Groundwater

Using $\delta^{18}O$ and $\delta^{2}H$ to Detect Hydraulic Connection Between a Sinkhole Lake and a First-Magnitude Spring

by Nur Ahmed[1], Ming Ye[1,2], Yang Wang[1], Tom Greenhalgh[3†], and Karlee Fowler[4]

Abstract

Oxygen and hydrogen isotopes were used in this study to detect a hydraulic connection between a sinkhole lake and a karst spring. In karst areas, surface water that flows to a lake can drain through sinkholes in the lakebed to the underlying aquifer, and then flows in karst conduits and through aquifer matrix. At the study site located in northwest Florida, USA, Lake Miccosukee immediately drains into two sinkholes. Results from a dye tracing experiment indicate that lake water discharges at Natural Bridge Spring, a first-magnitude spring 32 km downgradient from the lake. By collecting weekly water samples from the lake, the spring, and a groundwater well 10 m away from the lake during the dry period between October 2019 and January 2020, it was found that, when rainfall effects on isotopic signature in spring water are removed, increased isotope ratios of spring water can be explained by mixing of heavy-isotope-enriched lake water into groundwater, indicating hydraulic connection between the lake and the spring. Such a detection of hydraulic connection at the scale of tens of kilometers and for a first-magnitude spring has not been previously reported in the literature. Based on the isotope ratio data, it was estimated that, during the study period, about 8.5% the spring discharge was the lake water that drained into the lake sinkholes.

Introduction

Karst aquifer systems, supplying potable water to about 25% percent of the world's population, strongly interact with surface water systems and are vulnerable to contaminants from surface water (Ford and Williams 2007). A common way for contaminants to enter karst aquifers is when lake, river, or stream water drains into sinkholes (Tihansky 1999; Moore et al. 2009). Metz and Lewelling (2009) estimated that the Peace River in southwest Florida, USA, may lose up to 121,133 m^3 (32 million gallons) of water per day to sinkholes in the riverbed. Our study is focused on sinkhole lakes, where lake water drains through sinkholes in the lakebed and flows in karst conduits and through matrix of underlying aquifers. Sinkhole lakes are common in Florida, USA (Kindinger et al. 1999), and it has been found that, for some sinkhole lakes, lake water discharges at karst springs. Since the lake water is organic- and oxygen-rich and calcite unsaturated, it affects carbonate rock dissolution, groundwater geochemistry, spring water quality, and spring ecosystems (Katz et al. 1995, 1997; Katz 2001; Tihansky and Knochenmus 2002). Assessing such impacts is critical to informing water resource managers and protecting spring water quality (Florida Springs Task Force 2000).

The impact assessment requires detecting a hydraulic connection between sinkhole lakes (or rivers) and karst springs, and this is commonly done by using dye tracing experiments or geophysical techniques (e.g., Kincaid et al. 2005; Doctor et al. 2011; Meyerhoff et al. 2012; Burnham et al. 2015; McGlynn 2017, 2018). An alternative approach is to use oxygen and hydrogen isotopes because lake water is generally isotopically heavier than groundwater due to evaporation (Krabbenhoft et al. 1990). It has been demonstrated that the isotopes are highly effective for identifying a hydraulic connection between sinkhole lakes and karst springs (Criss

[1]Department of Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, FL 32306; na16g@my.fsu.edu; mye@fsu.edu; ywang@magnet.fsu.edu

[2]Corresponding author: Department of Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, FL 32306; mye@fsu.edu

[3]Florida Geological Survey, 3000 Commonwealth Blvd #1, Tallahassee, FL, 32303; thgreenhalgh@gmail.com

[4]Site Investigation Section, Florida Department of Environmental Protection, Tallahassee, FL, 32399; karlee.fowler@floridadep.gov

†retired

Article impact statement: $\delta^{18}O$ and $\delta^{2}H$ data identified hydraulic connection between a sinkhole lake and a first-magnitude karst spring tens of kilometers away from the lake.

Received December 2020, accepted April 2021.

doi: 10.1111/gwat.13105

et al. 2001; Torak et al. 2006; Day and Poeter 2009; Leng et al. 2010) and between sinking streams and karst springs (Greene 1997; Frisbee et al. 2019; Rusjan et al. 2019). While isotope approaches are less quantitative than dye tracing approaches (especially on the aspects of estimating hydrogeological parameters of solute transport), isotope approaches have an advantage that isotope ratios can be measured from water samples collected over a long period of time, for example, months and potentially years. The isotope ratios data can better reveal long-term (e.g., seasonal and annual) variations of groundwater and surface water characteristics, if the data can be appropriately interpreted.

Previous isotope studies have been conducted on sinkhole lakes and karst springs that are separated by a distance of 0.5 to 4 km, and there is no literature for first-magnitude springs that have average discharge rates larger than 2.83 m^3/s (100 ft^3/s) (Scott et al. 2004). This study aims to answer the following two questions: (1) Can oxygen and hydrogen isotopes be used to detect hydraulic connection between a sinkhole lake and a karst spring with a distance of tens of kilometers? (2) Can oxygen and hydrogen isotopes be used to detect hydraulic connection between a sinkhole lake and a first-magnitude spring? To answer the two questions, we collected water samples from Lake Miccosukee and Natural Bridge Spring, which are 32 km apart in the St. Marks River Watershed, Florida, USA. Natural Bridge Spring is a first-magnitude spring with an average discharge rate of 3.98 m^3/s measured over the period of September 2016 to May 2018 (M. Khadka, personal communication). Groundwater samples were also collected from a nearby well that penetrates the Upper Floridan aquifer, which supplies drinking water to more than 90% of the Florida population (Bush and Johnson 1988; Williams and Kuniansky 2015). Oxygen and hydrogen isotope compositions of the water samples were measured and used to detect a hydraulic connection between the lake and spring. The hydraulic connection was confirmed by a dye tracing experiment conducted at the lake sinkhole. Our hypothesis is that, if appropriate data are used, oxygen and hydrogen isotopes can be used as natural tracers for identifying the hydraulic connection between a sinkhole lake and first-magnitude spring tens of kilometers away from the lake.

