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by

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BACHELOR OF SCIENCE, BIOLOGY, THE UNIVERSITY OF NEW MEXICO, 2020

THESIS

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ABSTRACT

In 2022 I investigated niche partitioning among native and invasive freshwater turtles across three sites in the Middle Rio Grande using bulk stable isotope analysis. I found high degrees of niche partitioning at sites in which Red-eared sliders (*Trachemys scripta*) have yet to establish. However, at sites in which red-eared sliders are now the most abundant species they overlapped greatly in isotopic niche with all native turtles, in particular the painted turtle (*Chrysemys picta*). Using these same techniques, I then examined the trophic interactions between red-eared sliders and their acanthocephalan parasites. My results suggest that acanthocephalans occupy a lower trophic position relative to their hosts, and much like cestodes are likely reliant on nutrients derived from the host's liver.

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CHAPTER 1

INTRODUCTION

The idea of the niche is one of the most important and pervasive concepts within the field of ecology. In his seminal 1927 work *Animal Ecology*, Charles Elton describes the niche of a species as “the status of an animal in its community”, focusing primarily on how a species interacts with other organisms both as both predator and prey (1). Elton then uses this concept to draw parallels between species from distinct biological communities in a way not dissimilar to how a modern researcher might compare the relative trophic positions of two or more species within their respective food webs (i.e., primary producers, secondary consumers etc.). Three decades later, Hutchinson characterized the niche as an “n-dimensional hypervolume” in which each point corresponds to an environmental variable which allows an organism to exist indefinitely (2, 3). Hutchinson emphasized that the actual niche space (the realized niche) occupied by a species would in fact be much smaller than this theoretical space (the fundamental niche) due to competitive interactions with other species. We see then that from the beginning, that competition has long been central to the concept of the ecological niche.

In the early twentieth century Joseph Grinnell suggested that species with similar dietary niches could not co-exist over long timescales. Writing on the distribution of the chestnut-backed chickadee (*Poecile rufescens*), Grinnell states “Two species of approximately the same food habits are not likely to remain long evenly balanced in numbers in the same region.” (4). Modern ecological theory supports Grinnell’s hypothesis, and it is thought that competitor species can co-exist through the process of niche partitioning (5), whereby species split available resources along one or more niche axes such as diet, or activity period.

Niche partitioning can be driven and maintained through several mechanisms. One of the most intuitive is through interference competition, typically in the form of direct physical aggression (6). For example: laboratory experiments conducted on three sympatric species of kangaroo rat found aggressive behaviors between individuals to be the norm, and researchers hypothesized that these behaviors likely kept these species ecologically distinct in the wild (7). Competition can also occur indirectly when sympatric species both make use of a limited resource. Such interactions typically have a greater impact on the fitness of one species over another due to differences in resource use efficiency (6).

While the theoretical study of niche space and niche partitioning remains important, it also holds practical implications for dealing with the increasingly pressing issue of invasive species. Dietary niche studies in particular are an important means of examining competition between sympatric species (8, 9). The introduction of invasives species may result in greater interspecific competition for dietary resources, or potentially trophic divergence, allowing invader species to successfully integrate into ecological communities (10). Traditionally, these studies have relied on methods such as gut content and fecal sample analysis. Though these techniques have been widely used, they do have drawbacks. Perhaps the most significant is the fact that such techniques provide a limited temporal window into the feeding habits of consumers (11).

Additionally, it has been noted that differences in the digestibility of various food items can have the effect of overrepresenting or underrepresenting their importance in the diet of a consumer (12). Finally, without dissecting the animal in question it is impossible to determine the efficacy of techniques used to obtain such samples (13). Many of these issues are avoided through the use of stable isotope analysis (SIA).

Stable Isotopes

In recent years stable isotope analysis has emerged as a useful tool for investigating dietary niche overlap between sympatric species. Bulk isotope analysis of carbon and nitrogen has been increasingly utilized in the study of consumer diets and trophic interactions within ecological communities. This method holds advantages over other techniques typically used to study consumer diets in that isotopic ratios represent long-term, averaged feeding behavior. Ratios of nitrogen ($\delta^{15}N$) are typically used to estimate trophic positions as the difference between a consumer and its diet vary in a fairly predictable manner (approximately 3-4‰) (14). Carbon isotope ratios ($\delta^{13}C$) however, typically change little as carbon is transferred from diet to consumer (15). Due to this relative stability, $\delta^{13}C$ values are useful in determining the ultimate carbon source upon which a consumer depends.

The Middle Rio Grande

At 3,029 km long, the Rio Grande is among the top 25 longest rivers in the world. It drains 72,000 km, or approximately or about one quarter of New Mexico's total land area (16). Due to its historic importance as an arid land watershed, the river has been highly modified and has been both straightened and diverted in an attempt to hasten water delivery (17). In the 1500's Spanish explorers entered the region hoping to find large, wealthy cities such as the Aztec capital of Tenochtitlan which had been discovered less than two decades prior. What they found instead was a group of linguistically distinct people which nonetheless shared many important cultural traits such as large, well-built homes and the use of woven cotton clothing. During this time the Rio Grande Region would actually contain the greatest concentration of farming villages in the American Southwest (18). These settlements likely had little impact on the ecology of the region but would later be replaced by larger, more intrusive projects such as dams. Elephant Butte in New Mexico and La Boquilla in Chihuahua, Mexico were the first major dams constructed on the Rio Grande and were both completed in 1916 (17).

The construction of these dams greatly impacted the regions riparian vegetation and fisheries (16, 19). Historically, the Rio Grande supported between 21 and 24 native fish species. However, since European settlement in the region several have been extirpated including the Shovelnose sturgeon (*Scaphirhynchus platorynchus*), longnose gar (*Lepisosteus osseus*), American eel (*Anguilla rostrata*), and Rio Grande shiner (*Notropis jemezanus*) (16). Dam construction also led to the intrusion of several invasive plants such as Salt cedar (*Tamarix ramosissima*), and Russian olive (*Eleagnus angustifolia*). Along with these plants several invasive vertebrates have found their way into the Rio Grande including carp (*Cyprinus carpio*), bullfrogs (*Rana catesbeiana*) and the red-eared slider (*Trachemys scripta*).

The Red-eared Slider

Among the most successful invasive species is the Red-eared or pond slider (*Trachemys scripta*). Though native to North America, these turtles have been introduced across the globe due to their popularity in the pet trade and now occur on every continent except Antarctica (20). In Japan for example, a 2003 survey found non-native turtles to comprise the bulk (90%) of all turtles, with red-eared sliders accounting for 62% of all turtles captured (21). So widespread is this problem in fact, that the red-eared slider has now become the default species depicted in many popular images of turtles in Japan (22).

Though the red-eared slider is the most well studied turtle in North America, little is known about the impact that they may be having on native turtle communities (23). Of particular importance is an understanding of how the invasive red-eared sliders may be impacting native turtles found in regions already threatened by climate change and anthropogenic disturbance. Recently, there have been several studies examining the interactions between invasive red-eared sliders and native turtles in Southern Europe. This research suggests that red-eared sliders may compete with certain life stages of the European pond (*Emys orbicularis*) turtle for food as well as basking sites (24, 25).

In New Mexico red-eared sliders have been encountered in the Rio Grande since at least the 1970's, though in 1974 Degenhardt and Christiansen mention their lack of success in finding this species(26). Within the Middle Rio Grande the red-eared slider has been found to co-occur with at least four other turtle species (figure 1) including the Western painted turtle (*Chrysemys picta belli*), spiny softshell (*Apalone spinifera*), the threatened Big Bend slider (*Trachemys gaigeae*), and the common snapping turtle (*Chelydra serpentina*) (27).

