (SWIM) priority water body in 1989 to identify actions needed to improve and protect the river (SWFWMD 2015b). In 1995, the Florida Park Service opened the very popular Rainbow Springs State Park, which includes the area around the springs and much of the east bank of the river. The river is a major outdoor recreation area, and annual attendance in the state park in 2016-17 was 316,796 people (Mattson et al. 2019).

Materials and Methods

The Rainbow River System was divided into two zones, Upper River (Zone 1) and Lower River (Zone 2), based on differences in habitat and water clarity (Anastasiou 2006, SWFWMD 2015b, Water & Air 2020) (Figure 2). Submerged aquatic vegetation (SAV) coverage, biomass, and diversity are greater in the upper section of the river as compared to the lower portion. Strap-Leaf Sagittaria (*Sagittaria kurziana*) is the most common SAV species found in the Upper River (Mattson et al. 2019, Water & Air 2020). Nine other species of native SAV, exposed sand, and periphyton-covered limestone add to the habitat complexity of the Upper River, and filamentous algae and invasive species are largely absent (Water & Air 2020). In the Lower River, total SAV coverage, biomass, and diversity diminishes towards the confluence of the Withlacoochee River, and the habitat of this reach is dominated by filamentous algae, Hydrilla (*Hydrilla verticillata*), Eurasian Watermilfoil (*Myriophyllum spictatum*), and exposed sand and mud. Water clarity in the Upper River varies from about 20 to >60 m, while in the Lower River, which is contiguous with historical phosphate mining pits, clarity is typically <20 m (Anastasiou 2006).

The Florida Fish and Wildlife Conservation Commission (FWC) standardized river sampling protocols (Strickland et al. 2011, Bonvechio 2017) were applied to establish and sample transects using the centerline technique for site selection. Transect points were assigned at 25-m intervals throughout the center of the river. Prior to each sampling event, transect sites and riverbank side (left or right) were randomly selected. A total of 307 transects was established in the two zones; Transects 1 through 142 were located in the Lower River (Zone 2), while Transects 143 through 307 were located in the Upper River (Zone 1) (Figure 2). About 0.5 km of the Upper River, from the headsprings to Transect 307, was not included in the study since that area is located within Rainbow Springs State Park, and motorized boats are prohibited. In addition, approximately 1.3 km of the Lower River were not included since this area is a mixing zone; the influence of the tannic Withlacoochee River water varies depending on flows and seasons.

Thirty transects (each measuring 100 m in length) were randomly selected for every sampling event. Since the zones were similar in length, an equal number of transects were randomly selected in each zone in order to obtain the best representation of the overall fish assemblage (e.g., 165 or 54% of the transects were located in the Upper River and 142 or 46% of the transects were located in the Lower River). This was performed because randomly selecting transects from the entire river could result in the likelihood that the resulting transects might be weighted towards certain areas of the river, which might not accurately represent the species composition from the entire river. Adequately sampling each zone likely improved the ability to detect meaningful changes in the zones since the year-to-year variation in species presence/absence and abundance within zones should be less than the variation from combined zones.

Once at the transect centerline point, a starting GPS location was recorded after traveling to the randomly selected riverbank, and after sampling each transect, an ending GPS location was recorded. If obstacles arose during sampling (e.g., inclement weather, obstructions in selected transect, or navigational restraints), the completion of 20 electrofishing transects was considered a standard of success.

To characterize the fish communities and relative abundance, transect sampling was conducted using a 5.5-m aluminum electrofishing boat with a Smith-Root™ 9.0 Generator Powered Pulsator.