Study Site

The study area is located in the northwest of Florida, USA (Figure 1a). This region has a humid subtropical climate and average temperature of 20.7°C for the period of January 1, 2016 to August 10, 2020 (https://leon.weatherstem.com/, accessed as of March 31, 2021). Based on rainfall data available from three weather stations, Lake Miccosukee Park (30.5944, −84.0386), Monticello (30.4405, −83.9858), and Natural Bridge Road (30.2936, −84.1678) shown in Figure 1a, the average annual rainfall was estimated to be 1373.2 mm for the period of 2017 to 2019. Physiographically, the study area is divided into the Northern Highlands and the Woodville Karst Plain by the Cody Scarp, an east-west escarpment that roughly approximates an ancient shoreline of Florida when sea level was significantly higher than present (White 1970; Upchurch et al. 2019). As shown in Figure 1b, north of the Cody Scarp, the highly permeable carbonate rock of the Oligocene Suwannee limestone and the Miocene St. Marks formations are overlain by the low-permeability Miocene Torrreya and Plio-Pleistocene Miccosukee formations, which consist of undifferentiated clays, silts, sands, gravels, and minor amount of carbonate sediments (Scott 1988). The Torreya and Miccosukee Formations do not exist south of the Cody Scarp. The Suwannee limestone and St. Marks formations are part of the Upper Floridan aquifer, where karst conduits and sinkholes have developed and formed to shape the geomorphologic landscape of the study area.

While sinking streams and small sinkhole lakes are common in the Woodville Karst Plain, large sinkhole lakes are common in the Northern Highlands (Kindinger et al. 1999). Lake Miccosukee (Figure 1a) is approximately 33 km northeast of Tallahassee, Florida, and is one such large sinkhole lake with an approximate length and width of 10 and 4 km, respectively. The Lake Miccosukee watershed is about 65,000 ha in area and lies in Leon and Jefferson counties, Florida, and Thomas County, Georgia. The lake is perched on the low-permeability sediments of the Miccosukee and Torreya formations (Figure 1b). It is shallow with a depth of less than 2 m, and swampy with the majority of the lake area covered by aquatic plants. A study of lake sediments by Donoghue et al. (1998) shows little evidence of anthropogenic input of trace metals to the lake. There are, however, concerns on lake water quality. Continuous monitoring of lake water quality since 2006 by Leon County indicates that dissolved oxygen saturation of the lake does not meet Class III (fish consumption; recreation, propagation, and maintenance of a healthy, well-balanced population of fish and wildlife) water quality criteria (http://cms.leoncountyfl.gov/Home/Departments/Public-Works/Engineering-Services/Stormwater-Management/Water-Quality-Data, accessed as of March 31, 2021). In addition, the lake is verified as impaired for fecal coliform, according to the Florida Department of Environmental Protection (https://floridadep.gov/dear/watershed-assessment-section/content/assessment-lists, accessed as of March 31, 2021).

The lake has a sinkhole in its northern end near Reeves Landing shown in Figure 1a. In 1950, the lake drained through the sinkhole and completely dried up (Tarver 1980). After this sinkhole event, an earthen dike was built around the sinkhole to prevent additional lake water drainage. The sinkhole still receives lake water due to a lake overflow drainage, natural lake seepage through the dike, and occasional opening of a water level control gate at the dike, and lake water drains into the Upper Floridan aquifer through the sinkhole. The groundwater and lake water interaction is evidenced by the isotope data discussed below. In 2010, two sinkholes at the southern end of the lake appeared (Figure 1a), and have periodically

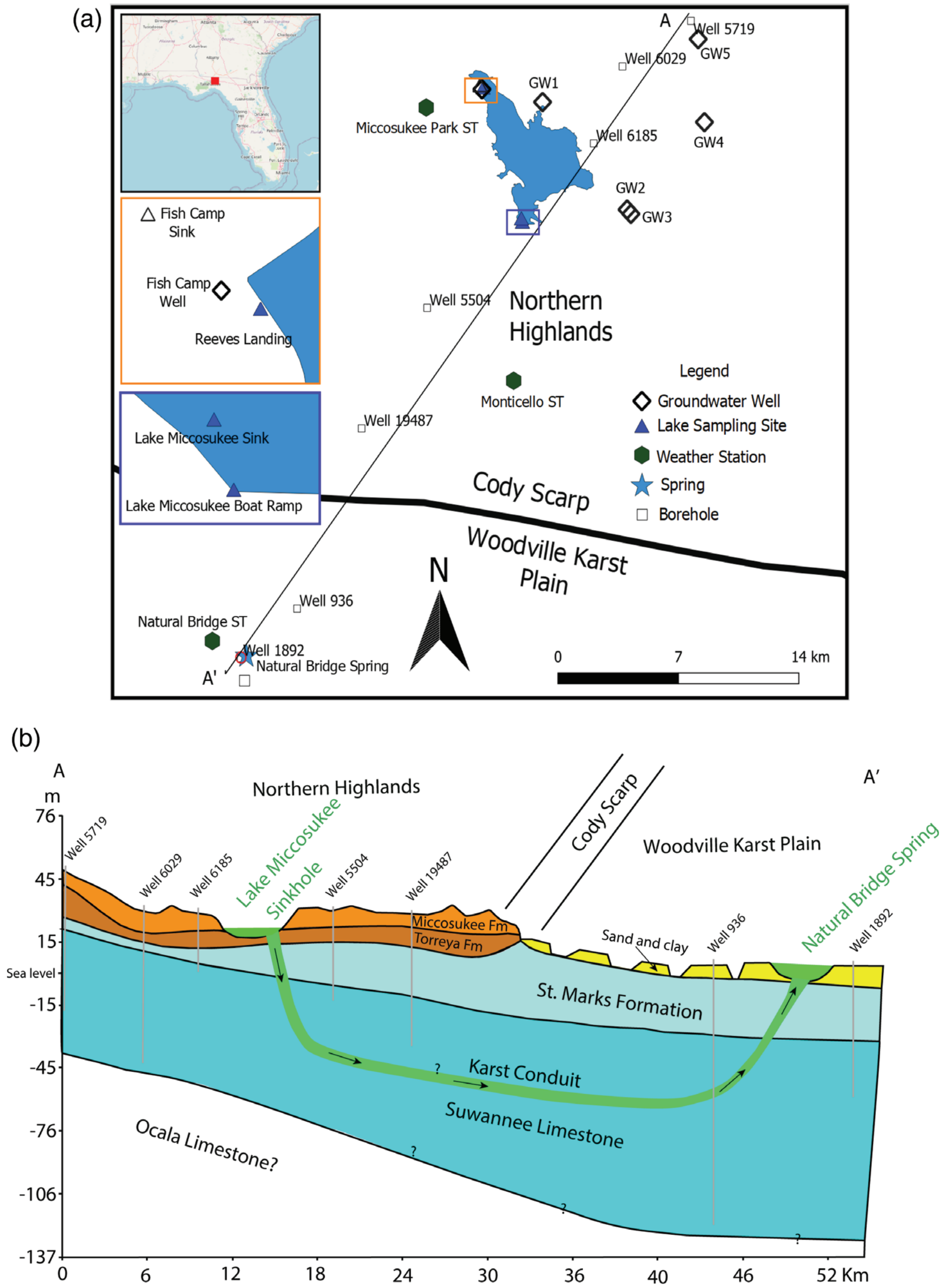

Figure 1. (a) Study area showing the locations of Lake Miccosukee, Natural Bridge Spring, groundwater and surface water sampling locations; (b) Generalized north-south lithostratigraphic cross-section through the study area. The tracer flow path from the lakebed sinkhole to the spring is also schematically represented bae on the 2018 dye tracing experiment. Locations of the boreholes used to generate (b) are shown in (a), and borehole data are from FDEP Well Database (https://geodata.dep .state.fl.us).