Painted turtles belong to the monotypic genus *Chrysemys* and are closely related to sliders (*Trachemys*) and cooters (*Pseudemys*), being the smallest of the three (28).

Native Turtles of the Middle Rio Grande

Like red-eared sliders, painted turtles have a large geographic range, occurring from Northern Mexico into Ontario, Canada. The species is considered native to New Mexico where it can be found in the Rio Grande, San Juan, and Pecos Rivers (29). Painted turtles have a strong preference for still water habitats with muddy bottoms and aquatic vegetation (28). In New Mexico they are frequently captured alongside *Trachemys scripta*, *Trachemys gaigeae*, *Chelydra serpentina*, *Apalone spinifera*, *Psuedemys gorzugi*, and *Kinosternon flavescens*. Like sliders, painted turtles are thought to be opportunistic omnivores, with young turtles being more carnivorous than adults (29). This hypothesis is supported by field observations from Algonquin Provincial Park in Ontario, Canada of the Midland painted turtle (*Chrysemys picta marginata*) suggesting rapid growth rates in the first year of life (30).

Though well studied as a whole, within New Mexico painted turtles are not well researched, and their interactions with other freshwater turtles are poorly understood. Based on observations in the mid 90’s Stuart hypothesized that larger red-eared slider may outcompete painted turtles for basking sites in the Middle Rio Grande (31) More recently, studies on turtle communities in the Midwest show a strong negative correlation between the presence of red-eared sliders and three species, including the painted turtle (32).

With its unique morphology and highly aquatic habits, the spiny Softshell is among the easiest freshwater turtles to identify in the Middle Rio Grande (Figure 1). While found largely East of the Rockies, the species does range into Northern Mexico and Southeastern Canada (29). While the state of New Mexico is home to two native species of softshell turtle (*Apalone spinifera* and *Apalone mutica*), only the spiny softshell occurs in the Rio Grande. The species is typically a river dweller, but can also be found in lakes, ponds and ditches. Regardless of where it is found, spiny softshells require soft, muddy substrates in which to bury themselves. The spiny softshell is among the most carnivorous turtle species found in the Rio Grande and is thought to feed primarily on aquatic invertebrates, but fish and carrion are also taken. As with the painted turtle Dreslik and Phillips found there to be a strong negative correlation between the presence of red-eared sliders and spiny softshells (32).

The common snapping turtle (*Chelydra serpentina*) is one the largest species of freshwater turtle in North America. Primarily found East of the Rockies and ranging into Nova Scotia, the species has been widely introduced outside its native range. Recently, common snapping turtles have been reported across Asia, including Korea, Japan, and Vietnam (33–36). Within New Mexico the native status of this species within the Middle Rio Grande has long been disputed (29, 37). However recent zooarchaeological remains recovered from Isleta Pueblo in Central New Mexico suggest that the common snapping turtle has been present in the region since at least the 17th century (38). For the purposes of this study, I assume the common snapping turtle to be a native chelonian species. These turtles are typically described as highly omnivorous. Studies from Florida found aquatic plants, flatworms, annelids, and snails to comprise up the bulk of the diet with smaller amounts of vertebrate material also being consumed (39). More recent field observations from Algonquin Provincial Park reveal invertebrate and amphibian larva to be important prey items for young snapping turtles, while adults typically fed on seeds and aquatic vegetation (30). Despite being two of the well-studied turtles in North America, there is little known or even conjectured on the interactions between common snapping turtles and red-eared sliders (23).

Objectives

In this study I use bulk isotope analysis of carbon and nitrogen to examine the dietary niche of freshwater turtles in the Middle Rio Grande and seek to determine what impact the presence of a highly successful invader may have on niche partitioning within these communities. My primary questions are 1). Do native turtles partition their dietary niche (using $\delta^{15}N$ and $\delta^{13}C$ as proxies) in the absence of red-eared sliders? 2.) Do Red-eared sliders and painted turtles in New Mexico show any evidence of ontogenetic shifts in diet? 3.) Do native species in the Middle Rio Grande vary in their isotopic niche between sites?

STUDY SITES

During the Summer of 2022, I captured turtle at three localities along the Middle Rio Grande in Central New Mexico (figure 1). These sites differ greatly in their degree of anthropogenic disturbance and distance from major population centers. Two of these localities (Rio Grande Nature Center and Bosque del Apache NWR) were previously surveyed for turtles by Stuart between 1994-1998 (31).

Rio Grande Nature Center State Park

This site is located in Albuquerque, the most populous city in the state of New Mexico. The park is located less than one quarter kilometer from the Rio Grande. With an area of approximately 109 hectares, the park has three artificial ponds which are home to large numbers of turtles, including *C. picta*, *C. serpentina*, *A. spinifera* and *T. scripta*. The park was originally built in 1980 and is a popular recreational spot featuring walking trails and nature viewing windows. Due to the large number of visitors and proximity to water, the park is a popular site for the disposal of unwanted pets including freshwater turtles. Along with Bosque del Apache, this site was previously surveyed for freshwater turtles by Stuart from 1994-1998. At the time of writing, Stuart estimated that there were perhaps only 50 adult to subadult sliders at the Nature Center (31).

Sevilleta National Wildlife Refuge

Located approximately 85 km south of Albuquerque, New Mexico in Socorro County, this locality is both a National Wildlife Refuge and Long-Term Ecological Research (LTER) site. The refuge sits in the Middle Rio Grande Valley at the northern edge of the Chihuahuan Desert. Although there have been previous studies on the on the Desert box turtle (*Terrapene ornata luteola*), our study represents the first investigation into the refuge's freshwater turtle community. Since this site serves primarily as an ecological research station, it is largely unfrequented by the public, making this site an unlikely locality focal point for introductions of invasive species including the red-eared slider, relative to sites closer to large urban centers.

Bosque del Apache National Wildlife Refuge

Created in 1939, this site lies approximately 156 km South of Albuquerque. It is bordered by the Chupadera and San Pascual Mountains and covers an area of approximately 57,000 acres, with approximately 12,000 acres consisting of seasonally flooded marshes. These marshes are artificially maintained through a system of canals and levees, allowing park officials to replicate the seasonal flooding that was historically seen in the Middle Rio Grande (31). Although most well-known for its large Fall gathering of sandhill cranes (*Antigone canadensis)* that stop at the refuge during their migration, the site is also home to four species of freshwater turtle. These include the painted turtle, spiny softshell and Big Bend slider, as well as smaller numbers of common snapping turtles. Introduced red-eared sliders have also been reported and are known to hybridize with the local Big Bend slider population (40).