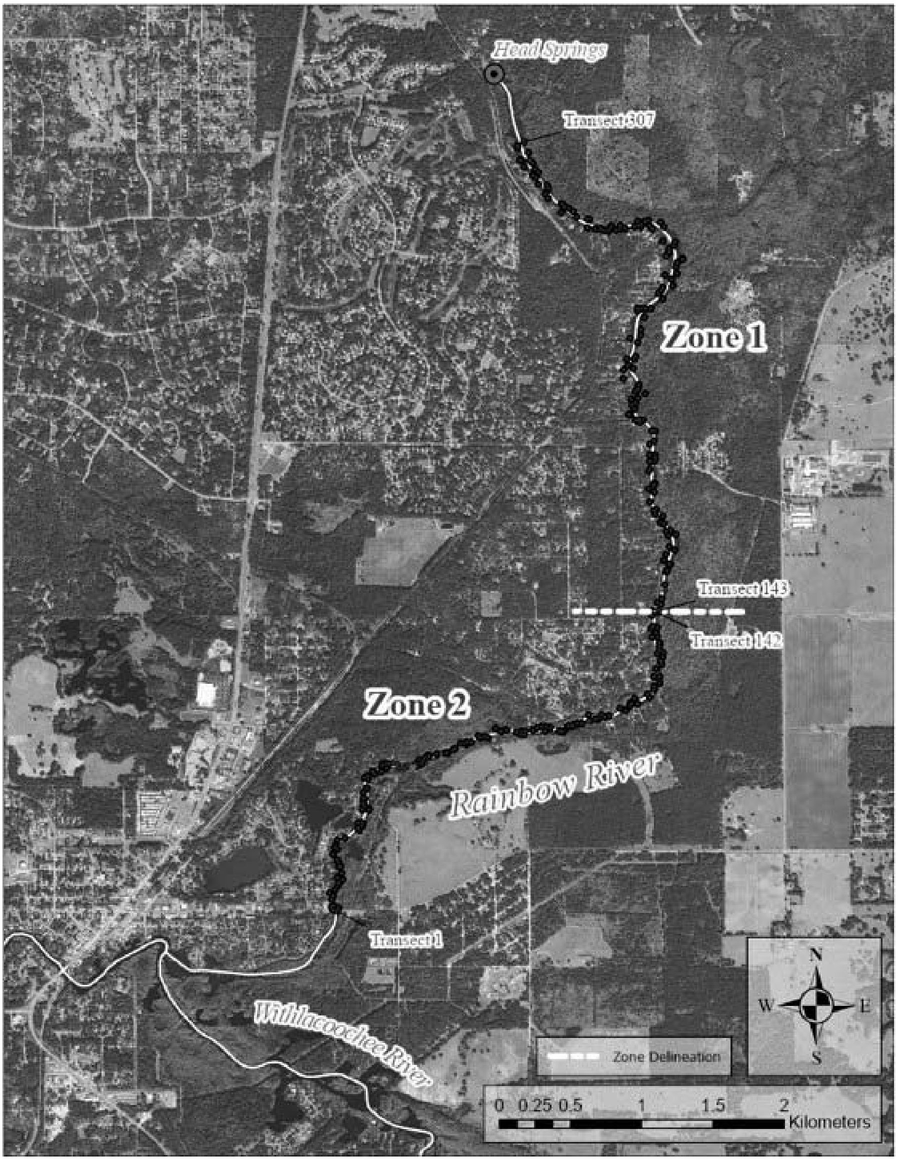

Figure 2. Location of the sampling transects in the Rainbow River System. Zone 1 (Upper River) = Transects 143-307 and Zone 2 (Lower River) = Transects 1-142.

Electrofisher output was standardized by transferring a target of 3,000 W of electrical power to the fish set to emit at 60 pulses per second, which was achieved by adjusting the voltage and amperage according to the temperature and specific conductivity of the water (Table 1) (Burkhardt and Gutreuter 1995, Miranda and Boxrucker 2009, Bonvechio 2017).

Electrofishing transects were conducted following the shoreline, moving at a speed of 2.4 to 4 km/h, while one dipper collected all fish with a 6-mm mesh net from the bow. Due to the efficiency threshold

Table 1. Details of Rainbow River System sampling events.