been exposed to air since then when lake stage is low in spring and fall, but covered by water when lake stage is high in summer. In January 2018, the sinkholes were exposed again, and lake water drained into them. The drainage has been continuous since January 2018.

The Natural Bridge Spring is located downgradient of Lake Miccosukee as indicated in the potentiometric surface map shown in Figure S1. The spring run flows westward approximately 200 m and into a siphon. It then flows underground a short distance and re-emerges in the St. Marks River at Natural Bridge. At Natural Bridge, the combined flow of Natural Bridge Spring and the St. Marks River flows through a series of siphons and rises for about 0.7 km, and finally emerges at the St. Marks River Rise. The river flows south to Apalachee Bay. The Florida Geological Survey measured the discharge of Natural Bridge Spring during the period of September 2016 to May 2018, and the average, minimum, and maximum discharge rates were 3.98 m^3/s, 3.54 m^3/s (in March 2018), and 4.51 m^3/s (in May 2018), respectively (M. Khadka, personal communication). The spring water is clear and blue-greenish in general, but becomes tannic after large rainfall events due to tannic surface water draining into sinkholes. While there is no water quality monitoring program for the spring, it was reported that the concentration of total NO_x ($NO_3 + NO_2$) as N increased from 0.27 mg/L in 2002 (Scott et al. 2004) to 1.30 mg/L in 2005 (Pratt 2006). Increasing nitrate concentrations adversely affects the spring ecosystem. The St. Marks River, where the spring ultimately discharges, is verified as impaired for fecal coliform, according to the Florida Department of Environmental Protection (https://floridadep.gov/dear/watershed-assessment-section/content/assessment-lists, accessed as of March 31, 2021).

Dye Tracing Experiment, Water Sampling, and Isotope Analysis

A dye tracing experiment was conducted in April 2018 at one of the two sinkholes when they were exposed. The experiment was conducted during dry hydrologic conditions to avoid a strong variability of flow rate at both Lake Miccosukee sinkhole and Natural Bridge Spring. On April 6, 2018, approximately 13.6 kg (30 lbs) of fluorescein dye was introduced into a sinkhole at the south end of Lake Miccosukee, while lake water was directly draining into the sinkhole at a flow rate of 0.35 m^3/s. Charcoal packets were deployed at a number of karst spring vents downgradient of Lake Miccosukee, and dye was detected at several sites including Natural Bridge Spring. The dye concentrations were analyzed using a Spectro-fluorophotometer (Model RF-5000 U) at Ozark Underground Laboratory.

Water samples were periodically collected from March 2018 to January 2020 at the following 10 locations: Natural Bridge Spring, lake water locations (Lake Miccosukee Boat Ramp, Reeves Landing, Lake Miccosukee Sink to which the dye tracer was introduced), and groundwater locations (Fish Camp Well and wells GW 1 to 5). The sampling locations are shown in Figure 1a, and their latitudes and longitudes are listed in Table S1. The groundwater samples are from the Upper Floridan aquifer that is comprised of the St. Marks formation and Suwannee limestone lithostratigraphic units in this region, and Wells GW 1 to 5 are located upgradient of the lake (Figures 1 and S1). During the period of October 1, 2019 to January 31, 2020, water samples were collected weekly from the lake, the spring, and Fish Camp Well. The stable isotope ratios of oxygen ($^{18}O/^{16}O$) and hydrogen ($^2H/^1H$) of the water samples were analyzed using a Finnigan MAT DELTAplus XP stable isotope ratio mass spectrometer (via a Finnigan GasBench II) at the National High Magnetic Field Laboratory of the Florida State University. The results are presented in the standard δ (delta) notation (Gonfiantini 1981) as $\delta^{18}O$ and δ^2H values in per mil (‰) relative to VSMOW (Vienna Standard Mean Ocean Water). The 1σ analytical precision based on replicate analyses of laboratory standards processed with each batch of samples is ±0.1‰ or better for $\delta^{18}O$ and ± 1‰ for δ^2H, indicating that the $\delta^{18}O$ data are more accurate than the δ^2H data.

Table S2 lists the $\delta^{18}O$ and δ^2H values of the lake, spring, and groundwater samples collected for this study. The isotope data are used to determine the relative proportional contributions of lake drainage to spring discharge. For a two-component mixture of lake water and groundwater, the mixing fraction (F_l) of lake water in spring water is determined by the following mass balance equations (Sklash et al. 1976; Klaus and McDonnell 2013)

$$F_l + F_{gw} = 1 \tag{1}$$

$$\delta^{18}O_l F_l + \delta^{18}O_{gw} F_{gw} = \delta^{18}O_{sp} \tag{2}$$

where F_{gw} is the mixing fraction of groundwater, and $\delta^{18}O_l$, $\delta^{18}O_{gw}$, and $\delta^{18}O_{sp}$ are the $\delta^{18}O$ of lake water, groundwater, and spring water, respectively.

Results

Figure 2a plots the breakthrough curve at the spring obtained from the dye tracing experiment. The figure presents a direct evidence that lake water drains into the sinkhole and then flows to the Natural Bridge Spring through karst conduits, as illustrated in Figure 1b. It however should be noted that the breakthrough curve is semi-quantitative, because a dye concentration measured from a charcoal packet only reflects an average concentration for the period between charcoal packet deployment and collection. The time (days) corresponding to the concentration was selected as the middle of the period (e.g., day 5 for a period of 10 days). The breakthrough curve suggests the following: (1) the introduced dye arrived at the spring after about 18 days, (2) the peak arrival time was about 34 days, and (3) the departure time was about 50 days. These time intervals were used below to determine paired data of isotopic ratios measured from the lake and spring water samples.

