

Dietary Niche of Three Omnivorous Turtle Species in a Northern Florida River: Insights from Stable Isotope Analysis

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Abstract - *Macrochelys suwanniensis* (Suwannee Alligator Snapping Turtle) coexists with 2 other native large omnivorous turtle species (*Chelydra serpentina* [Snapping Turtle] and *Trachemys scripta scripta* [Yellow-bellied Slider]) in a 9-km section of the Santa Fe River in northern Florida. A major shift in dominant submersed aquatic vegetation prompted us to quantify trophic position and niche overlap among these 3 species. Here, we examine carbon and nitrogen isotopic values of these turtles and their potential food resources within the changing riverine system. We provide evidence of low isotopic niche overlap between *M. suwanniensis* and *C. serpentina*, whereas *T. s. scripta* occupied a discrete niche having lower carbon and nitrogen values.

Introduction

Macrochelys suwanniensis Thomas, Granatosky, Bourque, Krysko, Moler, Gamble, Suarez, Leone, Enge, and Roman (Suwannee Alligator Snapping Turtle) is endemic to the Suwannee River drainage in northern Florida and southern Georgia, and is currently a candidate for listing as threatened under the Endangered Species Act (Enge et al. 2021, Thomas and Enge 2019, Thomas et al. 2014). Most studies of *M. suwanniensis* have focused on its distribution and population ecology (Enge et al. 2021; Johnston et al. 2015b; Moler 1996; Thomas et al. 2022, 2023 [this issue]). Comparatively little is known about its ecological interactions with other freshwater turtle species that occupy common habitat. For example, 2 other large native omnivorous species, *Chelydra serpentina* (L.) (Snapping Turtle) and *Trachemys scripta scripta* (Thunberg in Schoepff) (Yellow-bellied Slider) are also known to inhabit the Suwannee River and its tributaries (Ernst and Lovich 2009; Johnston et al. 2015b, 2016), but dietary niche overlap among the 3 species has not been studied. The only study of dietary niche overlap among the 3 species was focused on their common consumption of *Diospyros virginiana* L. (Common Persimmon) fruit (Johnston et al. 2015a). These 3 species coexist in a 9-km section of the lower Santa Fe River at higher abundances than elsewhere within the river system (Fig. 1; Johnston et al. 2012, 2015b, 2016).

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Johnston et al. (2016) hypothesized that artesian springs in the lower Santa Fe River create a unique riverine environment with a variety of substrates and an abundance of submerged aquatic macrophytes that facilitate the coexistence of these 3 species. The macrophyte community was historically dominated by native aquatic plants that presumably formed the foundation of the food web (Johnston et al. 2016, 2018). However, declines in the quality and quantity of spring water contributed to a massive die-off of these plants in 2012 and 2013, followed by a proliferation of macroalgae from 2014 to the present day (G.R. Johnston, pers. observ.; Johnston et al. 2018). Similar shifts in aquatic vegetation in other spring-fed Florida rivers have affected the diversity of small-bodied fishes and macroinvertebrates (Camp et al. 2014). These findings suggest that the vegetation shift in the Santa Fe River could affect organisms throughout the food web and profoundly impact the trophic dynamics and structure of the turtle assemblage. Competitive interactions among species may occur when trophic niches overlap, and resources are limited or decrease (Pianka 1974, 1981). Therefore, assessment of the potential food-web-mediated impacts to the *M. suwanniensis* population is warranted as is an evaluation of its trophic interactions with *C. serpentina* and *T. s. scripta*.

Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are intrinsic biomarkers used to trace foraging habits and movements of wildlife populations. The simultaneous analysis of carbon and nitrogen isotope tracers (i.e., isotopic niche) can describe the trophic position of organisms over space and time (Layman et al. 2007, Newsome et al. 2007). Changes in size or position of a consumer's isotopic niche within δ -space are reflective of changes or differences in both a consumer's dietary and ecological niche (Jackson et al. 2011). Carbon isotopes in animal tissues reflect the primary producers in the area of feeding, whereas nitrogen isotopes can be used to determine the trophic level of a species (Fry 2006) and to detect dietary shifts (Hobson and Wassenaar 2008). Animal tissues reflect the isotopic composition of their diets in a predictable manner, with consumers that feed on the same prey having similar $\delta^{13}\text{C}$ values with only a slight enrichment of $<1\%$ (DeNiro and Epstein 1978). Nitrogen undergoes $\sim 2\text{--}4\%$ enrichment with each trophic level and can vary among species and tissue types (Hobson and Wassenaar 2008). For example, nitrogen discrimination values from *T. s. scripta* tissues varied between claw tissue ($+2.3\%$), blood plasma ($+3.8\%$), and red blood cells ($+1.9\%$) (Aresco et al. 2015, Seminoff et al. 2007). Therefore, to accurately interpret stable isotope values in ecological studies, it is important to use the appropriate discrimination factors, especially when using different types of tissue interchangeably in an analysis or if the aim is to compare trophic position results amongst previous studies.

Here we calculated the trophic position and examined the isotopic niche relationships among these 3 species within the Santa Fe River. We analyzed stable carbon and nitrogen isotopes obtained from turtle claws (*M. suwanniensis*, *C. serpentina*, and *T. s. scripta*), vegetation, and potential prey within this river. Our objectives for this study were to (1) establish isotopic baselines in a changing riverine system and (2) quantify isotopic niche overlap among the 3 coexisting, large, omnivorous turtles in the Santa Fe River. We hypothesized that adult *M. suwanniensis*, *C. serpentina*, and *T. s. scripta* would each occupy a discrete niche due to their different

foraging strategies and body sizes. High niche overlap between *M. suwanniensis* and either of the other species would suggest the potential for interspecific competition and the need for a more thorough examination of resource availability and limitation. Information collected during this study can help elucidate the current trophic interactions among these omnivorous turtle species within this riverine system, providing a baseline for future studies.