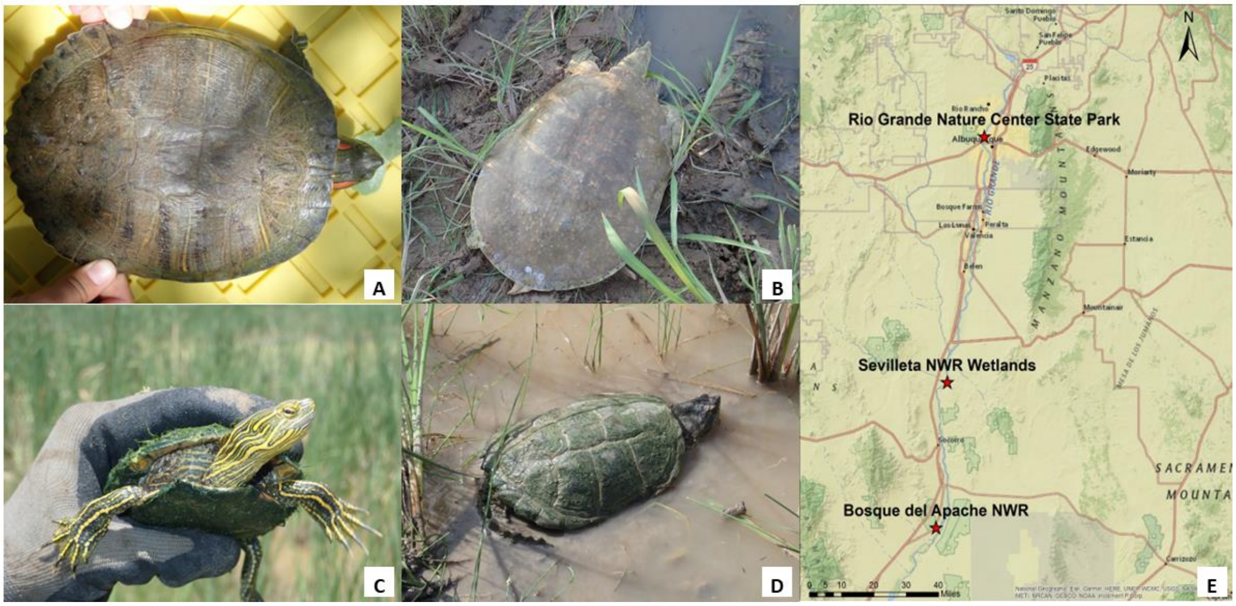


Figure 1: Turtle species and sites examined in this study. (**A**) *Trachemys scripta*, (**B**) *Apalone spinifera*, (**C**) *Chrysemys picta*, (**D**) *Chelydra serpentina*, (**E**), Map of study sites along the Middle Rio Grande.

MATERIALS AND METHODS

Turtle Sampling

To capture turtles, I utilized large hoop net style traps anchored to shore. I baited traps with canned fish in oil (sardines or herring) and placed traps in such a way that several inches were left between the surface of the water and the top of the trap, allowing individuals to freely surface for air (41). Traps were left for no more than twenty-four hours after which time turtles were removed and placed in large plastic containers for processing. Turtles were segregated by size and species to prevent aggressive behaviors between individuals. Native turtle species were processed immediately in the field, while a small number of sliders were removed and taken back to the University of New Mexico's (UNM) Animal Research Facility (ARF) where they were briefly housed for no more than 48 hours before being euthanized and processed. As red-eared sliders have been shown to hybridize with Big Bend Sliders in Central New Mexico, any *red-eared sliders* captured at the Sevilleta NWR or Bosque del Apache NWR were immediately removed and taken to the UNM ARF where they were humanely euthanized. Turtles were euthanized by first injecting them with 50% Tricaine (MS-222) solution and then placing them in a -20 C˚ freezer. All turtle tissue which was not used for stable isotope analysis were deposited in the Museum of Southwestern Biology.

With the exception of snapping turtles, all hard-shelled turtles were marked using the North American system which consists of a unique three letter code notched into the marginal scutes (42). Soft-shelled turtles were marked using a non-toxic, oil-based paint marker. Snapping turtles were marked by filing horizontal bars across two central scutes. Although this did not allow me to distinguish individual snapping turtles, it did allow me to know if a snapping turtle had been previously captured and processed. Standard shell measurements including straight plastron length (SPL), straight carapace length (SCL), and curved carapace length (CCL) were taken for each turtle using adjustable calipers to the and a soft tape measurer to the nearest 0.1 cm. Individuals were also weighed using adjustable hanging spring scales. Mass, plastron length, and curved carapace length were not obtained for most snapping turtles as individuals typically exceeded the range of both our calipers and scales.

I collected tail tissue by removing up to five millimeters (the amount of tissue removed was adjusted based off the size of the individual) of the tail using stainless steel scissors which had been sterilized with a 10% bleach solution followed by 70% ethanol rinse. Tail tissue was stored in 1.5 mL cryotubes and kept on ice for transport. Tail tips were then frozen at -20 C˚ prior to preparation for stable isotope analysis.

Sample Preparation

I prepared tissue samples for isotope analysis by rinsing them with distilled water and then oven drying at 60 C˚ for 24-48 hours (43). I did not utilize lipid extraction as prior studies have shown turtle tissues to fall below the 5% threshold recommended for this process (44). I then homogenized samples using a sterilized stainless steel razor blade and packed approximately 0.5 - 0.6 mg of tissue into 4x6 mm tin capsules.

Stable Isotope Analysis

Samples were analyzed at the UNM Center for Stable Isotopes (CSI) using a Costech ECS-4010 elemental analyzer (Valencia, CA) coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (Bremen, Germany). I report isotope values using δ notation using the equation: $\delta X = [(R_{sample}/R_{standard} - 1] \times 1000$, where X is ^{13}C and ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (45).

STATISTICAL ANALYSIS

Analysis of Isotopic Niche

For each turtle I characterized isotopic niche by plotting the values for $\delta^{15}N$ versus $\delta^{13}C$ (24, 46). I analyzed isotopic niche space for each species using the R package SIBER. Ellipse area, including 95% of the data, was calculated using Bayesian analyses as a robust way to compare the predicted niche breadth of species with different sample sizes (47). Individuals of generalist species may partition resources and have distinct isotopic niches, with the extent of variability expressed in the species total isotopic niche area (48). Overlap between isotopic niche space was calculated as the size of the intersection between the standard ellipse area of each species (47). Previous studies have determined that the degree of isotopic niche overlap between two species can serve as a useful proxy for potential dietary competition (49).

Body Size vs. Trophic Level

I utilized $\delta^{15}N$ values and morphological data obtained from red-eared sliders collected at Nature Center and painted turtles from the Sevilleta NWR to investigate the relationship between body size and trophic position in two species presumed to be ecologically similar. These two populations were chosen due to the relatively large sample sizes I was able to obtain at both sites. As a proxy for body size I used straight carapace length (SCL) as it is considered the standard when measuring freshwater turtles (28). These relationships were examined using simple linear regression models carried out in the statistical computing language R. For the final analysis, I removed three red-eared sliders as one individual was significantly enriched in $\delta^{15}N$ relative to other specimens (nearly 2‰ higher than the next most enriched individual). Additionally, I also removed the only hatchling obtained during my study as this individual's $\delta^{15}N$ values likely had not had sufficient time to equilibrate to its diet. Finally, one individual was released before I was able to obtain all shell measurements. In total I utilized 32 red-eared sliders and 30 painted turtles in my final analysis.

RESULTS

Community Composition

At the Rio Grande Nature Center (RGNC) I captured a total of 54 turtles. *Trachemys scripta* was by far the most frequently captured species (N=36). One of these individuals was excluded from our SIA as I was unable to collect tail tissue due to an apparent deformity. All individuals were collected from hoop traps except for a single *T. scripta* hatchling which was found dead near the water's edge. Though I found the remains of several nests, this was the only hatchling that I detected. Despite their large size, common snapping turtles were also relatively abundant at the park (N=10) and were frequently seen surfacing for air. I also captured a similar number of painted turtles (N=8). Finally, although I did not capture any spiny softshells I did witness a small individual basking.

At the Sevilleta NWR I captured a total of 51 turtles. Although I captured large numbers of painted turtles (N=38), only 30 of these individuals were used for SIA due to permit restrictions. I also captured four large snapping turtles at my primary site (Unit B Wetlands) and opportunistically collected one juvenile from a nearby mud hole. Finally, I captured seven spiny softshells and a single *Trachemys* specimen which appears to be a hybrid *T.scripta x T.gaigeae*. This individual was the only evidence I found of *Trachemys* at the refuge.

At the Bosque del Apache NWR my captures consisted primarily of spiny softshells (N=10) and one painted turtle. Although I witnessed several *Trachemys* basking, I was unable to determine if these were *T. scripta* or *T. gaigeae*. Finally, while there have been previous reports of snapping turtles from the refuge (50), I found no evidence of this species.