Sampling Event Dates	Number of Transects Sampled	Transect Temperature Range (°C)	Transect Specific Conductivity Range (μS/cm)
February 10-13, 2014	30	21.3-23.4	188-259
August 4-7, 2014	26	23.0-24.4	192-271
December 8-11, 2014	25	21.5-22.9	105-270
January 26-28, 2015	30	21.2-23.1	223-271
August 3-6, 2015	30	23.1-25.0	209-289
February 1-4, 2016	30	22.3-24.2	201-307
July 25-28, 2016	30	22.2-25.4	207-300
February 6-9, 2017	30	21.4-23.8	194-373
September 5-7, 2017	23	23.1-24.2	108-298
January 30-February 2, 2018	30	20.9-23.5	160-275
August 6-9, 2018	29	23.2-25.0	181-303
February 12-15, 2019	30	21.5-23.7	171-364

of electrofishing gear, areas exceeding 2 m in depth were not sampled. After fish collection was completed at each transect, fish held in the live well were identified to species level, measured (total length, nearest mm), individually weighed (wet weight, nearest gram) or batch weighed when applicable (e.g., large numbers of very small fish), and then released. Fish that could not be accurately identified in the field were placed on ice and brought back to the lab for identification.

The Rainbow River System was sampled seasonally during winter (December through February) and summer (July through September) from February 2014 through February 2019 (Table 1). Two sampling events occurred during the winter of 2014-2015.

Data exploration and statistical analyses were conducted using the R language for statistical computing, Version 3.5.2 (R Core Team 2018). Fish community composition was analyzed by calculating the Shannon Diversity Index (H'), species richness (R), Pielou's evenness (J), percent composition, and species accumulation curves. The normality of data subsets was tested by Shapiro-Wilks. Comparisons between seasons were performed by t-tests or Mann-Whitney tests depending upon normality of the data. Comparisons between sampling years were performed by either analysis of variance (ANOVA) or Kruskal-Wallis testing depending upon data normality. The Shannon Diversity Index was calculated using the equation: $H' = \sum(n/N)\mathrm{Ln}(n/N)$, where n is individual species abundance and N is total abundance. Species richness reflects the number of unique species found within the data subset. Evenness was calculated by the equation: $J = H'/\ln R$. Percent composition was calculated as the sum of individuals of each species divided by the sum of individuals from the respective data subset, multiplied by 100. Species accumulation curves were calculated using the Specaccum function and random method in the Vegan package in R (Oksanen et al. 2019). Species abundances were square root transformed and averaged by season and year prior to analyzing patterns of fish assemblages from Bray-Curtis similarity matrices. Analysis of similarity (ANOSIM) was used to determine if spatial patterns were distinguishable between seasons and zones, and non-metric multidimensional scaling (NMDS) was performed to visualize dispersion between groups. Similarity percentage analysis (SIMPER) showed which fish species contributed most to seasonal and zonal dissimilarity, and SIMPER outputs were then used to determine which species had the greatest influence on the observed differences. Statistical significance was determined at the level of $p < 0.05$.

Results

A total of 37 fish species, consisting of more than 34,000 individuals, was collected from the Rainbow River System during the 12 sampling events included in the study, with more than 13,000 Spotted Sunfish (*Lepomis punctatus*) making up 39% of the total individuals captured (Table 2). The next five most common species

Table 2. Total number of fish caught for each survey conducted in the Rainbow River System from February 2014 through February 2019 arranged in the order of the most individuals caught by species.