Field-Site Description

The Santa Fe River is the largest tributary of the Suwannee River in Florida (Fig. 1). It originates as a blackwater stream but receives substantial input from artesian springs in its lower reaches (Hornsby and Ceryak 1998, Johnston et al. 2016). We sampled turtles within the 9-km reach of the Santa Fe River immediately downstream from Poe Spring. Johnston et al. (2012, 2016) referred to this sampling area as the “high-density spring area” and “spring-influenced river” because it receives direct input from ≥ 21 artesian springs. Relative to the upstream reaches of the Santa Fe River, this 9-km reach has higher water clarity and supports a greater abundance of submersed aquatic vegetation. The majority of the Santa Fe River *C. serpentina* population occurs in this reach, perhaps due to a greater availability of soft sand/organic substrate along the edge of the river and in spring runs (Johnston et al. 2012). *Trachemys s. scripta* abundance in this reach is ~ 3 times greater than in blackwater reaches farther upstream (Johnston et al. 2016). *Macrochelys suwanniensis* abundance is generally low and evenly distributed throughout the Santa Fe River (Enge et al. 2022), but most large adult males of >600 mm straight-midline

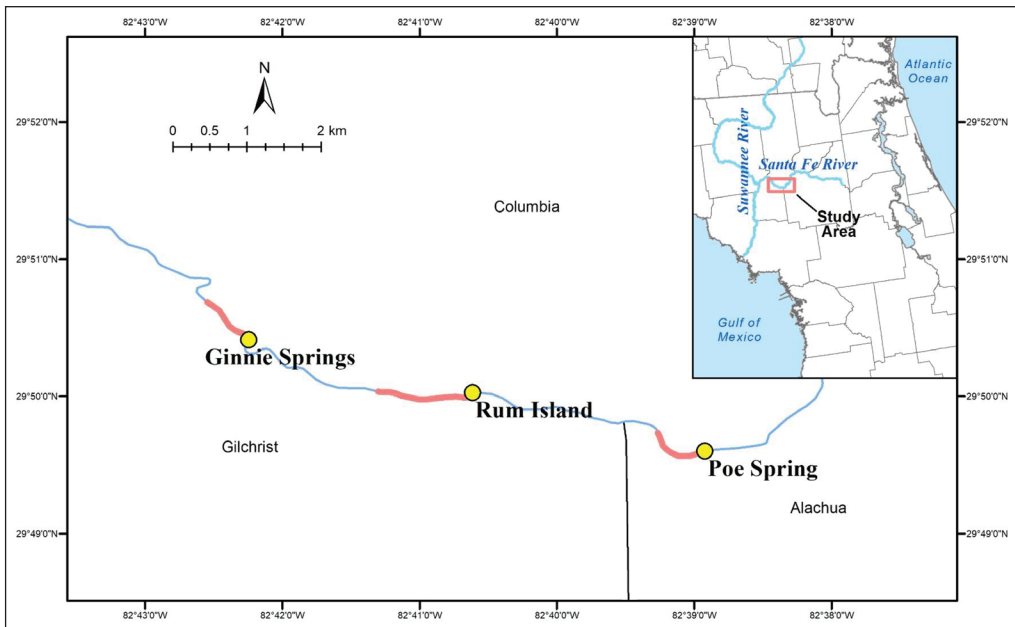


Figure 1. Map of the lower Santa Fe River with sampling sites shaded red. Major springs that flow into each site are represented by circles. Insert shows location within Florida.

carapace length (CL) occur in the “spring-influenced river” (Johnston et al. 2015b).

Johnston et al. (2012, 2016, 2018) described a plant-dominated macrophyte assemblage (primarily *Hydrilla verticillata* L. [Hydrilla], *Hygrophila polysperma* Roxburgh [Indian Swampweed], *Sagittaria kurziana* Glück [Strap-leaf Sagittaria], and *Vallisneria americana* Michaux [Tapegrass]) prior to 2013, followed by a die-off of plants in 2013–2014 and subsequent shift to primarily macroalgae. Population densities (individuals/ha) of *C. serpentina*, *M. suwanniensis*, and *T. s. scripta* were approximately 3.2, 0.5, and 11.8, respectively, prior to the plant die-off (G.R. Johnston, unpubl. data; Johnston et al. 2012, 2016). Ongoing studies are evaluating long-term variation in population dynamics of these species (G.R. Johnston, unpubl. data). Our study was conducted during 2019–2020, and we collected our samples from 3 separate sections of the river (0.73 km downstream from Poe Spring [29.8265°N, 82.6488°W], 1.14 km downstream from Rum Island [29.8337°N, 82.6770°W], and a 0.74 km reach bordering Ginnie Springs Park downstream from Sawdust Spring [29.8402°N, 82.7041°W]; Fig. 1). The water discharging from the primary springs that feed each of these river sections varies in nitrate levels (Johnston et al. 2016, Suwannee River Water Management District 2022). Gilchrist Blue Springs, which flows into the Rum Island sampling area had the highest nitrate values (1.6–2.3 mg/L). Nitrate levels were intermediate in Ginnie Springs (1.0–1.8 mg/L) and lowest in Poe Spring (0.1–1.3 mg/L).

Methods

Sample collection and stable isotope analysis

We captured turtles by hand while snorkeling and by using hoop-net traps baited with canned sardines (Johnston et al. 2012, 2015b, 2016). We measured, weighed, and marked each turtle following Johnston et al. (2012, 2015b, 2016). We categorized each turtle as immature, adult female, or adult male following Johnston et al. (2012, 2015b, 2016). During 2019–2020, we sampled claws from 39 individual turtles (5 immature and 3 adult male *M. suwanniensis*, 4 adult female and 4 adult male *C. serpentina*, 7 adult female and 14 adult male *T. s. scripta*). Straight-midline carapace lengths (mm) of *M. suwanniensis*, *C. serpentina*, and *T. s. scripta* were 34–620, 276–422, and 144–248, respectively. We also collected a small (≤ 5 mm) tip from a claw on the hindfoot of each turtle to be used for isotope analysis. We released all turtles at the capture site on the same day of capture.