Figure 2b plots the δ^2H and $\delta^{18}O$ values discussed above as well as the global and local meteoric water lines and evaporation line. The local meteoric water line was adopted from Bugna et al. (2020) based on a linear regression analysis of δ^2H and $\delta^{18}O$ data of rain samples collected from 2014 to 2017 on the campus of the Florida Agricultural and Mechanical University in Tallahassee, FL. The evaporation line was established using isotope data from the water samples collected at the Lake Miccosukee Boat Ramp. Figure 2b shows that, due to evaporation, the isotope ratios of all the lake water samples (collected at Reeves Landing, Lake Miccosukee Boat Ramp, and Lake Miccosukee Sink) are significantly higher than those of groundwater and rain water (the weighted mean rainfall isotope ratios were adopted from Bugna et al. (2020)). Groundwater samples from wells upgradient of the lake were derived from precipitation recharge, and their isotope ratios are remarkably uniform (Table S2) with the $\delta^{18}O$ values of −4.1‰.

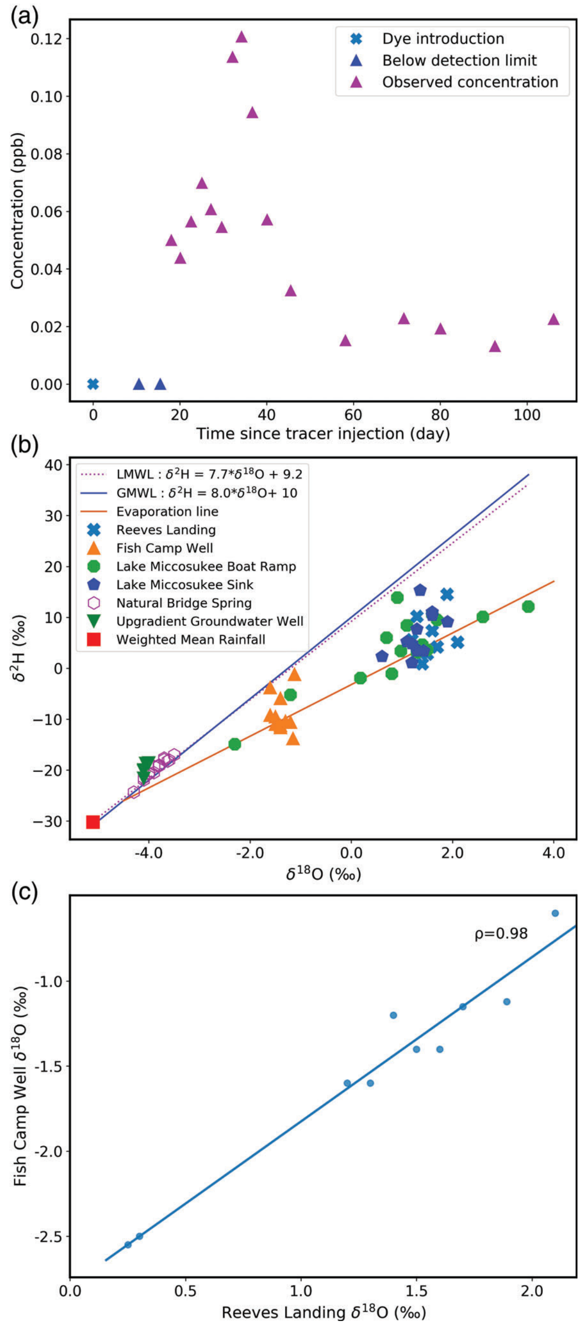

Figure 2. (a) Tracer breakthrough curve at Natural Bridge Spring with time zero being April 6, 2018, when dye was introduced at Lake Miccosukee sinkhole. (b) δ^2H and δ^{18}O isotopic compositions of groundwater, lake water, and spring water samples, relative to the global and local meteoric water lines and evaporation line. Upgradient groundwater wells are wells GW1-GW5 shown in Figure 1a. (c) Correlation of δ^{18}O isotope between lake water and groundwater samples collected at the Reeves Landing and the Fish Camp Well, respectively.

Discussion

Evidence of Lake Water and Groundwater Mixing at Fish Camp Well

The Fish Camp Well is a domestic well with its screen set in the St. Marks Formation. The δ^{18}O and δ^2H values at the well (Figure 2b) are significantly higher than those of wells GW 1 to 5 upgradient of the lake, but significantly lower than those of lake water collected at Reeves Landing. This suggests the mixing of lake water and groundwater, given that the Fish Camp Well is about 10 m away from the lake (Figure 1a). The mixing between lake water and groundwater does not have a delay, as shown by the strong correlation of the δ^{18}O values of the water samples collected in the same days at the Reeves Landing and Fish Camp Well (Figure 2c). The same mixing betwen lake water and groundwater is expected to occur at the sinkhole in the southern part of the lake where the dye tracing experiment was conducted.

Evidence of Lake Water and Groundwater Mixing at Natural Bridge Spring

The evidence of mixing between lake water and groundwater at the sinkhole may become obscured as lake water flows through karst conduits for at least 32 km to discharge at Natural Bridge Spring. The signature of heavy lake water isotopes may be diluted not only by regional groundwater that enters into the conduits between the sinkhole and the spring, but also by rain water entering the sampled sinkhole and other sinkholes before being discharged at the spring, considering that there are a number of sinkholes in the study area. To detect the lake water isotope signature requires: (1) assuming that the isotope ratios in regional groundwater are relatively uniform in space and time, which appears the case based on isotope data of wells GW1 to 5; and (2) removing rainfall effects by selecting appropriate pairs of lake water samples and spring water samples. The latter requirement is satisfied in this study, because during the sampling period between October 2019 and January 2020, drought conditions prevailed and only a small amount of rainfall was recorded in the study area (Figure S2). As a result, the lake sinkholes were exposed, and continuous lake water draining through the sinkholes was observed. It is thus possible to select a data pair from a time period when spring water collected at time t_0 was from the lake at time $t_0 - \Delta t$, where Δt is the travel time from the lake to the spring that was determined based on the dye tracing experiment. It is also required that zero or negligible amount of rainfall entered the aquifer during the period of Δt. For such data pairs, a strong correlation in δ^{18}O data between lake water and spring water is expected, similar to the correlation shown in Figure 2c. To determine the appropriate time interval Δt of data pairs, it is necessary to use the breakthrough curve of the dye tracing experiment, because of groundwater flow and hydrodynamic dispersion of solute (isotopes) transport. We assumed that the hydrodynamic conditions during the dye tracing experiment (April to June, 2018) were similar to those during the period (October 2019 to January 2020) of weekly water sampling. This assumption appears to be reasonable, since precipitation in the two periods is similar, as shown in Figure S2.