	Feb. 2014	Aug. 2014	Dec. 2014	Jan. 2015	Aug. 2015	Feb. 2016	Jul. 2016	Feb. 2017	Sept. 2017	Feb. 2018	Aug. 2018	Feb. 2019	Total Individuals Captured	Percent of Total Captures
Spotted Sunfish (*Lepomis punctatus*)	1071	1124	800	1062	1082	738	1133	1158	1384	1243	1358	1205	13358	39
Coastal Shiner (*Notropis petersoni*)	459	338	211	300	507	349	165	303	445	205	432	316	4030	12
Florida Bass (*Micropterus salmoides floridanus*)	344	258	243	236	345	190	250	342	235	312	372	355	3482	10
Eastern Mosquitofish (*Gambusia holbrooki*)	126	471	149	185	191	189	101	114	122	211	86	279	2224	6
Bluegill (*Lepomis macrochirus*)	215	183	94	98	249	210	257	163	206	107	83	71	1936	6
Redbreast Sunfish (*Lepomis auritus*)	232	154	115	141	92	78	172	93	199	154	136	163	1729	5
Seminole Killifish (*Fundulus seminolis*)	142	94	142	91	79	68	74	105	134	162	168	169	1428	4
Redear Sunfish (*Lepomis microlophus*)	80	106	86	95	80	45	150	109	231	176	129	89	1376	4
Inland Silverside (*Menidia beryllina*)		166	162	169	160	11	83	64	216	115	122	62	1330	4
Bluefin Killifish (*Lucania goodei*)	151	201	93	126	93	80	42	66	44	109	36	55	1096	3
Warmouth (*Lepomis gulosus*)	111	57	54	43	50	48	69	128	111	111	58	66	906	3
Redeye Chub (*Notropis harperi*)			15	52	29	44	19	30		65	30	22	306	1
Lake Chubsucker (*Erimyzon sucetta*)	4	3	5	9	23	24	17	18	12	19	57	46	237	0.7
Brook Silverside (*Labidesthes sicculus*)	63	2	1		8	2		6	1		11	55	149	0.4
Sailfin Molly (*Poecilia latipinna*)	12	18	6	1	24	10	3	5	22	3	4	1	109	0.3
Least Killifish (*Heterandria formosa*)	2	18	1	12	7	11	5		2	4	4	5	71	0.2
Dollar Sunfish (*Lepomis marginatus*)	7	12	1	2	5	1	10	10	2	2	7	6	65	0.2
Yellow Bullhead (*Ameiurus natalis*)	7	3	5	8	7	6	3	3	2	3	4	7	58	0.2
Bowfin (*Amia calva*)	2	3	1	1	3	6	4	10		6	1	2	39	0.1
Tadpole Madtom (*Noturus gyrinus*)	4	6	3	3	2	4	1	1	2	5	4	2	37	0.1
Golden Shiner (*Notemigonus crysoleucas*)	1			5	3			1	5	6	5	6	32	0.1
Metallic Shiner (*Pteronotropis metallicus*)				3	2	24							29	0.1
Taillight Shiner (*Notropis maculatus*)			1		1	1	7	1	5	3	1	5	25	0.1
Pygmy Sunfish (*Elassoma* spp.)	4	4	1								1		10	<0.1
Swamp Darter (*Etheostoma fusiforme*)		1			1	2			2	2	1		9	<0.1
Longnose Gar (*Lepisosteus osseus*)	1				1		1			2		4	9	<0.1
Pirate Perch (*Aphredoderus sayanus*)		1							6				7	<0.1

Table 2. Continued.

	Feb. 2014	Aug. 2014	Dec. 2014	Jan. 2015	Aug. 2015	Feb. 2016	Jul. 2016	Feb. 2017	Sept. 2017	Feb. 2018	Aug. 2018	Feb. 2019	Total Individuals Captured	Percent of Total Captures
Ironcolor Shiner (*Notropis chalybaeus*)								6					6	<0.1
White Catfish (*Ameiurus catus*)				2	1			1				1	5	<0.1
American Eel (*Anguilla rostrata*)					1			1		2			4	<0.1
Florida Gar (*Lepisosteus platyrhincus*)		1						1	1				3	<0.1
Atlantic Needlefish (*Strongylura marina*)				2									2	<0.1
Brown Bullhead (*Ameiurus nebulosus*)					1		1						2	<0.1
Gizzard Shad (*Dorosoma cepedianum*)		1					1						2	<0.1
Black Crappie (*Pomoxis nigromaculatus*)											2		2	<0.1
Rainwater Killifish (*Lucania parva*)									1				1	<0.1
Golden Topminnow (*Fundulus chrysotus*)									1				1	<0.1
Total Number of Fish Caught													34115	