Niche Partitioning by Site

Results from the Sevilleta NWR indicate a relatively high degree of dietary niche partitioning among the native turtle community (Figure 2). Slight isotopic niche overlap was seen between *C. picta*, and C. serpentina (2.6%), as well as *C. picta* and *A. spinifera* (4.1%).

Moderate isotopic niche overlap was seen between *A. spinifera* and *C. serpentina* (17.5%). Finally, the single *Trachemys* that I captured falls well within the niche space of *C. picta*.

My results from Rio Grande Nature Center show a high degree of isotopic niche overlap between invasive red-eared sliders, painted turtles, and snapping turtles. *T. scripta* and *C. picta* were found to have the greatest overlap (70.1%). Niche overlap between *T. scripta* and C. *serpentina* was nearly equal to the overlap between *C. picta* and *C. serpentina* (31.6% and 30.4%, respectively).

At this time, I am unable to draw any meaningful conclusions about niche partitioning among turtles at the Bosque del Apache NWR due to the small sample size for all species other than the spiny softshell. However, the single painted turtle that I captured falls well outside of the niche space of *the* spiny softshells, which is consistent with my results from the nearby (approximately 56 km North) Sevilleta NWR.

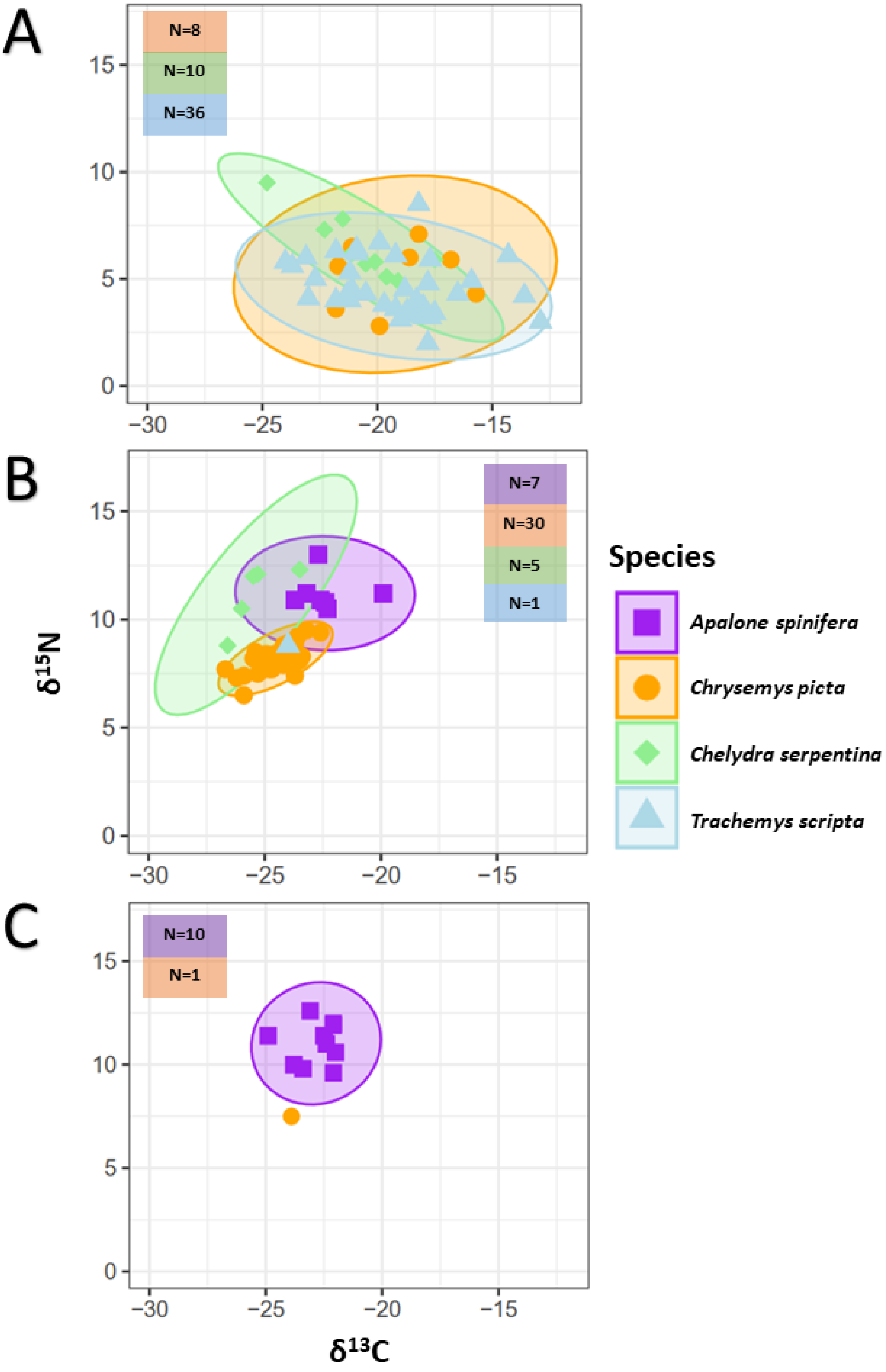


Figure 2: Predicted isotopic niche space for turtle species at three different sites across the Middle Rio Grande. (**A**) Rio Grande Nature Center State Park, (**B**) Sevilleta NWR, (**C**) Bosque del Apache NWR. Ellipses include 95% of data. Colored boxes indicate sample size by species.

Table 1: Summary of average δ13C, δ15N and ellipse area for the Rio Grande Nature Center turtle community.

Species	Average δ^{13}C (‰) (range)	Average δ^{15}N (‰) (range)	Corrected Standard Ellipse Area (‰^{2}) (95% confidence interval)
Trachemys scripta	-19.3 (-24.0 to -12.9)	4.7 (2.0-8.5)	67.4
Chrysemys picta	-19.2 (-21.8 to -15.7)	5.2 (2.8-7.1)	74.4
Chelydra serpentina	-20.6 (-24.8 to -17.5)	6.3 (4.9-9.5)	33.7

Table 2: Summary of average δ13C, δ15N and ellipse area for the Sevilleta NWR turtle community.

Species	Mean δ^{13}C (‰) (range)	Mean δ^{15}N (‰) (range)	Corrected Standard Ellipse Area (‰^{2}) (95% confidence interval)
Trachemys scripta	na	na	Na
Chrysemys picta	-24.5 (-26.7 to -22.6)	8.2 (6.5-9.5)	9.2
Chelydra serpentina	-25.4 (-26.6 to -23.5)	11.1 (8.8-12.3)	27.4
Apalone spinifera	-22.4 (-23.7 to -19.9)	11.2 (10.5-13.0)	22.4

Table 3: Summary of average δ13C, δ15N and ellipse area for the Bosque del Apache NWR turtle community.

Species	Mean δ^{13}C (‰) (range)	Mean δ^{15}N (‰) (range)	Corrected Standard Ellipse Area (‰^{2}) (95% confidence interval)
Apalone spinifera	-22.8 (-24.9 to -22.0)	11.0 (9.6-12.6)	20.4
Chrysemys picta	na	na	na

Intraspecific Isotopic Niche Variation

Turtles at Rio Grande Nature have far greater isotopic niche areas (Figure 3). Painted turtles have a roughly 800% greater isotopic niche than those seen at Sevilleta NWR. This pattern is also seen in the snapping turtle communities across the two sites, though not as dramatically with snapping turtles at Nature Center having approximately 123% of the isotopic niche space as those at Sevilleta.

The softshell communities studied at Sevilleta and Bosque del Apaches NWR differ little in isotopic niche area, with Sevilleta softshells having approximately 110% of the niche area as those captured at nearby Bosque del Apache.

