DIET OF A POPULATION OF PRAIRIE RATTLESNAKES (*CROTALUS VIRIDIS*) IN KANSAS

COLLEEN ROTHE-GROLEAU[1] AND JAMES D. FAWCETT[2]

[1]Nebraska Natural Heritage Program, Nebraska Game and Parks Commission, Lincoln, NE 68503, USA; corothe_groleau1@yahoo.com
[2]University of Nebraska at Omaha, Department of Biology, Omaha, NE 68182, USA. Deceased prior to publication

Correspondence: corothe_groleau1@yahoo.com

ABSTRACT: This study examined prey items consumed and seasonal feeding events within a Prairie Rattlesnake (*Crotalus viridis*) population near Ulysses, Kansas, USA. A total of 183 specimens were collected over a period of three consecutive years (2012–2015) during the active season from April to October. Most prey items consumed by this population were taken in spring between emergence from hibernacula and mating in early summer. Gravid females continued to take prey, likely opportunistically, while gestating. The only ontogenetic shift in diet composition was related directly to prey size. Larger snakes exhibited a preference for larger prey items. The two most abundant taxa in the diet of this population were *Peromyscus* spp. (29.8%) and *Sylvilagus* spp. (17.0%). Only one juvenile had consumed any prey within a week of capture.

Key Words: Crotalus viridis*, diet, Prairie Rattlesnake, prey, rodent*

INTRODUCTION

Rattlesnakes are unique to the Americas and inhabit a variety of ecosystems, including deserts, wetlands, forests, and open grasslands. Several species have seen dramatic contractions in range due to habitat loss/fragmentation (Wittenberg and Beaupre 2014) and persecution by humans (Martin et al. 2008). Four species of rattlesnakes can be found in Kansas, USA. One, the Prairie Rattlesnake (*Crotalus viridis*), ranges across the western half of the state (Collins et al. 2010). The Prairie Rattlesnake's range overlaps with those of several rodent species often regarded as pests and/or disease vectors by humans (Reid 2006, Kays and Wilson 2009).

Many snake species, including rattlesnakes, exert a measure of population control over pest species such as rats, mice and rabbits, as well as limit the quantity and scope of diseases spread by these prey species (Bouskila 1995). Deer mice (*Peromyscus* spp.) are reservoirs for a number of diseases that affect humans such as Hantavirus (Centers for Disease Control 2021), Ehrlichiosis and Babesiosis (Cronin 2014). *Peromyscus* species are a major part of the diet of many *Crotalus* species, including the Prairie Rattlesnake (Fitch 1998). Control of these pest species can in part be assisted by their known predators (Collins et al. 2010, Fogell 2010).

Several studies and field guides have identified diet composition in populations of Prairie Rattlesnakes and other crotalid species (Conant and Collins 1998; Clark 2002; Collins et al. 2010; Fogell 2010). Diet items reported mostly include small mammals and birds with some geographic variation in composition (Conant and Collins 1998, Fitch 1998). Prairie Rattlesnakes have been identified as generalist predators that tend to utilize the most abundant prey species within the area (Holycross 1993). Diet composition within and between populations of Prairie Rattlesnakes can provide researchers with information on prey presence in a study area, as well as identify shifts associated with growth and development of individual predators (ontogenetic shifts).

Prairie Rattlesnake diet varies across time and space, as well as across life stages. As individuals grow, they are able to consume larger prey, while younger, smaller individuals are limited by gape size to correspondingly smaller prey items. The goal of this study was to identify what and when this population of Prairie Rattlesnakes was eating and to compare those data with those previously reported.

While data for adult Prairie Rattlesnakes are readily available, no other published study has examined such a large portion of a single population (183 individuals) from

a single area (approx. 129.5 hectares) collected over three consecutive short active periods. There are records of many large individuals collected from single populations, as in rattlesnake round-ups (Fitch 1998, Schmidt 2002), but there are no collections currently available for study that are as comprehensive as that upon which this paper is based. This unique collection is particularly valuable from an ecological standpoint in that it represents all age classes of a single population and may be used to identify ontogenetic changes in prey consumption.