included Coastal Shiner (*Notropis petersoni*), Florida Bass (*Micropterus salmoides floridanus*), Eastern Mosquitofish (*Gambusia holbrooki*), Bluegill (*Lepomis macrochirus*), and Redbreast Sunfish (*Lepomis auritus*), which together made up 39% of the total fish captured. Except for two Atlantic Needlefish *(Strongylura marina*) collected in January 2015, all fish collected from the Rainbow River System during the study were freshwater species. During the surveys, no introduced fish species were collected (Table 2).

The species accumulation curves suggested that sufficient sampling was performed in this study. According to the calculated curves, 152 transects were needed to document 100% of species in the system, and 220 transects were sampled.

With the exception of Flagfish (*Jordanella floridae*) and Bluespotted Sunfish (*Enneacanthus gloriosus*), all fish species included in the FLMNH's fish collection for the Rainbow River System were collected during this study. In addition, all species collected in past surveys were collected during this investigation except for Striped Mullet (*Mugil cephalus*) (FWC 1992, Walsh and Williams 2003, WSI 2010). This study also documented eight fish species which had not historically been documented in the Rainbow River System: American Eel (*Anguilla rostrata*), Golden Topminnow (*Fundulus chrysotus*), Ironcolor Shiner (*Notropis chalybaeus*), Metallic Shiner (*Pteronotropis metallicus*), Rainwater Killifish (*Lucania parva*), Swamp Darter (*Etheostoma fusiforme*), Tailight Shiner (*Notropis maculatus*), and White Catfish (*Ameiurus catus*).

Overall, there was no significant seasonal difference ($p = 0.225$) in species diversity of the Rainbow River System fish community (Table 3). Diversity was significantly higher ($p = 0.003$) in Zone 2 (Lower River) as compared to Zone 1 (Upper River). Species diversity overall was not significantly different between years ($p = 0.099$). There were no seasonal differences of species diversity within either Zone 1 ($p = 0.050$) or Zone 2 ($p = 0.929$) (Table 3).

Fish assemblages were better distinguished by zone (ANOSIM: $R = 0.550$, $p = 0.001$; NMDS: $p = 0.001$, Stress = 0.140, Homogenous Groups $p = 0.641$) than by season (ANOSIM: $R = 0.038$, $p = 0.269$; NMDS: $p = 0.211$, Stress = 0.108, Homogenous Groups $p = 0.708$). The zonal differences in fish assemblages are further demonstrated in the NMDS plot (Figure 3).

Differences in species composition between zones were largely driven by the abundances of ten species: Spotted Sunfish (10.7%), Coastal Shiner (8.6%), Inland Silverside (*Menidia beryllina*) (7.7%), Warmouth (*Lepomis gulosus*) (6.7%), Eastern Mosquitofish (6.6%), Redeye Chub (*Notropis harperi*) (6.5%), Bluegill (5.3%), Redbreast Sunfish (4.7%), Redear Sunfish (*Lepomis microlophus*) (4.6%), and Florida Bass (4.1%) (Figure 4). Species that were significantly more abundant in Zone 1 (Upper River) as compared to Zone 2 (Lower River) included Spotted Sunfish ($p < 0.001$), Redeye Chub ($p = 0.006$), and Florida Bass ($p = 0.020$), while significantly more abundant species in the Lower River compared to the Upper River included Coastal Shiner ($p < 0.001$), Warmouth ($p < 0.001$), Bluegill ($p = 0.030$), Redbreast Sunfish ($p < 0.001$), and Redear Sunfish ($p < 0.001$).

No significant seasonal differences were observed in the fish community within Zone 1 (Upper River) ($R = 0.003$, $p = 0.432$) or Zone 2 (Lower River) ($R = -0.005$,

Table 3. Indices describing the fish community of the Rainbow River System as a result of surveys conducted from February 2014 through February 2019.