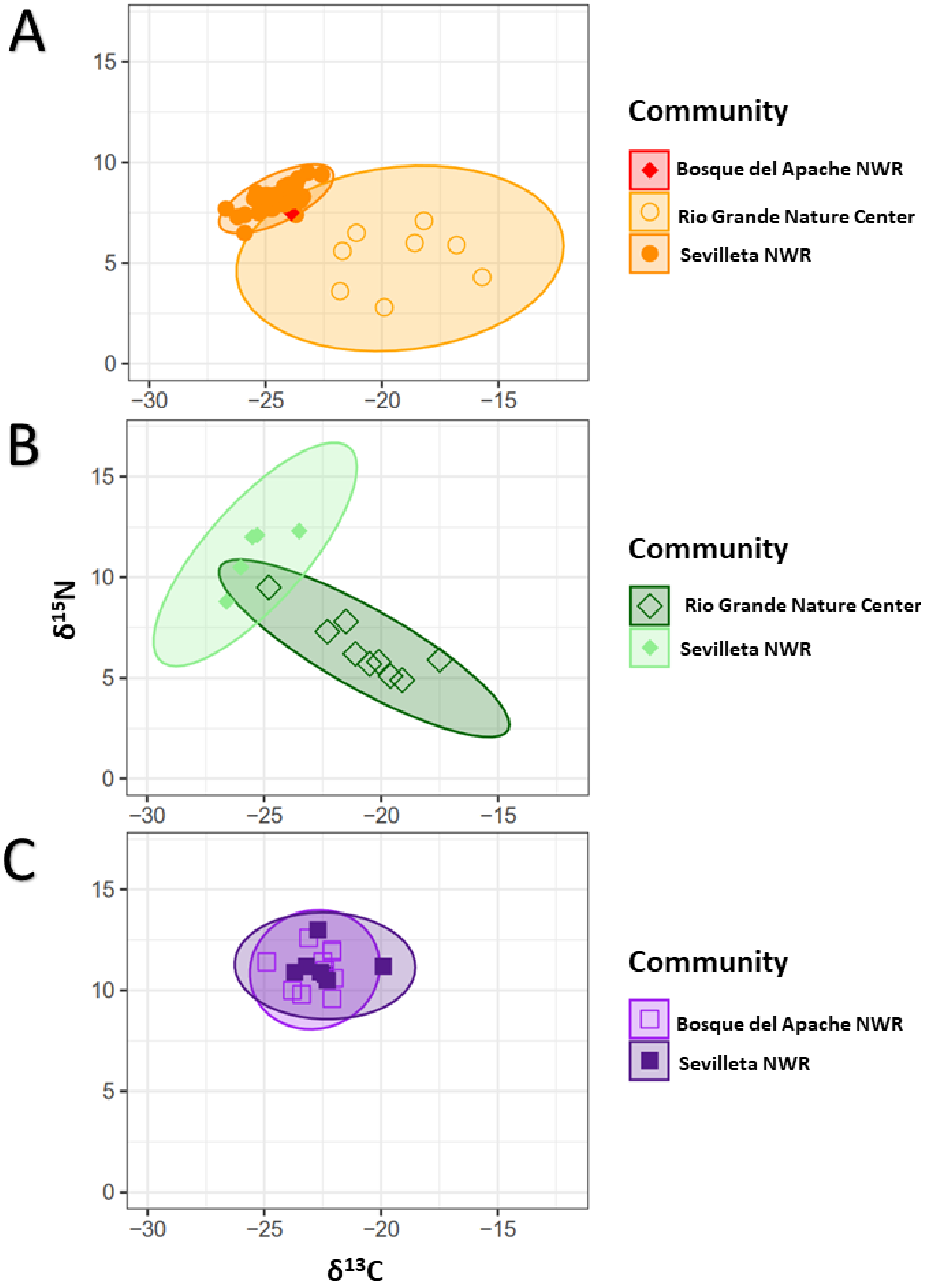
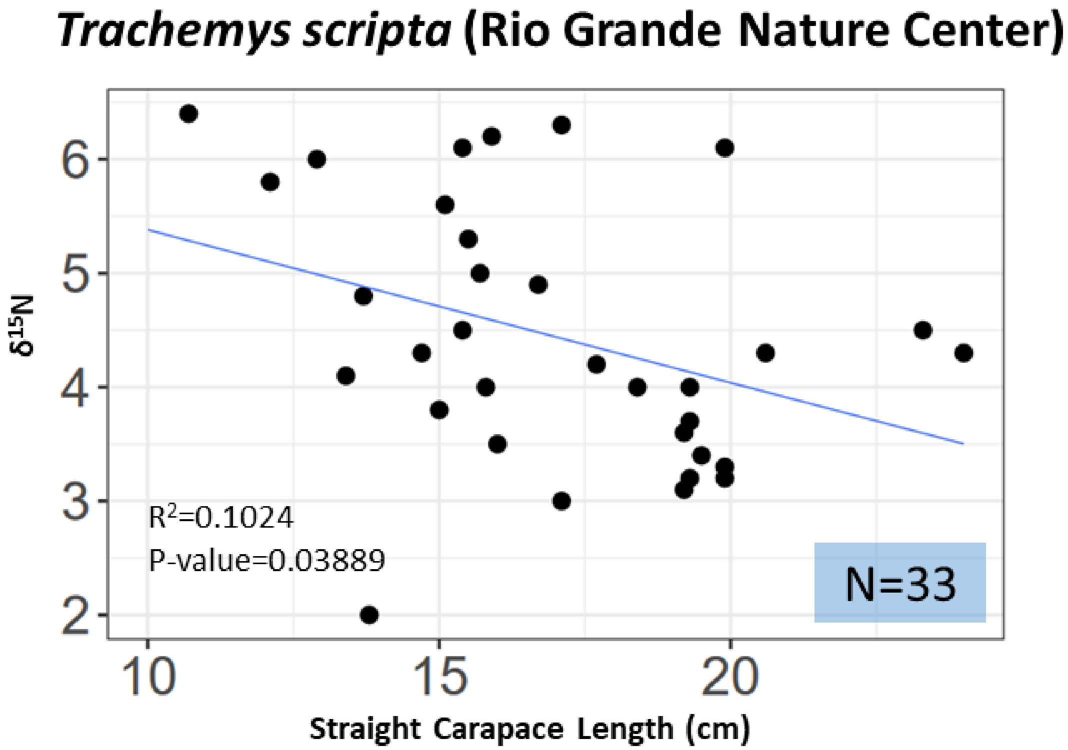


Figure 3: Predicted Isotopic niche space within turtle species across the Middle Rio Grande.