Turnover rates can vary among tissues and have not been determined for all species we examined; thus, it is important to consider the type of tissue analyzed when interpreting isotopic niches (Newsome et al. 2007, Seminoff et al. 2007). Moreover, ectotherms have some of the slowest turnover rates, so more time is needed for shifts in carbon sources to be detected in the turtle’s tissues (Vander Zanden et al. 2015). Seminoff et al. (2007) estimated that nitrogen turnover time in whole blood from *T. s. scripta* was ~ 5 –7 months and carbon turnover time was >146 days. Aresco et al. (2015) determined claw tissue can reflect dietary changes of freshwater turtles after 6 months for $\delta^{15}\text{N}$ and greater than 6 months for $\delta^{13}\text{C}$. Therefore, even if carbon turnover rates were greater than 1 year, our samples were collected 5 years

after the aquatic plant die-off was documented, and thus turnover should have occurred. Another possibility could be that the turtles we sampled were transient, so their isotopic values may not be representative of the sampling locations. However, movements of these turtle species within the Santa Fe River are typically <5 km (G. Johnston, unpubl. data; Johnston et al. 2012), and several of the individuals we sampled have been captured within the same reach of river multiple times as part of previous research, further suggesting residency (Johnston et al. 2012, 2015b, 2016). Thus, we chose to sample claws to investigate long-term dietary trends. Future studies may benefit from analyzing blood-plasma fractions in addition to claw, due to the more rapid turnover in plasma (Rosenblatt and Heithaus 2013, Seminoff et al. 2007) providing both shorter- and longer-term dietary information.

Riverine ecosystems are complex with biological and biochemical processes (i.e., respiration and flow velocity) acting to regulate a range of ecosystem characteristics, from water clarity to productivity, which can lead to strong spatial/temporal variability in basal $\delta^{13}\text{C}$ values (Finlay and Kendall 2008, Mariash et al. 2018, Singer et al. 2005). Variations in plant $\delta^{15}\text{N}$ values can occur at relatively small scales; thus, it is important to clearly define and apply nitrogen baseline corrections at scales that account for spatial variation in plant $\delta^{15}\text{N}$ values (Woodcock et al. 2012). Reliable baselines are required for correct interpretation of isotopic values, such as for assessing relatively small-scale movements between a main river and a tributary (Durbec et al. 2010) or for determining trophic position (Post 2002). Freshwater-system food webs may be based on macrophytes, macroalgae, particulate organic matter (POM), phytoplankton, detritus, or any combination of these sources. To investigate whether macrophytes or macroalgae were the more important basal resource in the turtles' food web, we sampled vegetation including macroalgae (green filamentous algae, diatoms, red algae, and *Vaucheria* sp.) and aquatic plants (*Ludwigia repens* J.R. Forst. [Red Ludwigia], *Hydrocotyle* sp. [marsh pennywort], *Eleocharis* sp. (spikerush), and Indian Swampweed), along with potential prey items such as *Procambarus* spp. [crayfish], fish (*Lepomis* sp. [sunfish], *Percina* sp. [roughbelly darter], *Notropis* sp. [eastern shiner], and *Gambusia* sp. [topminnow]), and snails (*Viviparus* sp. [mysterysnail] and *Elimia* sp. [elimia]) from sites proximal to turtle-capture locations to establish baseline values within this section of the Santa Fe River. We utilized opportunistic collections of resources sampled from close proximity of turtle captures from Poe Spring (October 2019: fish, snails, crayfish, plants, macroalgae), and Rum Island (June, October 2018: fish, snails; October 2019: macroalgae). We were unable to sample Ginnie Springs in 2019 and all sites in 2020 owing to COVID-19 pandemic restrictions. After collection, we stored samples in plastic bags or 50-ml centrifuge tubes on ice until freezing. In the laboratory, we processed samples following Révész et al. (2012). We thawed and rinsed vegetation samples with distilled water and used a dissecting microscope to remove all non-vegetation material. We analyzed macroalgae mats as whole clumps, and again after separating into individual taxa when possible. Vegetation samples were then rinsed again and placed in a sterile vial to dry. In addition, we rinsed all prey specimens with distilled water and dissected

muscle tissue. Finally, we rinsed muscle tissue samples again with distilled water and placed them in individual vials to dry. We dried all samples at 60 °C for up to 48 hours before grinding them into a powder.

Isotope analysis

We weighed homogenized samples (claw tissue/prey: 0.5–1.0 mg; vegetation: 2.3–2.5 mg) into 5 mm x 9 mm pressed tin capsules, then sealed them. We then sent all samples to the University of California Davis Stable Isotope Facility to be analyzed for ^{13}C and ^{15}N isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Results are presented as “delta” (δ) values in parts per thousand (denoted as ‰) enrichments or depletions relative to international standards for carbon (Vienna Pee Dee Belemnite [VPDB]) and nitrogen (referenced to atmospheric N_2), where $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = ([R_{\text{sample}} / R_{\text{standard}} - 1]) \times 1000$, with $R = (^{13}\text{C} / ^{12}\text{C})$ or $(^{15}\text{N} / ^{14}\text{N})$ (Peterson and Fry 1987). The SD of internal reference materials were ± 0.04 for $\delta^{13}\text{C}$ and ± 0.07 for $\delta^{15}\text{N}$. Duplicates of every 10th sample were analyzed to ensure homogenization.

There was no correlation between C:N and ^{13}C among turtle claws ($P = 0.3848$, $t = 0.8801$, $\text{df} = 35$), or prey taxa ($P = 0.7721$, $t = -0.2914$, $\text{df} = 47$), and mean C:N ratios for all animal tissue in this study was 3.6 ± 0.3 SD. In aquatic animals with low C:N ratios (< 3.5), lipid removal has little effect on $\delta^{13}\text{C}$ signatures (Post et al. 2007). Therefore, since there was no correlation between C:N and ^{13}C and C:N ratio was low, we did not chemically remove or account for lipids mathematically (Sweeting et al. 2006, Logan et al. 2008, Post et al. 2007).

Calculation of baseline $\delta^{15}\text{N}$ values and trophic position. We estimated trophic position of turtles by correcting for the variation in $\delta^{15}\text{N}$ values of the identifiable primary producers (trophic level 1; Vander Zanden and Rasmussen 1999). Resources from Poe Spring were found to be higher in $\delta^{15}\text{N}$ values than from Rum Island, and we were unable to collect vegetation samples from Ginnie Springs. Therefore, we calculated baseline $\delta^{15}\text{N}$ values using the primary producers (aquatic plants and macroalgae) and a primary consumer (snails) from 2 of the 3 sites and calculated 1 baseline to be used throughout. We estimated the trophic position of turtles relative to the baseline $\delta^{15}\text{N}$ value with the equation $\text{TP} = ([\delta^{15}\text{N} - \delta^{15}\text{N}_{\text{baseline}}] / 2.3) + 1$, where TP is trophic position and 2.3‰ is the mean enrichment of $\delta^{15}\text{N}$ between trophic levels as determined by Aresco et al. (2015). Mean nitrogen enrichment was not known for all remaining fish species or crayfish, and this study was focused on interactions among turtles, thus we did not calculate trophic position for prey taxa.