	Number of Individuals Collected	Species Richness (R)	Average Shannon Diversity (± SE), (H')	Evenness (J)	Number of Transects Sampled
All (n = 12)	34115	37	2.087 ± 0.017	0.58	343
Summer (n = 5)	15342	34	2.058 ± 0.033	0.58	138
Winter (n = 7)	18773	32	2.107 ± 0.015	0.61	205
Zone 1/Upper River (n = 12)	17215	30	1.961 ± 0.026	0.58	176
Zone 2/Lower River (n = 12)	16900	35	2.121 ± 0.028	0.60	167
2014 (n = 3)	8452	28	2.126 ± 0.015	0.64	81
2015 (n = 2)	5693	29	2.116 ± 0.012	0.63	60
2016 (n = 2)	4709	26	2.084 ± 0.080	0.64	60
2017 (n = 2)	6129	29	2.042 ± 0.001	0.61	53
2018 (n = 2)	6140	28	2.051 ± 0.074	0.62	59
2019 (n = 1)	2992	24	2.075	0.65	30
Zone 1/Upper River, Summer (n = 5)	7088	27	1.899 ± 0.037	0.58	67
Zone 1/Upper River, Winter (n = 7)	10127	27	2.005 ± 0.027	0.61	109
Zone 2/Lower River, Summer (n = 5)	8254	32	2.118 ± 0.046	0.61	71
Zone 2/Lower River, Winter (n = 7)	8646	30	2.123 ± 0.038	0.62	109

$p = 0.493$). The percent composition of the ten species most contributing to the differences in fish communities between zones did not vary significantly by season (p values ranged from 0.145 to 0.844).

Fish assemblage composition did not differ significantly between years ($R = 0.068$, $p = 0.23$). The relative abundance of the ten species that most contributed to the dissimilarity between the upper and lower river system did not vary

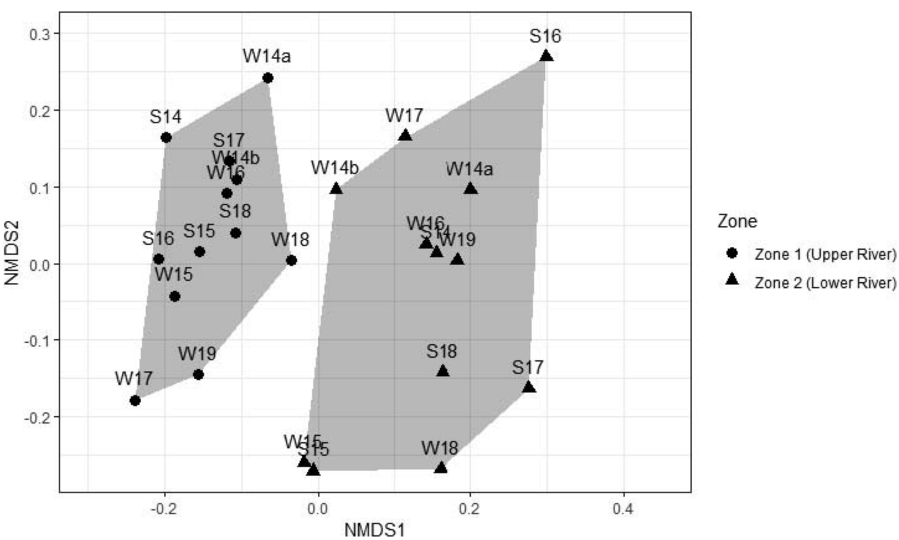

Figure 3. Non-metric multidimensional scaling (NMDS) plots for Rainbow River System fish abundance data by zone (S = Summer, W = Winter, numbers indicate year).