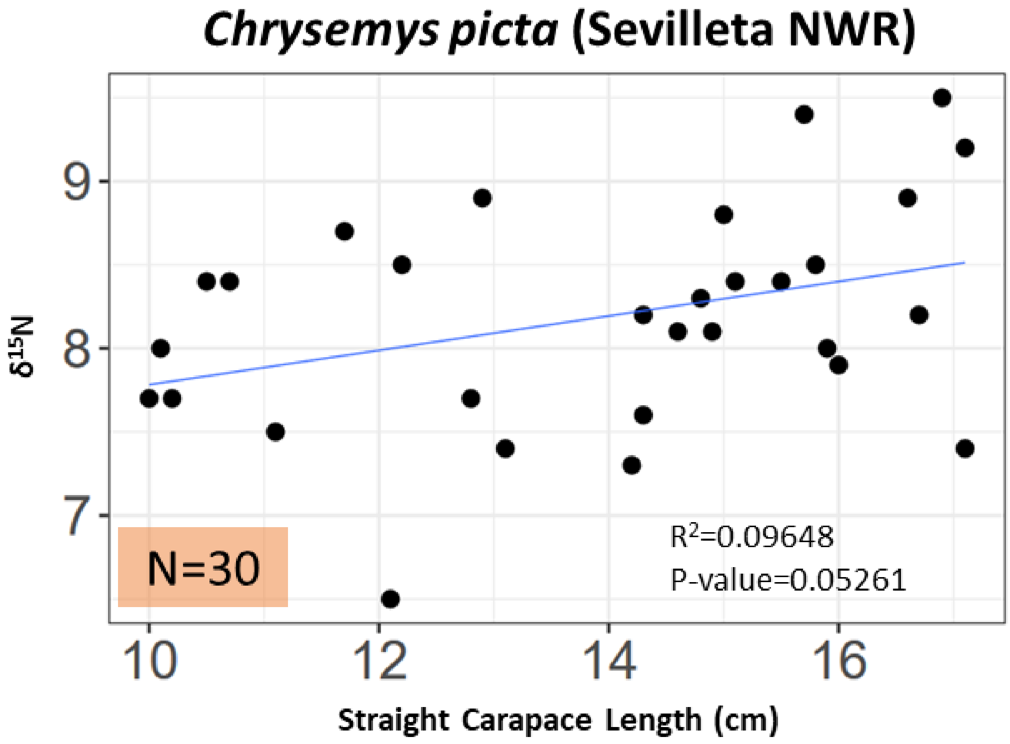
Body Size vs. Trophic Level

Analysis of the red-eared slider turtle community at Rio Grande Nature Center (Figure 4) shows some support for an ontogenetic dietary shift in this species within the Middle Rio Grande. In red-eared sliders there is a small but statistically significant trend (p-value of approximately 0.04) suggesting that turtles with a greater straight carapace lengths feed at lower tropic levels. However, red-eared sliders at a given size can and do eat at much higher trophic levels as well.

Painted turtles also show a slight, but not statistically significant trend (p-value of approximately 0.05) for an ontogenetic shift in diet towards. As with red-eared sliders it should be noted that individual turtles within a given size can feed at drastically different trophic levels.



Trachemys scripta (Rio Grande Nature Center)



Chrysemys picta (Sevilleta NWR)

Figure 4: Trophic position as a function of straight carapace length two populations of freshwater turtles.

DISCUSSION

While the turtle species examined in this study have been well studied overall, little is known about their ecology and natural history within New Mexico (51). Although my data is drawn primarily from two sites, my results suggest that the introduction of the red-eared slider into the Rio Grande has significant impacts on community dynamics of freshwater turtles in the region. The presence of this species within the Rio Grande seems to decrease niche partitioning among native turtles, even those whose taxonomic distance and seemingly disparate ecological needs (i.e., snapping turtles) would seem to preclude significant levels of competition with this species.

At the Rio Grande Nature Center, red-eared sliders overlap strongly in isotopic niche space with at least two species of native freshwater turtle. Additionally, based off the high proportion of total captures this species represents, along with the large number of disturbed nests that I observed, red-eared sliders appear to now be the dominant turtle species at the park. Though I didn't collect any softshell turtles at this sight, they appear to be uncommon relative to other species. A key question that remains is how red-eared sliders may impact this species in the Middle Rio Grande. Given that even in regions where the species is native, the presence of red-eared sliders seems to negatively correlate with the presence of spiny softshells (32), it is not unlikely that introduced sliders are negatively impacting this species as well.

Unfortunately, my results from Bosque del Apache are of little use in shedding light on the issue of potential resource overlap within the Middle Rio Grande. However, results from the Sevilleta NWR do suggest that under certain conditions native turtles in the Rio

Grande can indeed exist within the same habitat with little to no overlap in dietary resource use. Given the increased pressures of anthropogenic disturbance and climate change, such communities will likely become increasingly rare. Though I only managed to confirm the presence of a single individual *Trachemys* at the refuge, the fact that this individual fell well within the isotopic niche space of the native painted turtle is telling and does suggest the potential for resource competition between the two species, much like what is seen at the Nature Center. Additionally, if this individual is indeed a hybrid, it would represent the Northern most record of genetic admixture between the red-eared and Big Bend slider to date. While genetic admixture between these two species has been well documented in the Middle Rio Grande (40, 52), competitive interactions between red-eared sliders and Big Bend sliders have received little to no attention. Given the taxonomic proximity of these two species it would be reasonable to expect the invasive red-eared slider to overlap greatly in niche space with native Big Bend sliders.

In addition to biological invaders, turtle communities in New Mexico are also susceptible to the effects of a changing climate. The Southwestern United States continues to experience massive drought associated with climate change, but the situation will only worsen as temperatures, aridity, and increased human demand for water continues to increase. Given the state’s already arid climate and high elevation, these problems are likely to be particularly acute in New Mexico (53). The Middle Rio Grande will be especially impacted as its flow is largely derived from snowpack (54). High levels of modification and decreased surface water area seen across the Middle Rio Grande will likely only compound this issue, as more and more turtles are increasingly concentrated into smaller areas of suitable habitat.

Already, the flow of the river has been substantially reduced and altered due to the creation of reservoirs and increased channelization throughout the twentieth century. However, recent restoration efforts are seeking to recreate the historical Rio Grande floodplain environment (55, 56).

The continued study of all these sites can help yield insights into the success of these projects and how they may impact freshwater turtles in the region. Though New Mexico has the lowest percentage of water area of any US state (0.2%), with a total of nine species, the state has a surprisingly diverse freshwater turtle fauna (29, 57). A knowledge of how these animals will be impacted by not just invasive species but also habitat disturbance and climate change will become increasingly important for ecologists, conservationists, and resource managers. This study can serve as an important baseline to understand the long-term impacts the red-eared slider may be having on the freshwater turtles of Central New Mexico.

CHAPTER 2

INTRODUCTION

Stable isotope analysis (SIA) has become an increasingly popular tool for investigating resource use and competition in free-living organisms. Bulk SIA of carbon and nitrogen in particular has proven useful in studying the feeding ecology of many species (58, 59). Carbon isotope ratios ($\delta^{13}C$) change little as they pass from prey to consumer and are thus a useful tool for determining the carbon source(s) upon which an organism ultimately relies. In contrast, nitrogen isotope ratios ($\delta^{15}N$) tend to differ in a fairly predictable manner as energy is transferred from diet to consumer (typically by 3-4‰) making them a useful indicator of trophic position (15).

These patterns, however, do not seem to hold for many endo-parasites (60, 61). For example: absorptive feeders such as acanthocephalans and cestodes are often depleted in $\delta^{15}N$ relative to their hosts (62, 63). On the other hand, nematodes show great variability, with some species being enriched relative to their host, while others are relatively depleted. The fact that many endoparasites also go through several life stages with vastly different ecologies only adds to the potential complexity. Despite the uniqueness of these patterns, stable isotope studies of parasites remain scarce and to our knowledge, only fish parasites have been examined in the past (61). Additionally, previous studies have typically utilized parasites from widely divergent taxonomic groups, often representing entirely separate phyla (i.e., trematodes and cestodes). Though studies utilizing parasites which represent such highly distinct evolutionary histories and ecologies are indeed valuable, they make it difficult to address the question of whether bulk SIA is a useful method for examining resource competition in parasites as it has been in free living species.

Competitive interactions between co-occurring endoparasites have long been of interest to researchers but have proved difficult to study (64). The acanthocephalans (thorny-headed worms), particularly those infecting freshwater hosts may be a useful model to examine these interactions due to their relatively simple life cycles (65). Previous studies have examined the distribution and site preferences of each species along the host intestine as a means of elucidating how these organism were able to infect the same host (66, 67). However, stable isotopes could prove a useful tool for investigating whether co-occurring parasite species do indeed compete for similar resources.