Statistical analyses

We fit all models using the ‘lm()’ function in the base package of program R (v.4.1.2; R Core Team 2021). We tested all data for normality and homogeneity of variance using probability plots and multivariate normality tests where applicable. We conducted separate ANOVAs to test for differences in carbon and nitrogen values between turtle species and capture locations and assessed significance with

Tukey's honestly significant difference (HSD) test. We considered a probability level of $P < 0.05$ to be statistically significant for all tests. We used the R package 'ggplot' version 3.3.5 to create bi-plots of turtle and resources. We then calculated trophic position for each individual turtle and used the average for each species in the analyses. We employed ANOVA and Bonferroni pairwise comparisons to determine differences in trophic position among the 3 species of turtle.

Isotopic diversity indices were calculated for turtle species using the R package 'SIBER: Stable Isotope Bayesian Ellipses in R' (Jackson et al. 2011). We calculated the standard ellipse area (SEA) using the variance and covariance of bivariate isotope data to contain ~40% of the data to represent a core isotopic niche for each population. We then corrected the resulting SEA values (SEA_C) to minimize bias due to small sample size by using an $(n - 2)$ correction on the denominator (Jackson et al. 2011). We used the SEA_C to calculate the degree of isotopic niche overlap, representing a quantitative measure of dietary similarity between populations. We then used a Bayesian approach (SEA_B) as described in Jackson et al. (2011) to obtain highest density intervals for isotopic niche areas, allowing for statistical comparisons of the sizes of isotope niches among populations.

Results

Turtle claw samples

The $\delta^{13}C$ isotope values for turtle claws varied between -35.4‰ to -25.2‰, with $\delta^{15}N$ values varying from 6.5‰ to 13.0‰ depending on species (Table 1; Denton et al. 2022). Adult *M. suwanniensis* fed within a relatively narrow breadth of carbon sources (-27.8‰ to -26.7‰) as did adult *C. serpentina* (-30.6‰ to -27.9‰), whereas juvenile *M. suwanniensis* (-31.7‰ to -25.2‰) and adult *T. s. scripta* (-35.4‰ to -27.3‰) fed across relatively wider extent of carbon sources. Amongst all turtles a positive correlation was detected between CL and $\delta^{13}C$ values ($P = 0.0086$) and $\delta^{15}N$ values ($P < 0.0001$); however, within each species, there was no correlation between CL and $\delta^{13}C$ (*M. suwanniensis*: $P = 0.88$, *C. serpentina*: $P = 0.058$, *T. s. scripta*: $P = 0.61$) nor $\delta^{15}N$ (*M. suwanniensis*: $P = 0.58$, *C. serpentina*: $P = 0.79$, *T. s. scripta*: $P = 0.079$).

Trophic position and niche relationships among turtles

Pairwise comparisons indicated average trophic position of *M. suwanniensis* (TP = 5.4) was significantly greater than *C. serpentina* (TP = 4.6, $P = 0.0081$) and *T. s. scripta* was significantly below the TP of both snapping turtle species (TP = 3.0, $P < 0.0001$). Adult *M. suwanniensis* had a trophic position of 5.8, and juveniles had a position of 5.2, with no relationship between age class and trophic position ($r^2 = 0.3001$, $P = 0.0924$). Trophic position did not differ between sexes for *C. serpentina* (female = 4.6, male = 4.5, $P = 0.91$) nor *T. s. scripta* (female = 3.0, male = 3.1, $P = 0.68$).

Results of ANOVA revealed significant differences in the $\delta^{13}C$ values among the 3 turtle species ($F_{2,32} = 8.2$; $P < 0.0014$) but not among capture sites ($F_{3,32} = 1.8$; $P > 0.1778$). Tukey tests showed *T. s. scripta* had lower carbon values than both *M. suwanniensis* ($P < 0.0011$) and *C. serpentina* ($P < 0.0104$), whereas there was

Table 1. Comparison of mean \pm SD $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among turtle species and by sex/age class and among sampling sites. The min–max values of straight-midline carapace lengths (mm) are given in parentheses.

Species/sex	Carapace length (min–max)	n	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
			Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
<i>Macrochelys suwanniensis</i>								
Male	583–620	8	-28.3 \pm 2.0	-31.7	-25.2	11.7 \pm 1.2	9.5	12.9
Juvenile	34–307	3	-27.5 \pm 0.6	-27.8	-26.7	12.0 \pm 0.4	11.6	12.4
		5	-28.9 \pm 2.4	-31.7	-25.2	11.5 \pm 1.6	9.5	13.0
<i>Chelydra serpentina</i>								
Female	276–330	8	-28.9 \pm 0.9	-30.6	-27.9	10.1 \pm 1.50	8.4	12.1
Male	312–422	4	-29.2 \pm 1.0	-30.6	-28.3	10.3 \pm 1.9	8.4	12.1
		4	-28.6 \pm 0.7	-29.4	-27.9	9.9 \pm 1.3	8.8	11.0
<i>Trachemys scripta</i>								
Female	189–248	21	-31.3 \pm 2.1	-35.4	-27.3	7.5 \pm 0.92	6.5	9.6
Male	144–220	7	-31.6 \pm 1.7	-34.4	-29.3	7.5 \pm 0.6	6.5	8.4
		14	-31.2 \pm 2.3	-35.4	-27.3	7.5 \pm 1.1	6.5	9.6
Species	Location							
<i>Macrochelys suwanniensis</i>	Ginnie Springs	4	-28.2 \pm 2.8	-31.7	-25.2	11.2 \pm 1.7	9.5	13.0
<i>Macrochelys suwanniensis</i>	Poe Spring	3	-28.5 \pm 1.6	-29.9	-26.7	12.2 \pm 0.5	11.6	12.5
<i>Macrochelys suwanniensis</i>	Rum Island	1	-27.8	-27.8	-27.8	11.9	11.9	11.9
<i>Chelydra serpentina</i>	Ginnie Springs	2	-29.2 \pm 0.3	-29.4	-28.9	8.9 \pm 0.0	8.8	8.9
<i>Chelydra serpentina</i>	Poe Spring	3	-29.0 \pm 1.4	-30.6	-27.9	11.7 \pm 0.6	11.0	12.1
<i>Chelydra serpentina</i>	Rum Island	3	-28.6 \pm 0.7	-29.4	-28.2	9.4 \pm 1.4	8.4	11.0
<i>Trachemys scripta</i>	Ginnie Springs	6	-29.6 \pm 2.5	-34.3	-27.3	6.9 \pm 0.4	6.5	7.4
<i>Trachemys scripta</i>	Poe Spring	5	-31.6 \pm 2.5	-35.4	-28.8	8.9 \pm 0.7	8.1	9.6
<i>Trachemys scripta</i>	Rum Island	10	-32.1 \pm 0.7	-32.9	-30.9	7.1 \pm 0.4	6.5	7.7