Figure 3a illustrates an example of selecting such a data pair for the spring water sample collected on

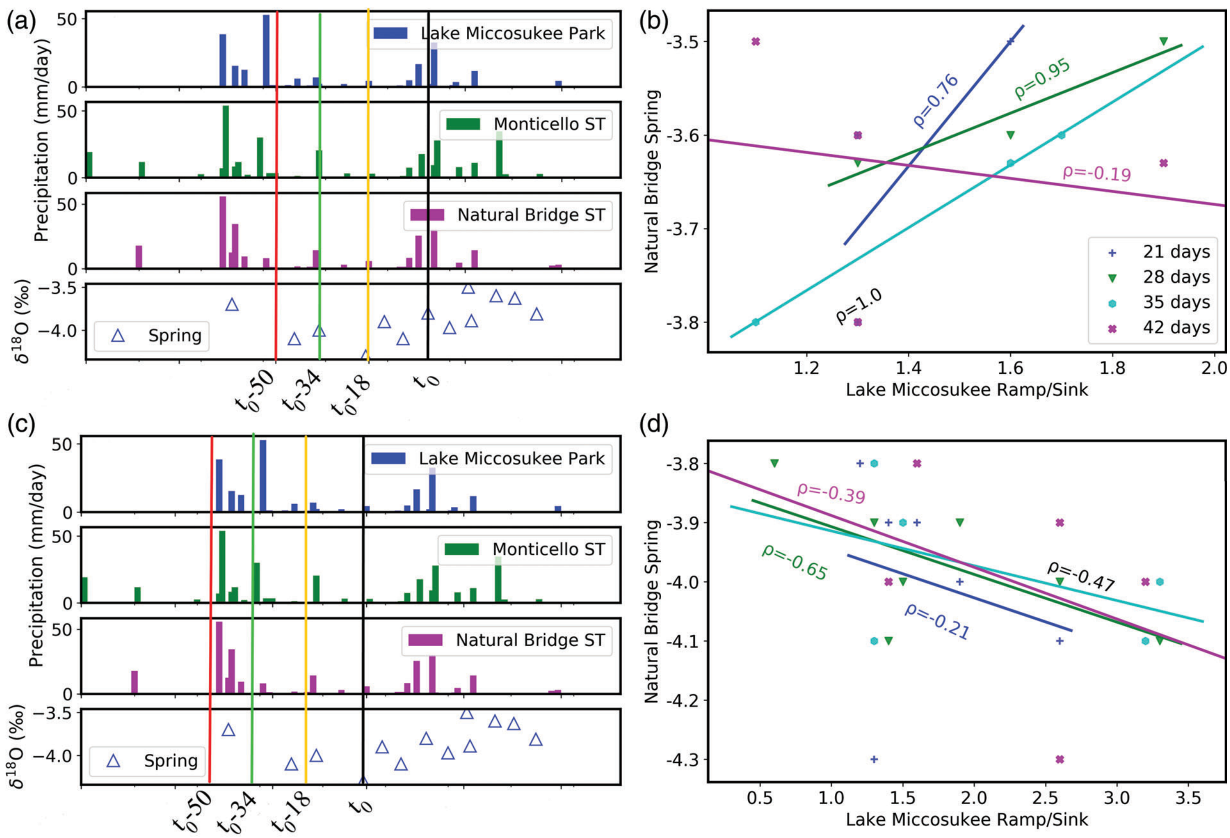

Figure 3. (a) Selecting δ^{18}O data pairs between spring water sample collected on December 20, 2019 (t_0) and lake water samples to remove rainfall effects on spring water δ^{18}O. The date of December 20, 2019 is denoted as time t_0, and 18, 34, and 50 days are estimated times of first arrival, peak arrival, and departure of dye tracer based on the breakthrough curve shown in Figure 2(a). (b) Correlation (quantified by the Pearson's correlation coefficient, ρ) between δ^{18}O data pairs between spring water and lake water with rainfall effects removed. Figures (c) and (d) were plotted for t_0 set as November 30, 2019 in the same manner of Figures (a) and (b), respectively, without rainfall impacts removed.

December 20, 2019, which is denoted as time t_0. The spring water sample has a δ^{18}O value of −3.8‰, heavier than the groundwater value of −4.1‰, indicating possible mixing of lake water and groundwater. Figure 3a plots the time series of the δ^{18}O value at the spring and rainfall at the three weather stations located between the lake and the spring (Figure 1a). Due to spatial variability of the rainfall, it is necessary to consider the rainfall at all the three weather stations. Since the dye trace breakthrough curve indicates that the early arrival time is 18 days (Figure 2a), the lake water and rainwater between t_0-18 days and t_0 do not contribute to the spring discharge at t_0. Therefore, the isotope ratios of spring water collected on December 12, 2019 should not be paired with those of lake water collected 7 and 14 days earlier. Considering that the dye tracer peak arrival time was 34 days and that there was a negligible amount of rainfall between t_0-34 days and t_0-18 days, it is anticipated that, during this period, rain water had negligible impacts on spring water isotopes and that spring discharge on December 12, 2019 consisted of only lake water and groundwater. Therefore, the isotopes in the spring water collected on December 20, 2019 can be paired with the isotopes in the lake water collected 21, 28, and 35 days earlier. Given that the dye tracer departure time was 50 days, the isotope ratios of spring water collected on December 20, 2019 should not be paired with the isotope ratios of lake water collected 50 days earlier. Between 34 and 50 days, the isotope data paring may be possible, depending on the magnitude of rainfall and hydrodynamic dispersion in karst conduits.