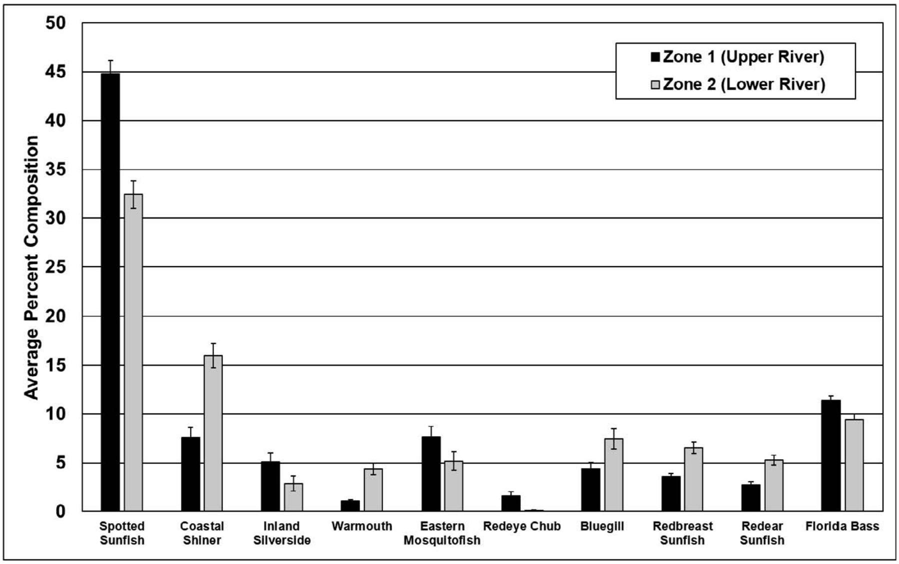

Figure 4. Average percent composition (± standard error) of the ten species most attributing to the differences in fish communities between zones captured in the Rainbow River System during the study by zone (Zone 1 = Upper River, Zone 2 = Lower River).

significantly between years (p values ranged from 0.143 to 0.961), with the exception of Bluegill, in which percent composition in 2016 (9.9 ± 0.10%) was significantly higher ($p = 0.0365$) than values for 2018 and 2019 (3.1 ± 0.43% and 2.37%, respectively).

Discussion

This study is the first long-term monitoring of the fish community of the Rainbow River System, which contributes to the improved understanding of this major freshwater resource and one of the crown jewels of Florida's springs systems. The Rainbow River System faces various complex threats, and this dataset serves as an important baseline to which future fish surveys can be compared to monitor changes in the fish community as a result of management actions, responses to environmental disturbances and human activities, and climate change, as well as the overall health of the Rainbow River System. The results of this study also demonstrated the effects of historical impacts to the Rainbow River System; for example, except for a few individuals, all fish collected were freshwater species due to the damming of the Withlacoochee River more than 100 years ago.

Our results demonstrated that Spotted Sunfish, an abundant species found in streams and rivers in the Southeast United States (Warren 1992), is an important component of the Rainbow River System fish community. This species made up almost 40% of the fish caught during this study, and typically had the highest percent composition by zone, season, or year. The Spotted Sunfish was also the most common fish species found in nearby Silver Springs (Odum 1957) and Gum Slough Spring Run (Nagid et al. 2014).

Because the Rainbow River System is dominated by groundwater rather than surface water, annual variations in water levels are usually less than 0.3 m, and water temperatures average around 23.3° C throughout the year (Holzwart et al. 2017); these factors most likely contributed to the lack of seasonal differences found in the fish assemblages. However, similar to what was found in a four-year study of Volusia Blue Spring Run (Work et al. 2010), fish density and species composition changed along the length of the spring run. Spotted Sunfish were more abundant in the Upper Rainbow River System, which has lush beds of SAV and complex physical habitat (Water and Air 2020), a finding that is consistent with an investigation conducted by Sears (2010). In Silver Springs, large numbers of Spotted Sunfish were found to reside in beds of Strap-Leaf Sagittaria (Odum 1957). In an investigation of Spotted Sunfish habitat selection in the Anclote, Little Manatee, and Manatee Rivers, this species generally selected habitats with greater structural complexity, including woody debris and aquatic plants, compared to the average available habitat (Dutterer and Allen 2008). In the Peace River, Spotted Sunfish were more abundant in the upper portion as compared to the lower portion and were associated with higher water velocity, greater macrophyte cover, and lower conductivity (Call et al. 2013). In Gum Slough Spring Run, adult Spotted Sunfish favored locations where the terrestrial vegetation hung over and into the stream, whereas juvenile Spotted Sunfish were most commonly associated with floating mats of vegetation (Nagid et al. 2014). McLane (1955) observed that Spotted Sunfish used fallen trees and dense vegetation along stream margins, possibly because they feed on invertebrates associated with submerged snags (Benke et al. 1985) and aquatic vegetation (VanderKooy et al. 2000).