no difference between *M. suwanniensis* or *C. serpentina* ($P > 0.7584$). In addition, ANOVA of $\delta^{15}\text{N}$ values revealed significant differences among species ($F_{2,32} = 75.7$; $P < 0.0001$) and sites ($F_{3,31} = 18.6$; $P < 0.0001$). Tukey tests showed differences in $\delta^{15}\text{N}$ values among all species (*M. suwanniensis* $>$ *C. serpentina*, $P < 0.0044$; *M. suwanniensis* $>$ *T. s. scripta*, $P < 0.0001$; *C. serpentina* $>$ *T. s. scripta*, $P < 0.0001$). Turtles sampled from Poe Spring had significantly higher $\delta^{15}\text{N}$ values than those from Ginnie Springs ($P < 0.0001$) or Rum Island ($P < 0.0001$), with no difference between those from Rum Island and Ginnie Springs ($P > 0.0567$).

Results from SIBER analysis indicate there was little overlap in isotopic niches between *M. suwanniensis* and *C. serpentina* ($\sim 10\%$), with no overlap between *T. s. scripta* and either *M. suwanniensis* or *C. serpentina* (Fig. 2a). Core isotopic niche position and orientation for *M. suwanniensis* and *C. serpentina* suggests they are third- and second-level consumers, respectively, while *T. s. scripta*'s position indicate it is a primary consumer with a higher level of herbivory compared to the 2 snapping turtles. Ellipse estimates (SEA_C) suggested *M. suwanniensis* (7.3‰^2) and *T. s. scripta* (6.2‰^2) had similar niche sizes that were larger than the niche of *C. serpentina* (4.8‰^2). Niche estimates for female *C. serpentina* (8.2‰^2) were larger than for males (1.3‰^2), male *T. s. scripta* (7.9‰^2) had a larger niche than females (2.9‰^2), and juvenile *M. suwanniensis* (9.2‰^2) was larger than adults (1.3‰^2 ; Fig 3a). There was more overlap in the niches between sexes of *C. serpentina* (female: 12%, male: 81%) and *T. s. scripta* (female: 95%, male: 35%), with less overlap ($< 1\%$) between juvenile *M. suwanniensis* and adult males. Bayesian estimates further suggest niche areas for all 3 turtle species were similar

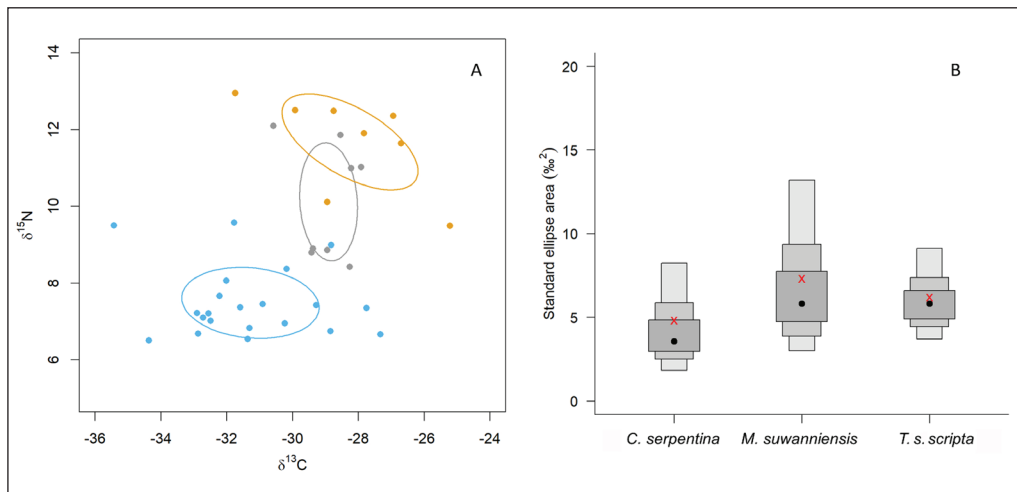


Figure 2. (a) Stable isotope bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each species, with associated ellipses illustrating the 40% core isotopic niches of the 3 species of turtles within the Santa Fe River, FL.. The yellow circles and ellipse represent *Macrochelys suwanniensis*, gray circles and ellipse represent *Chelydra serpentina*, and blue circles and ellipse represent *Trachemys s. scripta*. (b) Bayesian standard ellipse area (SEA_B) estimated posterior distribution estimates show 50% (dark gray), 75% (medium gray), and 95% (light gray) credible intervals. The black dots indicate mode, with the x indicating estimated mean from SEA_C .