Table 1 lists the oxygen isotope data pairs selected in the procedure described above that considers rainfall data and dye tracer residence time in the karst conduits. For the lake isotope components, the measurements at the Lake Miccosukee Sink were used when they were available; otherwise, the measurements at the Lake Miccosukee Boat Ramp were used. Figure 3b plots the paired data with time lags of 21, 28, 35, and 42 days. The figure shows the correlation (quantified by Pearson correlation coefficient, ρ) of the paired data increases from 0.76 to 1.0 when the time lag increases from 21 to 35 days. The correlation is weak and negative for the data pairs with a time lag of 42 days. It is thus concluded that the evidence of lake water and groundwater mixing can be found in spring water if isotope data pairs of lake water and spring water are carefully selected to remove rainfall effects. It however should be noted that the

Table 1

δ^{18}O of Spring Water Samples (Collected at Date t_0) and δ^{18}O of Lake Water Samples (Collected at Date $t_0-\Delta t$, Δt Being Approximately 21, 28, 35, and 42 Days due to Weekly Sampling)

Sampling Date (t_0)	Spring δ^{18}O	Lake δ^{18}O t_0-21 days	Lake δ^{18}O t_0-28 days	Lake δ^{18}O t_0-35 days	Lake δ^{18}O t_0-42 days
December 20, 2019	−3.8	1.3 (November 30, 2019)		1.1 (November 15, 2019)	1.3 (November 7, 2019)
January 2, 2020	−3.5	1.6 (December 12, 2019)	1.9 (December 6, 2019)		1.1 (November 15, 2019)
January 11, 2020	−3.6	1.3 (December 20, 2019)	1.6 (December 12, 2019)	1.7 (December 6, 2019)	1.3 (November 30, 2019)
January 17, 2020	−3.6		1.3 (December 20, 2019)	1.6 (December 12, 2019)	1.9 (December 6, 2019)

Note: The data pairs were selected to remove effects of rainfall on spring discharge.

Table 2

δ^{18}O of Spring Water Samples (Collected at Date t_o) and δ^{18}O of Lake Water Samples (Collected at Date $t_0-\Delta t$, Δt being Approximately 21, 28, 35, and 42 Days due to Weekly Sampling)

Sampling Date (t_0)	Spring δ^{18}O	Lake δ^{18}O t_0-21 days	Lake δ^{18}O t_0-28 days	Lake δ^{18}O t_0-35 days	Lake δ^{18}O t_0-42 days
November 7, 2019	−4.1	2.6 (October 18, 2019)	3.3 (October 11, 2019)	3.2 (October 4, 2019)	
November 15, 2019	−4.0		2.6 (October 18, 2019)	3.3 (October 11, 2019)	3.2 (October 4, 2019)
November 30, 2019	−4.3	1.3 (November 7, 2019)			2.6 (October 18, 2019)
December 6, 2019	−3.9	1.4 (November 15, 2019)	1.3 (November 7, 2019)		2.6 (October 18, 2019)
December 12, 2019	−4.1		1.4 (November 15, 2019)	1.3 (November 7, 2019)	
December 27, 2019	−4.0	1.9 (December 6, 2019)	1.5 (November 30, 2019)		1.4 (November 15, 2019)
January 3, 2020	−3.9	1.6 (December 12, 2019)	1.9 (December 6, 2019)	1.5 (November 30, 2019)	
January 24, 2020	−3.8	1.2 (January 3, 2020)	0.6 (December 27, 2019)	1.3 (December 20, 2019)	1.6 (December 12, 2019)

Note: The data pairs were selected without removing effects of rainfall on spring discharge.

correlation is based on a small number of paired data, and a longer period of water sampling is warranted in a future study.

It also happened that the rainfall effects cannot be removed for certain water samples, and this is illustrated in Figure 3c for the spring water sample collected on November 30, 2019, denoted as t_0 in the figure. The spring water sample has a δ^{18}O isotope value of −4.3‰, which is lighter than the δ^{18}O of −4.1‰ in upgradient groundwater samples (Figure 3c). During the period of t_0-10 days and t_0-34 days, a 53 mm rainfall event occurred on October 29, 2020 at the lake, and the rain water may have drained into the lake sink and other sinkholes. As a result, the isotope ratios of lake water during the period cannot be paired with the isotopes of spring water collected on November 30, 2019. Table 2 lists the oxygen isotope data pairs with the time interval of approximately 21, 28, 35, and 42 days due to weekly sampling. These data pairs were selected without removing effects of rainfall on spring discharge. Figure 3d shows the correlation of the data pairs are negative and weak, indicating that, when lake water oxygen isotope ratios increase, the spring oxygen isotope ratios decrease due to the rainfall effects.

Mixing Fraction Estimation

Based on the data listed in Table 1, the lake water/groundwater mixing fraction was estimated using the two-component isotope mass balance model given in Equations 1 and 2. Using −4.1‰ for groundwater $\delta^{18}O_{gw}$ and the average values of spring water $\delta^{18}O_{sp}$ and lake water $\delta^{18}O_l$ listed in Table 3, the δ^{18}O-based mixing fraction, $F_l(\delta^{18}O)$, of lake water was calculated as 8.5%, 9.2%, and 7.5% for the data pairs of 21, 28, and 35 days, respectively. These values are consistent with the range of 7.8% to 9.9%, calculated using the flow rate of 0.35 m^3/s for lake water flowing into the sinkhole measured on April 6, 2018 and the minimum (3.54 m^3/s) and maximum (4.51 m^3/s) spring discharge rate given in the section of Study Site. When all the values listed in Table S2 were used, for the average $\delta^{18}O_l$ value of 1.3‰ and average $\delta^{18}O_{gw}$ value of −4.1‰, the highest $\delta^{18}O_{sp}$ value (−3.5‰) yielded a mixing fraction of 11.1%, and the average $\delta^{18}O_{sp}$ value (−3.9‰) gave a fraction of 4.4% due to rain water effects.

Similar mixing fraction estimates were also obtained by using hydrogen isotopes, and Table 3 lists the δ^2H-based mixing fractions, $F_l(\delta^2H)$, which were calculated in the way of calculating $F_l(\delta^{18}O)$. The $F_l(\delta^2H)$ values are slightly smaller than the $F_l(\delta^{18}O)$ values. The difference is not surprising, because lab measurements of hydrogen isotope ratios are less accurate than those of oxygen isotope ratios. If the isotope data are measured at multiple lakes and springs, and the data are used together with a numerical model of isotope transport in a context of inverse modeling to delineate the subsurface conduit network (Perrin et al. 2003; Husic et al. 2020), it is feasible to characterize spatial distributions of karst conduits at a large scale. This is warranted in a future study.

Table 3
Average $\delta^{18}O$ and δ^2H Values of the Paired Spring Water Samples and Lake Water Samples Collected over the Time Intervals Approximately 21, 28, and 35 Days

Time interval (daya)	Average $\delta^{18}O_{sp}$ (‰)	Average $\delta^{18}O_l$ (‰)	F_l ($\delta^{18}O$) (%)	Average δ^2H_{sp} (‰)	Average δ^2H_l (‰)	F_l (δ^2H) (%)
21	−3.6	1.4	8.5	−18	8	7.1
28	−3.6	1.6	9.2	−18	8	7.1
35	−3.6	1.5	7.6	−18	9	6.8

Notes: The subscriptions *sp* and *l* are for spring water and lake water, respectively. The values were used to calculate the mixing fraction, F_l, of lake water in spring discharge, using the $\delta^{18}O$ values of −4.1 ‰ (and δ^2H value of −20‰) for regional groundwater.