Florida Bass also were more abundant in the Upper Rainbow River System than the Lower River likely due to the same reasons as described above for the Spotted Sunfish. However, four other centrarchids, including Bluegill, Warmouth, Redbreast Sunfish, and Redear Sunfish, were more abundant in the Lower River. In a study of *Lepomis* spp. of the Rainbow River System, Spotted Sunfish were dominant upstream, while Bluegill were most abundant downstream near the Withlacoochee River (Sears 2010); this led to the conclusion that Bluegill preferred the downstream habitat dominated by chironomids, whereas Spotted Sunfish preferred the upstream habitat dominated by amphipods.

Even though the Lower River has reduced habitat complexity and more invasive aquatic plant species, filamentous algae, and exposed sand and mud than the Upper River, the fish community diversity was higher, possibly due to the migration of fish from the Withlacoochee River. Work et al. (2010) found that larger species, such as centrarchids, were more abundant in the lower reach of Volusia Blue Spring Run, most likely due to the influence of the St. Johns River.

Redeye Chub is endemic to springs, spring runs, and groundwater-dominated stream reaches in parts of Alabama, Georgia, and North Florida (Meffe 1989, Walsh 2001, Boschung and Mayden 2004, Nagid et al. 2014). Therefore, it was not surprising that this small fish was one of the species that contributed most to the fish community differences between the Upper and Lower River and was more abundant in the Upper River than in the Lower River. The temperature preference of Redeye Chub is 21° C (Nagid et al. 2014), which is typical of freshwater springs, including the Rainbow Springs Group. Its limited distribution to spring systems make this species worthy of consideration as a focal species for future Rainbow River System investigations.

Since introduced, non-native fish species are now common in most flowing systems in Florida (Robins et al. 2018), it was encouraging that none were collected from the Rainbow River System during the study. Introduced Vermiculated Sailfin Catfish (*Pterygoplichthys disjunctivus*) were previously documented in the Rainbow River System in 2002; however, they were successfully eradicated by hand and fish spear from 2006 through 2008 (Hill and Sowards 2015).

The five-year dataset described in this study is the most comprehensive information available on the fish community of the Rainbow River System. It is recommended that future fish surveys use the same monitoring protocol to evaluate the status and trends in the fish community and the health of the Rainbow River System. Since the results of our study indicated no seasonal or yearly differences in the fish community, conducting a survey once a year every three to five years is adequate to characterize changes in the Rainbow River System fish assemblage. However, because differences in the Upper and Lower River fish communities were significant, continuing to sample each zone using the representative transects defined in the protocol is recommended. Rogers et al. (2005) identified a positive correlation between Spotted Sunfish abundance and overall fish richness in Florida streams and suggested that Spotted Sunfish could serve as an indicator of ecosystem health. Since our study demonstrated the dominance of Spotted Sunfish in the Rainbow River System, this species could serve as an indicator for ecosystem health in this system as well. The possible decline of Bluegill since 2016 should be investigated in future surveys. Continued fish surveys could also evaluate the success of restoration and nutrient reduction projects that are listed in the SWIM Plan (SWFWMD 2015b). In addition, these surveys could be used to develop one of the many criteria for the re-evaluation of minimum flows and levels for the Rainbow River System (Miller et al. 2015, Work et al. 2017).

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