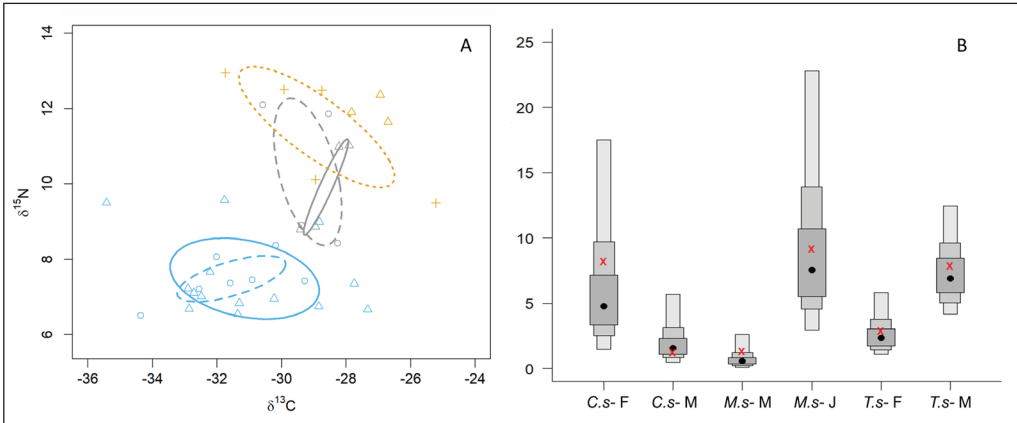


Figure 3. (a) Stable isotope bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each species by sex and age class, with associated ellipses illustrating the 40% core isotopic niche of the 3 species of turtles within the Santa Fe River, FL. Triangles and solid ellipses represent males (M), circles and dashed ellipses represent females (F), and plus sign and dotted ellipse represent juveniles (J). Yellow ellipse represents *Macrochelys suwanniensis* (M.s), gray represents *Chelydra serpentina* (C.s), and blue represents *Trachemys s. scripta* (T.s). (b) Bayesian standard ellipse area (SEA_B) estimated posterior distribution estimates show 50% (dark gray), 75% (medium gray), and 95% (light gray) credible intervals. The black dots indicate mode, with the x indicating estimated mean from SEA_C .

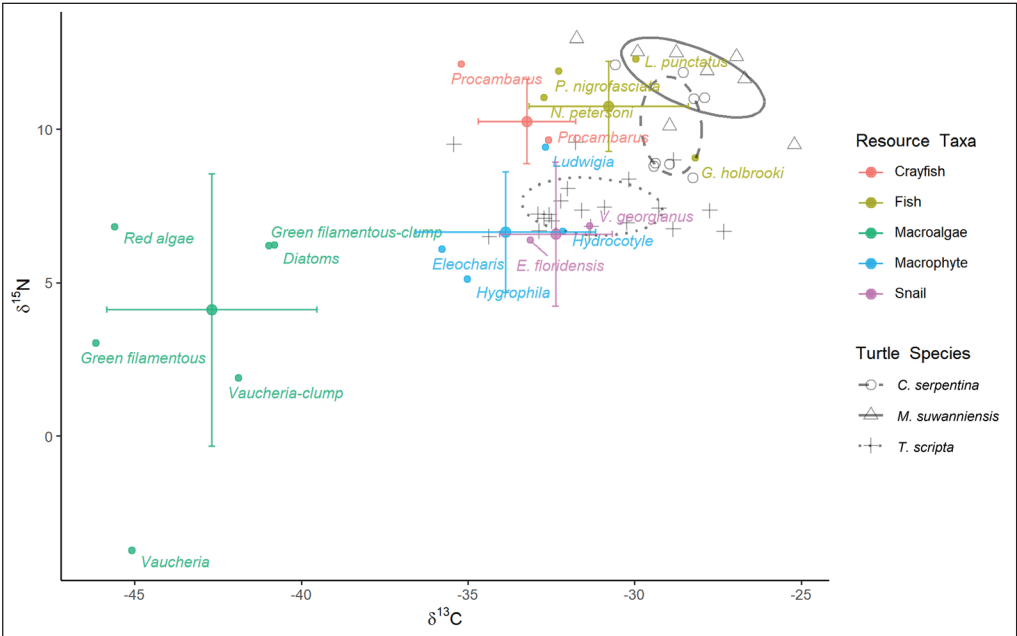


Figure 4. Stable isotope bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for resource taxa within our Santa Fe River, FL, study area. Large solid circles represent resource taxa means (± 1 SD), and small solid circles represent mean values for smaller taxonomic groupings within the main resource taxa. Ellipses represent the 40% core isotopic niche for each turtle species, *Macrochelys suwanniensis* (open triangles), *Chelydra serpentina* (open circles), and *Trachemys s. scripta* (plus sign).

Table 2. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (\pm SD) values for each resource taxon by sampling site.

Taxon	Location	n	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
			Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
Prey								
Crayfish	<i>Procambarus</i>	3	-32.6 \pm 0.8	-33.1	-31.6	9.6 \pm 0.8	8.9	10.4
Crayfish	<i>Procambarus</i>	1	-35.2	-	-	12.1	-	-
Fish	<i>G. holbrooki</i>	6	-28.2 \pm 1.5	-30.9	-26.6	9.1 \pm 0.8	8.0	10.1
Fish	<i>L. punctatus</i>	2	-30.0 \pm 0.7	-30.5	-29.4	12.3 \pm 0.4	12.0	12.6
Fish	<i>N. petersoni</i>	5	-32.7 \pm 1.7	-35.0	-30.3	11.0 \pm 1.0	9.4	12.2
Fish	<i>P. nigrofasciata</i>	5	-32.3 \pm 0.6	-33.1	-31.7	11.9 \pm 0.4	11.2	12.2
Snail	<i>E. floridensis</i>	6	-33.0 \pm 2.3	-35.5	-30.4	9.7 \pm 0.3	9.3	10.0
Snail	<i>E. floridensis</i>	10	-33.2 \pm 0.9	-34.3	-31.4	4.4 \pm 0.6	3.6	5.7
Snail	<i>V. georgianus</i>	4	-30.9 \pm 1.6	-33.3	-29.7	8.9 \pm 0.2	8.6	9.0
Snail	<i>V. georgianus</i>	8	-31.6 \pm 1.3	-33.1	-28.9	5.8 \pm 1.5	3.3	8.1
Vegetation								
Macroalgae	Diatoms	2	-41.0 \pm 0.9	-41.6	-40.3	6.2 \pm 0.6	5.8	6.7
Macroalgae	Green filamentous	2	-45.3 \pm 3.4	-47.7	-42.9	4.2 \pm 1.4	3.2	5.2
Macroalgae	Green macroalgae clump (green filamentous/diatoms)	5	-40.2 \pm 3.2	-43.7	-35.1	7.2 \pm 1.7	4.6	9.1
Macroalgae	Red algae	2	-45.6 \pm 0.3	-45.8	-45.4	6.8 \pm 0.2	6.7	6.9
Macroalgae	<i>Valucheria</i>	1	-45.1	-	-	-3.7	-	-
Macroalgae	<i>Valucheria</i> clump	2	-40.1 \pm 2.4	-41.8	-38.4	8.8	8.0	9.7
Macroalgae	<i>Valucheria</i> clump	3	-43.1 \pm 1.4	-44.2	-41.6	-2.7 \pm 1.1	-4.0	-1.9
Aquatic plant	<i>Eleocharis</i>	4	-35.8 \pm 2.2	-38.0	-32.7	6.1 \pm 0.6	5.6	6.9
Aquatic plant	<i>Hydrocotyle</i>	5	-32.2 \pm 3.4	-36.3	-29.1	6.7 \pm 1.4	5.1	8.6
Aquatic plant	<i>Hygrophila</i>	4	-35.0 \pm 1.6	-36.6	-33.3	5.1 \pm 1.2	3.9	6.8
Aquatic plant	<i>Ludwigia</i>	3	-32.7 \pm 0.9	-33.3	-31.6	9.4 \pm 2.3	7.7	12.0