Conclusions

We explored in this study the feasibility of using oxygen and hydrogen isotopes to detect the hydraulic connection between a sinkhole lake (Lake Miccosukee) and a first-magnitude karst spring (Natural Bridge Spring) located 32 km downgradient from the lake. The detection was facilitated by the dye tracing experiment conducted in April 2018 that provided physical evidence that lake water draining into the lake sinkhole discharges at the karst spring. Weekly samples were collected from the lake, spring, and a groundwater well 10 m away from the lake during the dry period between October 2019 and January 2020. The isotope ratios of lake water are substantially higher than those of groundwater due to evaporation. Mixing of lake water and groundwater significantly changes groundwater isotopes, evidenced by the increased isotope ratios of the groundwater samples. When rainfall effects on spring discharge are removed using dye tracer travel times estimated from the breakthrough curve, increased heavy isotope contents of spring water can be explained by the mixing of heavy-isotope-enriched lake water with groundwater, indicating hydraulic connection between the lake and the spring. This leads to a conclusion that it is promising to use oxygen and hydrogen isotopes for detecting hydraulic connection between a sinkhole lake and a first-magnitude karst spring at the scale of several tens of kilometers. Based on $\delta^{18}O$ isotope ratios of collected water samples, the calculated fraction of lake water in spring water varies between 7.5% and 9.2%, consistent with the range of 7.8% to 9.9% calculated using flow rate measurements. This suggests that the isotope ratios can be used for a quantitative analysis of groundwater and lake water mixing due to lake sinkholes in karst areas.

Acknowledgments

This research was supported by National Science Foundation grant EAR-1828827. We thank Yuch-Ping Hsieh for providing rainfall isotope data and are grateful to Jonathan Arthur, Harley Means, and Clint Kromhout for reviewing the paper and providing valuable inputs. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the Florida Geological Survey or the Florida Department of Environmental Protection. Stable isotope analyses were performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida. All the data used in this study are listed in the Supporting Information file associated with the paper.

Authors' Note

The authors do not have any conflicts of interest or financial disclosures to report.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article. Supporting Information is generally *not* peer reviewed.

Table S1. Locations of 10 sampling locations of lake water, groundwater, and spring water. Groundwater samples are from the Upper Floridan Aquifer.
Table S2. $\delta^{18}O$ and δ^2H values (‰) in water samples collected during this study.
Figure S1. Potentiometric surface of Upper Floridan Aquifer in the study area for April 2019 (http://aquarius-web.nwfwmd.state.fl.us/Data). The groundwater flow direction (indicated by the arrows in the figure) in the study area is from northeast to southwest across, that is, from Lake Miccosukee to Natural Bridge Spring. The potentiometric surface was generated by inverse distance weighting (IDW) using QGIS software.
Figure S2. Rainfall intensity at the Lake Miccosukee Park weather station and $\delta^{18}O$ data at five sampling locations for the period from March 2018 to January 2020.

References

Bugna, G.C., J.M. Grace, and Y. Hsieh. 2020. Sensitivity of using stable water isotopic tracers to study the hydrology of isolated wetlands in North Florida. *Journal of Hydrology* 580: 12432.

Burnham, T., I. Luffman, M. Whitelaw, and Y. Gao. 2015. Assessing structural control on groundwater flow in the Morrell Cave springshed, Sullivan County, Tennessee.

Geological Society of America Special Papers 516: SPE516–SPE513.

Bush, P.W., and R. Johnson. 1988. Ground-water hydraulics, regional flow, and ground-water development of the Floridan aquifer system in Florida and in parts of Georgia, South Carolina. *U. S. Geological Survey Professional Paper* 1403: 80.

Criss, R.E., S.A. Fernandes, and W.E. Winston. 2001. Isotopic, geochemical, and biological tracing of the source of an impacted karst spring, Weldon Spring, Missouri. *Environmental Forensics* 2: 99–103.

Day, K.W., and E. Poeter. 2009. Investigating hydraulic connections and the origin of water in a mine tunnel using stable isotopes and hydrographs. *Applied Geochemistry* 24: 2266–2282.

Doctor, D.H., N.C. Farrar, and J.S. Herman. 2011. Karst mapping, dye tracing, and geographic information systems. In *USGS Karst Interest Group Proceedings*, April 26–29, Fayetteville, Arkansas. U.S Geological Survey Scientific Investigation Report 2011-5031: 84–104.

Donoghue, J.F., P.C. Ragland, Z.Q. Chen, and C.A. Trimble. 1998. Standardization of metal concentrations in sediments using regression residuals: An example from a large lake in Florida, USA. *Environmental Geology* 36: 65–76.

Florida Springs Task Force. 2000. *Florida's Springs: Strategies for Protection and Restoration*. Tallahassee, Florida: Florida Department of Environmental Protection.

Ford, D.C., and P.W. Williams. 2007. *Karst Geomorphology and Hydrology*. West Sussex, England: John Wiley & Sons, Ltd.

Frisbee, M.D., Z.P. Meyers, J.B. Miller, C.L. Gleason, N.S. Stewart-Maddox, E.B. Larson, D.E. Granger, S. Saksena, S. Dey, and E.E. Frisbee. 2019. Processes leading to the reactivation of a sinkhole in buried karst and the subsequent drying of waterfalls in a small catchment located in northern Indiana, USA. *Journal of Cave and Karst Studies* 81: 69–83. https://doi.org/10.4311/2017ES0116

Gonfiantini, R. 1981. The δ-notation and the mass-spectrometric measurement techniques. In *Stable Isotope Hydrology: Deuterium and Oxygen-18 in the Water Cycle*, ed. J.R. Gat, and R. Gonfiantini, 35–84. Vienna, Austria: International Atomic Energy Agency.

Greene, E.A. 1997. Tracing recharge from sinking streams over spatial dimensions of kilometers in a karst aquifer. *Groundwater* 35, no. 5: 898–904.

Husic, A., J. Fox, E. Adams, E. Pollock, W. Ford, C. Agouridis, and J. Backus. 2020. Quantification of nitrate fate in a karst conduit using stable isotopes and numerical modeling. *Water Research* 170: 115348.

Katz, B.G. 2001. A multitracer approach for assessing the susceptibility of ground-water contamination in the Woodville Karst Plain, Northern Florida. In *Proceedings of U.S. Geological Survey Karst Interest Group*, Water Resources Investigations Report 01-4011. ed. E.L. Kuniansky. 167–176.