While reptiles have been shown to have relatively depauperate helminth communities, turtles seem to be an exception, particularly members of the family Emydidae (68). Members of the genus *Trachemys* often have relatively large helminth communities (typically 3-7 species) and are parasitized by a wide diversity of species including approximately 10 acanthocephalans of the genus *Neoechinorynchus* (69). These worms are frequently encountered in the hundreds within a single host and have historically drawn the interest of researchers from both herpetology and parasitology (70, 71). Up to four species of acanthocephalan may be attached to the small intestine of a single host (67, 72). This is in stark contrast to the acanthocephalan communities of fish, in which co-infection by multiple congeneric species is rare (65). These traits may make freshwater turtles a useful system in which to examine how closely related parasites partition their dietary resource use and how their trophic positions may vary relative to their host.

Turtles are believed to become infected through the accidental ingestion of the intermediate host (ostracods) as they graze on aquatic vegetation (73). As some freshwater turtle species have been shown to exhibit pronounced ontogenetic shifts in diet, with juveniles typically being more carnivorous, it is possible that adult turtles experience the highest rates of infection (74). While interest in the study of turtle acanthocephalans has experienced something of a revival (69), this host-parasite system remains poorly understood and despite a large number of suitable host species, acanthocephalans have only rarely been reported in the state of New Mexico (75). In this study we examine the trophic positions of two congeneric acanthocephalans collected from the invasive chelonian *Trachemys scripta* in the state of New Mexico.

MATERIALS AND METHODS

Host, Parasite, and Tissue Collection

In October 2022 I captured seven, adult *Trachemys scripta* at The Rio Grande Nature Center in Albuquerque, New Mexico. The park is a popular site for nature viewing among locals and is located less than one quarter kilometer from the Rio Grande. Three man-made ponds cover a large portion of the park (Figure 5) and are home to several species of freshwater turtle including the introduced red-eared slider (*T. scripta*), as well as smaller numbers of native turtles including the painted turtle (*Chrysemys picta*), spiny softshell (*Apalone spinifera*) and common snapping turtle (*Chelydra serpentina*).

Sampling activities were conducted in accordance with New Mexico Department of Game and Fish Permit #3854 and protocols approved by the University of New Mexico

(UNM) Institutional Animal Care and Use Committee. I captured turtles using a large hoop net trap baited with canned sardines, placed in the water of the Candelaria Wetlands Pond (35° 7' 50.736"N, 106° 40' 55.128"W) for 22 hours. Upon capture, I removed turtles and placed them in large plastic tubs. I then transported these animals to the UNM Animal Research Facility where they were humanely euthanized using tricaine methanesulfonate (76) followed by subsequent freezing at -20 C. After being euthanized, I weighed each turtle using hanging spring scales and then measured their carapace using a soft tape measure. I then removed the plastron of each turtle using an oscillating multi-tool to expose the inside of the animal. I collected tissues including tail tip, liver and intestine and then froze them at -20 C. I removed the entire gastrointestinal tract of each turtle which I then examined for helminths. Any acanthocephalans recovered were cleaned and placed in containers of deionized water and refrigerated for 24 hours to evert the proboscis of each specimen. These worms were then placed in 70% ethanol for long-term preservation. Previous studies have shown only slight alterations of $\delta^{13}C$ and $\delta^{15}N$ in animal tissues stored in 70% ethanol (77).

I prepared wet mounts of acanthocephalans by placing an individual worm on a microscopic slide with 70% ethanol and then a coverslip. These were then examined under a compound microscope to determine sex. As male acanthocephalans are difficult to distinguish from each other, these were set aside. Instead, all morphological species identifications were made using features of the gravid female and eggs (78). Eggs were obtained by making a small hole in the posterior end of the female using an insect pin and then lightly applying pressure to the worm.

Figure 5: Ariel view of Rio Grande nature Center State Park.

Stable Isotope Analysis

I prepared worms and host tissues for isotope analysis by first rinsing them with distilled water to remove excess ethanol and then oven drying them at 40 C for 24-48 hours (43). I then selected a small sub-sample (N=5) of *N. emyditoides* specimens from each of six host turtles for SIA, and opportunistically sampled smaller numbers of *N. pseudemydis*. One host lacked sufficient gravid, mature females (Table 1) and was thus not utilized for SIA (but see discussion). I did not use lipid extraction on host tissues as prior studies have shown lipid levels in turtle tissues fall well below the 5% threshold recommended for this process (44). I homogenized turtle tail samples using a sterilized stainless steel razor blade. Turtle liver and intestine were homogenized using a ceramic mortar and pestle. Acanthocephalans were not homogenized. I then packed approximately 0.1 - 0.6 mg of tissue into 5x9 mm tin capsules (Costech Analytical Technologies). Samples were analyzed at the UNM Center for Stable Isotopes using a Costech ECS-4010 elemental analyzer (Valencia, CA) coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (Bremen, Germany). Here I report isotope values using δ notation using the equation: $\delta X = [(R_{sample}/R_{standard} - 1] \times 1000$, where X is ^{13}C and ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (45).

DNA Extraction and Amplification

After an individual worm was subsampled for SIA, I then used the remaining tissue for DNA extraction. I extracted DNA using the Omega E.Z.N.A.® Mollusc DNA Kit. DNA was then quantified using a Qubit® dsDNA BR assay kit and Qubit 4 fluorometer. The entire internal transcribed spacer rDNA region (ITS1–5.8S–ITS2) was amplified by polymerase chain reaction (PCR) using a forward primer in the 18S region 5’-GTCGTAACAAGGTTTCCGT-3’ and reverse primer in the 28S region 5’-TATGCTTAAATTCAGCGGGT-3’ (79). PCR reactions were performed in a 25-ɥL total volume (13.375-ɥL molecular grade water, 5-ɥL Promega 5X GoTaq® Green Flexi Buffer, 1-ɥL 25 mM mgCl$_2$, 0.5-ɥL 10mM dNTPs, 1-ɥL each of 10 ɥM Forward and Reverse primer, 0.5-ɥL GoTaq® polymerase, and 3-uL of extracted DNA). Reactions were run on a BIO-RAD T100 thermal cycler under the following conditions: 94 C for 5 min; followed by 35 cycles of 94 C for 30 sec, 50 C for 1 min, and 72 C˚for 30 sec; and a final extension at 72 C for 7 min. PCR products were visualized on a 1% agarose gel run at 120 volts for 30-minutes. Single, solid bands were deemed successful, and the PCR product was then purified using ExoSAP-IT™ PCR Product Cleanup Reagent. DNA sequences were edited using the bioinformatics software Geneious Prime (Dotmatics). All acanthocephalan sequence data will be uploaded to the open access database GenBank so that it can be made freely available to the wider scientific community.

Statistical Analysis

All analysis was carried out using the statistical computing language R. For each sample type and host individual, I plotted the values for $\boldsymbol{\delta}^{15}$N versus $\boldsymbol{\delta}^{13}$C using the R package GGplot2. Isotopic niche areas were graphed for *N. emyditoides* by plotting ellipses using 95% of the data using the R package SIBER (47).

RESULTS

All seven turtles were found to be infected with *Neoechinorhynchus emyditoides*, but at differing intensities ranging from 2 to 570 individual worms (Table 4). Two turtles were also found to be infected with smaller numbers of *Neoechinorhynchus pseudemydis*. Additionally, leeches were recovered from six turtles (*Placobdella parasitica*) and a single nematode (*Serpinema trispinosum*) was recovered from the stomach of one host (MSB: Herp:107082). Another host individual (MSB: Herp:107086) was found to be infected by a single nematode (likely *Serpinema trispinosum*) but was lost during processing.