in size (Fig. 2b), with intraspecific variation in isotopic niche among sex/age classes (Fig. 3b).

Resource taxa

Vegetation resources exhibited a wider span in $\delta^{13}\text{C}$ isotope values (-47.7‰ to -29.1‰) than potential prey (-35.5‰ to -26.6‰; Table 2, Fig. 4), whereas they were similar in $\delta^{15}\text{N}$ values (vegetation: 3.2‰ to 12.0‰, prey: 3.3‰ to 12.6‰; Table 2, Fig. 4). Primary producers had $\delta^{13}\text{C}$ values that suggested 2 distinct groups of basal resources, the relatively more enriched macrophytes (-33.9‰) and more depleted macroalgae (-41.9‰). Macroalgae were lower in carbon than all other taxa, while macrophytes were more similar to crayfish and snails but were lower than fish. Nitrogen values did not differ among vegetation samples (macroalgae and macrophytes) or snails, whereas fish and crayfish differed from all taxa except each other. Nitrogen values from resource samples collected at Poe Spring were higher than those collected from Rum Island.

Discussion

Within our study site, each of the 3 turtle species occupied its own trophic position, with *M. suwanniensis* and *C. serpentina* being higher-level consumers, while *T. s. scripta* were primary consumers. Each species also occupied distinct but similarly sized core isotopic niches, and intraspecific variation between the sexes or age classes was detected in all 3 species. Despite the observed shift in dominant aquatic vegetation from macrophytes to macroalgae, macrophytes were, proportionately, a much greater source of primary production for turtles within this section of the Santa Fe River.

Niche relationships among turtles

Each turtle species within the river occupied its own TP, but there was a slight overlap in core isotopic niche between the 2 snapping turtles. *Macrochelys suwanniensis* occupied the largest niche out of the 3 sampled species; however, it is the only species with both adults and juveniles represented. Our analysis detected possible ontogenetic differences in foraging behavior that did not result in different trophic positions. This finding is consistent with that of Aresco et al. (2015), who reported that *T. s. scripta* from lakes in Leon County in north Florida exhibited ontogenetic shifts whereby they consumed aquatic insects as juveniles and then more aquatic vegetation and fish (carrión) as adults, but their trophic position remained the same. *Macrochelys temminckii* (Troost in Harlan) (Alligator Snapping Turtle) is known to be an opportunistic scavenger consuming a wide variety of aquatic invertebrates, fish, and crayfish (Elsey 2006), and it is reasonable to hypothesize that *M. suwanniensis* consumes similar prey. Our analyses suggest while juvenile *M. suwanniensis* is in the same trophic position as adults with no significant difference in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values, juveniles exhibited more variation with a wider niche and lower mean values for both, with no overlap in core niche space. Thus, similar to Aresco et al. (2015), we may be detecting an ontogenetic shift in the diet of *M. suwanniensis* that does not result in a change in trophic position, illustrating the

importance of utilizing multiple analyses. Comparing niche orientation/size/position/overlap enables researchers to identify potential differences between size/age classes not detected by ANOVA assessments of isotopic values or trophic position when overall mean values are similar. However, our sample size is small, and we were only able to sample 3 adult male *M. suwanniensis*, which may also differ from females. Therefore, further sampling is needed to investigate potential interactions or competition during the different life stages.

While *M. suwanniensis* and *C. serpentina* had overlapping isotopic niches, the overlap was with juvenile *M. suwanniensis*; all adult *M. suwanniensis* males fell outside of the *C. serpentina* core isotopic niche. Although our sample sizes are small, our results suggest greater overlap in core diet when *M. suwanniensis* are juveniles than as adults. As adults, *M. suwanniensis* potentially forage on a broader suite of larger prey unavailable to the smaller individuals (Ewert et al. 2006). Ontogenetic changes in diet have been well-documented in many other turtle species, including *Chelonia mydas* (L.) (Green Turtle; Morais et al. 2014), *Deirochelys reticularia miaria* Schwartz (Western Chicken Turtle; McKnight et al. 2015), *Emydura macquarii kreffii* (Gray) (Kreff's River Turtle; Georges 1982), *Kinosternon hirtipes* Wagler (Rough-footed Mud Turtle; Platt et al. 2016), *Sternotherus minor* (Agassiz) (Loggerhead Musk Turtle; Zappalorti and Iverson 2006), and *T. scripta* (Bouchard and Bjorndal 2006, Clark and Gibbons 1969). Isotope studies have also detected ontogenetic shifts in *Apalone ferox* Schneider (Florida Softshell Turtle) populations in lakes in northwestern Florida (Aresco et al. 2015), as well as in a *Malaclemys terrapin terrapin* Schoepff (Northern Diamondback Terrapin) population in southern Barnegat Bay, NJ (Denton et al. 2023). The more vertical orientation of the ellipse in *C. serpentina* in our study with its narrower range in carbon values indicates foraging on consistent basal resources, whereas the wider range in nitrogen values may reflect a diet spanning trophic levels (Fig. 4a). This finding contrasts with the wider carbon and narrower nitrogen niche of *C. serpentina* in a northern Florida lake (Aresco et al. 2015). However, diet of *C. serpentina* can be highly variable within and among populations, with some in northern Florida appearing to be more herbivorous (Aresco and Gunzburger 2007), and others being more carnivorous (Aresco and James 2005).