Katz, B.G., T.B. Coplen, T.D. Bullen, and J.H. Davis. 1997. Use of chemical and isotopic tracers and geochemical modeling to characterize the interactions between ground water and surface water in mantled karst. *Groundwater* 35, no. 6: 1014–1028.

Katz, B.G., E. Busenberg, K.M. Revesz, and B.F. Jones. 1995. Chemical evolution of groundwater near a sinkhole lake, northern Florida 2. Chemical patterns, mass transfer modeling, and rates of mass transfer reactions. *Water Resources Research* 31, no. 6: 1565–1584.

Kincaid, T.R., T.J. Hazlett, and G.J. Davies. 2005. Quantitative groundwater tracing and effective numerical modeling in karst: An example from the Woodville Karst Plain of North Florida. In *Sinkholes and the Engineering and Environmental Impacts of Karst*, ed. B.F. Beck, 114–121. Reston, Virginia: American Society of Civil Engineers.

Kindinger, J.L., J.B. Davis, and J.G. Flocks. 1999. Geology and evolution of lakes in northcentral Florida. *Environmental Geology* 38, no. 4: 301–321.

Klaus, J., and J.J. McDonnell. 2013. Hydrograph separation using stable isotopes: Review and evaluation. *Journal of Hydrology* 505: 47–64.

Krabbenhoft, D.P., C.J. Bowser, M.P. Anderson, and J.W. Valley. 1990. Estimating groundwater exchange with lakes 1. The stable isotope mass balance method. *Water Resource Research* 26, no. 10: 2445–2453.

Leng, M.J., I. Baneschi, G. Zanchetta, C.N. Jex, B. Wagner, and H. Vogel. 2010. Late quaternary palaeoenvironmental reconstructionfrom lakes Ohrid and Prespa (Macedonia/Albania border) using stable isotopes. *Biogeosciences* 7: 3109–3122.

McGlynn, S. 2018. Live and let dye: Researchers plumb depths of sinkhole to help heal Wakulla Springs. https://www.tallahassee.com/story/news/2018/04/10/live-and-let-dye-researchers-plumb-depths-sinkhole-help-heal-wakulla-springs/502743002/ (accessed August 16, 2020).

McGlynn, S. 2017. Dye release looks to track water from Lake Jackson to Wakulla Springs. https://www.tallahassee.com/story/news/2017/09/22/dye-release-looks-track-water-lake-jackson-wakulla-springs/694321001/ (accessed August 16, 2020).

Metz, P.A., and B.R. Lewelling. 2009. Hydrologic Conditions that Influence Streamflow Losses in a Karst Region of the Upper Peace River, Polk County, Florida. U.S. Geological Survey Scientific Investigation Report 2009-5140.

Meyerhoff, S.B., M. Karaoulis, F. Fiebig, R.M. Maxwell, A. Revil, J.B. Martin, and W.D. Graham. 2012. Visualization of conduit-matrix conductivity differences in a karst aquifer using time-lapse electrical resistivity. *Geophysical Research Letter* 39, no. 24: L24401. https://doi.org/10.1029/2012GL053933

Moore, P.J., J.B. Martin, and E.J. Screaton. 2009. Geochemical and statistical evidence of recharge, mixing, and controls on spring discharge in an eogenetic karst aquifer. *Journal of Hydrology* 376, no. 3–4: 443–455.

Perrin, J., P.-Y. Jeannin, and F. Zwahlen. 2003. Epikarst storage in a karst aquifer: A conceptual model based on isotopic data, Milandre test site, Switzerland. *Journal of Hydrology* 279, no. 1–4: 106–124.

Pratt, T.R. 2006. Groundwater chemical characterization of the St. Marks River Rise, Florida. North West Florida Water Managemnt District Special Report 06-01.

Rusjan, S., K. Sapač, M. Petrič, S. Lojenc, and N. Bezaka. 2019. Identifying the hydrological behavior of a complex karst system using stable isotopes. *Journal of Hydrology* 577: 123956.

Scott, T.M., G.H. Means, R.P. Meegan, R.C. Means, S.B. Upchurch, R.E. Copeland, J. Jones, T. Roberts, and A. Willet. 2004. Springs of Florida. Florida Geological Survey Bulletin No. 66. Tallahassee, Florida: Florida Geological Survey.

Scott, T.M. 1988. The lithostratigraphy of the Hawthorn Group (Miocene) of Florida. Florida Geological Survey Bulletin 59, Tallahassee, Florida: Florida Geological Survey.

Sklash, M.G., R.N. Farvolden, and P. Fritz. 1976. A conceptual model of watershed response to rainfall, developed through the use of oxygen-18 as a natural tracer. *Candian Journal of Earth Science* 13, no. 2: 271–283.

Tarver, D.P. 1980. Water level fluctuation and the aquatic flora of Lake Miccosukee. *Journal of Aquatic Plant Management* 18: 19–23.

Tihansky, A.B., and L.A. Knochenmus. 2002. Karst features and hydrology in west-Central Florida: A field perspective. In *United States Geological Survey Karst Interest Group Proceedings, Water-Resources Investigation Report 01–4011*, ed. E.L. Kuniansky, 198–211. Reston, Virginia, USA: U.S. Geological Survey.

Tihansky, A.B. 1999. Sinkholes, West-Central Florida: A link between surface water and ground water. In *Land Subsidence in the United States*, ed. D. Galloway, D.R. Jones, and S.E. Ingebritsen, 121–140. Tampa, Florida: U.S. Geological Survey Circular 1182.

Torak, L.J., D.M. Crilley, and J.A. Painter. 2006. Physical and hydrochemical evidence of lake leakage near Jim Woodruff Lock and Dam and ground-water inflow to Lake Seminole and an assessment of karst features in and near the lake, southwestern Georgia, and northwestern Florida. U.S. Geological Survey Scientific Investigations Report 2005–5084.

Upchurch, S., T.M. Scott, M.C. Alfieri, B. Fraesi, and T.L. Dobecki. 2019. *The Karst Systems of Florida: Understanding Karst in a Geologically Young Terrain*. Cham, Switzerland: Springer International Publishing.

White, W.A. 1970. The geomorphology of the Florida peninsula. Florida Bureau of Geology, Geological Bulletin No. 51. Tallahassee, Florida: Florida Geological Survey.

Williams, L.J., and E.L. Kuniansky. 2015. Revised hydrogeologic framework of the Floridan aquifer system in Florida and parts of Georgia, Alabama, and South Carolina. U.S. Geological Survey Professional Paper 1807.