Also known as the common turtle leech, *Placobdella parasitica* is frequently encountered on several species of North American freshwater turtle (80, 81). The nematode *Serpinema trispinosum* is also a common parasite of freshwater turtles and has been reported in *Trachemys* as far South as Cuba and the Yucatan Peninsula (82).

Table 4: Host morphology and parasite load for seven *Trachemys scripta* specimens. Note that MSB 107085 was not included in the final analysis and is included here to show the range in host size and parasite load.

Host ID	Curved Carapace Length (cm)	Mass (g)	Sex	Number of Acanthocephalans	Other Parasites Found
MSB:Herp:107081	26.2	1900	female	73	Hirudinea
MSB:Herp:107082	27.0	2050	female	90	Hirudinea Nematoda
MSB:Herp:107083	22.0	1050	male	35	None
MSB:Herp:107084	21.3	900	male	36	Hirudinea
***MSB:Herp:107085**	26.9	2200	female	2	Hirudinea
MSB:Herp:107086	25.1	1300	male	570	Hirudinea Nematoda
MSB:Herp:107087	25.2	1450	male	341	Hirudinea

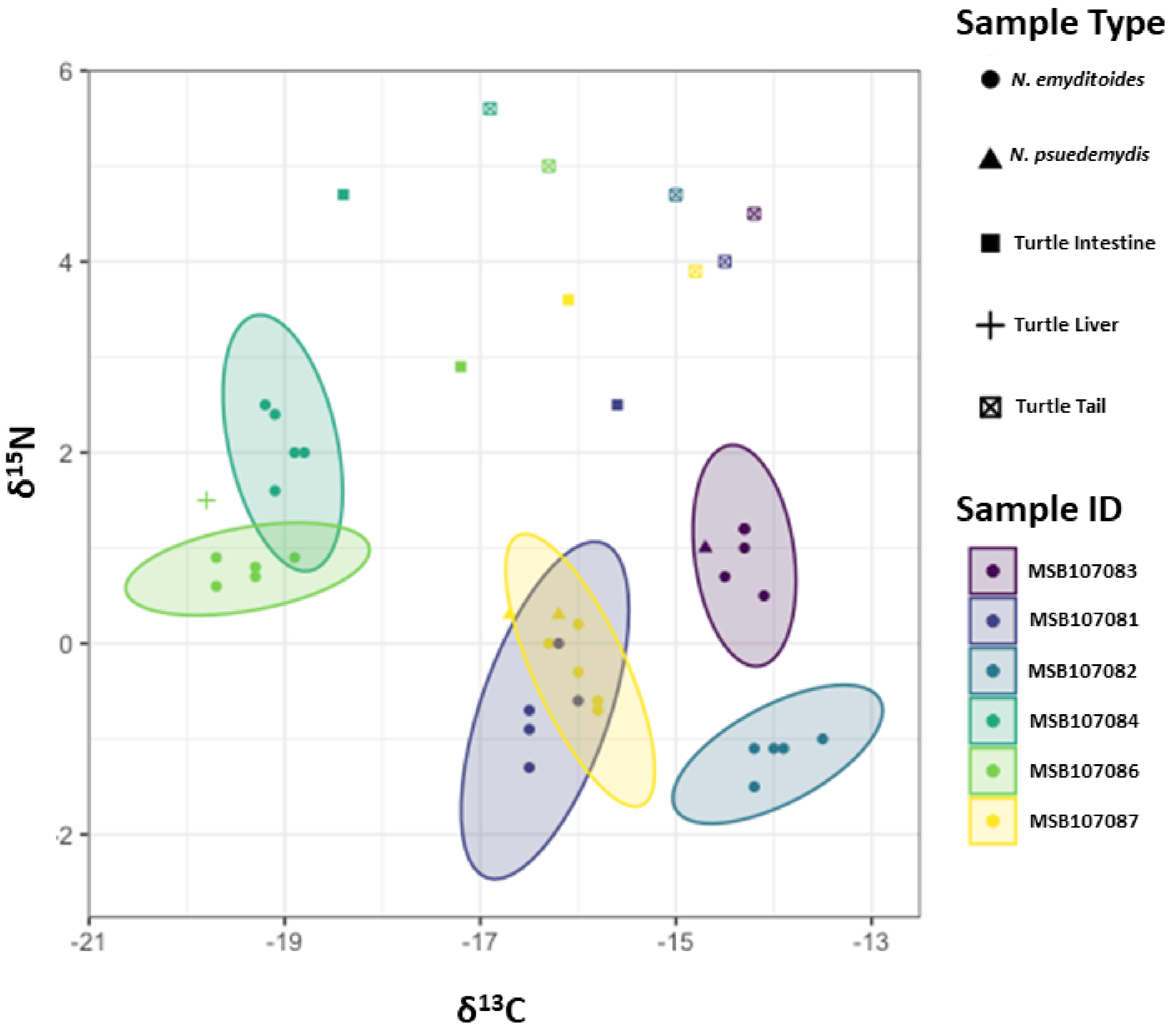


Figure 6: Plot showing isotopic signatures of acanthocephalans and host tissues.

Table 5: Summary statistics for samples used in this study.

		Mean $\delta^{13}C$ ± (sd)	**Mean $\delta^{15}N$ ± (sd)**
Trachemys scripta	Tail (n = 6)	-15.3 ± (1.0)	4.6 ± (0.6)
	Intestine (n = 4)	-16.8 ± (1.2)	3.4 ± (1.0)
	Liver (n=1)	-19.8	1.5
N. emyditoides	(n = 30)	-16.5 ± (2.1)	0.3 ± (1.2)
N. pseudemydis	(n = 3)	-15.9 ± (1.0)	0.53 ± (0.4)

DISCUSSION

This study represents the first investigation into the trophic position of turtle acanthocephalans using stable isotope analysis. I found both species of acanthocephalan were depleted in N^{15} relative to the tissues of their hosts. These findings are in line with previous investigations of acanthocephalans in fish hosts as well as cestodes (60). Examinations of N^{15} values in worms relative to host tail and intestine show acanthocephalans are approximately one trophic position (approximately 3-4‰) lower than their hosts. Though only one host liver was included in this study, differences between liver tissue and mean acanthocephalan N^{15} and C^{13} were found to be very low (0.72 ‰ and 0.42 ‰ respectively). This suggests that much like cestodes, acanthocephalans likely feed on secondary metabolites released from the host liver rather than host tissues.

At this time, it does not appear that there is sufficient evidence to suggest resource overlap between co-occurring acanthocephalan species. However, future research should examine the potential for competitive interactions using larger numbers of worms as well as hosts. It is possible that in most hosts dietary resources are not a limiting resource and there may be more direct interactions between acanthocephalan species which could explain differences in relative abundance.

While one host's acanthocephalans were excluded from my final analysis, I examined δN^{15} and δC^{13} from this individual, which revealed it be highly enriched in δN^{15}. This trend was seen in both tail tissue as well as intestine (18.8‰ and 10.5‰ respectively), suggesting that sample contamination was unlikely. In contrast, during my previous investigations into the stable isotope signatures of the same freshwater turtle community the most N^{15} enriched red-eared slider was an individual of similar size whose δN^{15} value was 8.5‰. Assuming a trophic enrichment factor of 3.4‰, this would place these individuals at nearly three full trophic levels apart. One scenario which could explain these findings is that this individual represented a recently released pet or at least an individual which had recently been in captivity. Previous SIA studies have determined that captive red-eared sliders are often highly enriched in N^{15} relative to wild sliders (83). Such a scenario would also explain the low acanthocephalan infection level of this individual as it likely would not have had time to acquire large numbers of these parasites. Future investigations should examine the utility of using endoparasite communities in conjunction with stable isotope analysis to determine the origin of potential invaders, including red-eared sliders.

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