Adults of both snapping turtle species exploited similar carbon sources, primarily macrophytes, while *T. s. scripta* likely utilized proportionally more macroalgae in addition to macrophytes. Our results suggest both *M. suwanniensis* and *C. serpentina* consume all of the sampled diet items but likely in different proportions (Fig. 4). From our data, adult *M. suwanniensis* appear to consume mostly fish including the larger *Lepomis*, with less consumption of crayfish and snails. Whereas juvenile *M. suwanniensis* appear to consume a higher proportion of more carbon-depleted resources than the adults, possibly consuming more crayfish and other small fish. *Chelydra serpentina*'s niche suggests foraging across trophic levels with consumption of a mix of smaller fish (*Gambusia* and *Notropis*) and crayfish in addition to the lower trophic-level snails, presumably the larger *Viviparus*. With their wider niche, female *C. serpentina* likely also consume a greater proportion of crayfish, *Percina*, or *Notropis* than males. *Trachemys s. scripta* appeared to

consume a mixture of all diet items, but at a lower trophic position with more snails and small fish consumed than were by either *M. suwanniensis* or *C. serpentina*. Thus, there was no overlap in core niche space with the other turtle species. The larger niche of male *T. s. scripta* suggests proportionally more contribution from more carbon-depleted macroalgae basal sources, and nitrogen-enriched prey such as crayfish and small fish, whereas females likely consume a higher proportion of macrophytes and snails. In this study, we did not sample all potential prey species; thus, we did not have the proper geometry necessary to apply mixing models as we were missing endmembers (resources higher in carbon and nitrogen that encompass all turtles). Mixing models enable researchers to estimate probability distributions of source contributions; however, these models can be misused and misinterpreted if the assumptions and limitations are not recognized (Phillips et al. 2014). Additionally, being unable to sample all locations each year prevented investigation of temporal variation; thus, future studies could build on our work by including seasonal sampling. Such seasonal sampling of additional prey taxa with higher carbon and nitrogen values could be included in mixing models to gain insight on the estimated contributions of each potential food source.

Resources

Vegetation. While macrophytes were the greatest source of primary production, the higher $\delta^{15}\text{N}$ values of the snapping turtles suggest less-direct contribution through consumption, and more indirectly through consumption of the invertebrate communities that feed on them. However, the *T. s. scripta* values, indicating greater herbivory, were more consistent with the direct consumption of aquatic plants. *Trachemys s. scripta* had the lowest carbon and nitrogen values of all the turtle species we sampled, suggesting proportionally more direct consumption of macrophytes compared with the more omnivorous snapping turtles (Fig. 4). Turtles' isotopic discrimination factors between trophic levels ($\sim 1\text{‰}$ $\delta^{13}\text{C}$ and $\sim 2.3\text{‰}$ for $\delta^{15}\text{N}$) suggest the relatively nitrogen-enriched macrophyte *Ludwigia*, and to a lesser extent *Hydrocotyle*, contributed more, either directly or indirectly, to the snapping turtle diet than the other less nitrogen-enriched macrophyte species, or macroalgae. The lower nitrogen values of *T. s. scripta* suggest they likely foraged on a higher proportion of the relatively less nitrogen-enriched *Hydrocotyle*, *Eleocharis*, and *Hygrophila* macrophytes. Mean carbon values for all turtles sampled were $>5\text{‰}$ larger than those of macroalgae, suggesting less direct consumption and more indirect influence through consumption of prey species that feed on macroalgae. While it is possible all turtles directly consumed all possible vegetative sources, niche results indicate the core source of primary productivity for all turtles within this site were macrophytes.

Prey. Similar to the vegetative resources, prey species' carbon values did not differ between locations. However, a difference in $\delta^{15}\text{N}$ values was detected between locations, with snails and small fish from Poe Spring having higher values than those from Rum Island. The variation in the turtles' isotopic $\delta^{15}\text{N}$ values followed similar patterns as those of the prey resources; thus, differences in turtle nitrogen values between locations can likely be attributed to differences in baseline levels at

each location, rather than turtles feeding on different trophic levels. Of the sampled prey, fish had the highest mean $\delta^{15}\text{N}$ values, with crayfish slightly lower, while snails had the lowest nitrogen values of all prey sampled. Both snail species sampled are primary consumer grazers feeding on algae, detritus, and diatoms (Duch 1976, Johnson et al. 2013). Crayfish, with the second highest nitrogen values, consume a variety of plants, crustaceans, insects, or detritus, similar to the smaller fish (USFWS 2020); with the larger *L. punctatus* feeding primarily on periphyton and macroinvertebrate species of benthic macrofauna (Sears 2010). Restrictions during our 2020 field season prohibited additional resource sampling, preventing further investigation into the spatial and temporal variability in basal resource baselines within this stretch of the river. Thus, the variation we detected in nitrogen isotope values among sampling locations could be due to temporal variations among sites. To answer these questions, future work to sample turtles and resources from each location on more regular intervals would be valuable.

Conclusion

Our results suggest each of the 3 turtle species occupies its' own trophic position with little overlap in core resource use within our study site. All 3 species primarily exploited the same source of carbon, aquatic plants, through both direct consumption and indirectly through invertebrate communities and higher-order animal taxa, which fed on the plants and then were subsequently consumed by the turtles. The lack of overlap detected between adult *M. suwanniensis* and either *C. serpentina* or juvenile *M. suwanniensis* may indicate resource partitioning by size, with the smaller juvenile *M. suwanniensis* having more overlap in core resource use with *C. serpentina* and thus higher potential for interspecies competition for similar resources when sizes are comparable. Additional research utilizing mixing models can help to enable a more robust comparison between estimated contributions of organic matter sources and prey, and thus competition for resources between species and size classes. This riverine system may be in the beginning of a food-web transition, and continued sampling of turtles and resources may allow for detection of a shift towards a macroalgae-driven food web. Alternatively, we may be detecting resilience within the system where there were enough macrophytes to continue supporting a high proportion of the basal resources for the turtles' food web. Future studies would benefit from sampling *M. suwanniensis* farther upstream where there is less abundance of submerged aquatic vegetation, as well as including more herbivorous turtle species, which may more quickly reflect changes in the basal resources than the more omnivorous species. In addition to identifying current critical habitat and resources, these types of additional data can provide a baseline for comparison with future samples to facilitate interpretation of demographic changes that may be detected during long-term capture-mark-recapture study of these populations.

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