MATERIALS AND METHODS

A preserved collection of 183 Prairie Rattlesnake specimens housed in the Sternberg Museum of Natural History at Fort Hays State University, Hays, Kansas, USA was used to collect data for this study. These specimens were collected by a group of environmental consultants contracted to facilitate a safe working environment, free of venomous snakes, for workers removing structures and materials from a decommissioned natural gas processing facility on privately owned property near Ulysses, Kansas. In compliance with contractual agreements specified by clients, all removed snakes were humanely euthanized. Specimens were deposited and catalogued in the Sternberg Museum of Natural History in Hays, Kansas to be used for future ecological studies (Dan Fogell, pers. comm. 2015).

Standard dissection techniques (Smith and Schenk 2014) were used to determine the presence/absence of prey items. Liver samples were removed and preserved in 95% ethanol for use in future genetic analysis studies. The stomach and intestines of each individual were dissected and examined for intact prey, partially digested prey, and indigestible mammalian guard hairs. Prey items were visually identified to lowest taxonomic level possible.

Whole or partial prey items were fixed in 10% neutral buffered formalin and stored in 70% isopropanol until they could be identified using body measurements, skull and bone characteristics, and hair samples. If only guard hairs were found, they were allowed to air dry and were stored for later identification in petri dishes marked with an alpha-numeric code associated with the individual from which the sample was removed.

Using a timeline for digestion based on methods adapted from Wallace and Diller (1990), we estimated the time elapsed between feeding and capture of individuals by documenting the location of prey remains within the digestive system of the snake. This method was used to estimate discrete numbers of prey items ingested by individual snakes. If a prey item was in the stomach, feeding was estimated to have occurred within one day of capture. If a prey item was in the small intestine, the snake was estimated to have fed three days prior to capture. If a prey item was in the large intestine, the snake was estimated to have fed four days prior. If scat was able to be palpated out, the snake was estimated to have fed seven days prior to capture. If prey and guard hairs were found in separate locations in the digestive tract (e.g. a hair sample in the stomach and another hair sample in scat from the cloaca), they were assumed to be two different prey items and identified accordingly. Prey items were recorded and prey species composition was analyzed. Feeding frequency and abundance of prey consumed were compared with results from other population diet studies.

Intact prey items were identified using field guides (Reid 2006, Kays and Wilson 2009, Collins et al. 2010). Partially digested prey was identified using skull and other skeletal features (Reid 2006, Kays and Wilson 2009). Often, skull characteristics could not be used for prey identification because the snake had ingested the prey head first and the head was the first part of the body dissolved by digestive fluids. In these cases, the hind feet and tail were examined and identified when possible using guidebooks on North American mammals (Reid 2006, Kays and Wilson 2009). Any remaining prey items that were not clearly identifiable were identified from guard hairs using techniques adapted from Moore et al. (1974). In mammals, dorsal guard hairs are unique to species Moore et al. 1974). They are largely indigestible by snakes and can be used to help identify prey (Clark 2002). Several characteristics are useful in identifying dorsal guard hairs, including basal configuration, hair color, band color and location, cortex, medullary configurations, shield configurations, scale patterns and margins, and hair strictures (Moore et al. 1974, Holycross 1993).

Hairs were isolated from digestive tract and cloacal samples and cleared of natural oils and debris in xylene for approximately one hour. Hairs were then placed on a glass slide marked with a number-letter combination unique to each individual and examined at 40x, 100x, and 400x magnification using a light microscope (Leica™). Characteristics visible at 40x magnification were hair strictures and length of hairs. At 100x magnification, color bands and basal configuration could be identified. Medullary configurations and scale patterns were not evident until they were examined under 400x magnification.

If a scale pattern could not be seen clearly and if identification relied solely on the scale pattern, a scale cast was made using techniques modified from the forensics website Identification of Human and Animal Hair (Accessed 3/18/2016). Scale casts were created by brushing a thin layer of clear nail polish (Sally Hansen – Hard as Nails™) onto a clean glass slide and placing a hair sample onto the polish. Once the polish was almost dry (tacky), the hair was pulled quickly off the polish, leaving an imprint of the scale pattern which could then be examined using light microscopy.

Table 1. Identification, frequency and percent occurrence of prey items found during dissection of a population of *Crotalus viridis*.

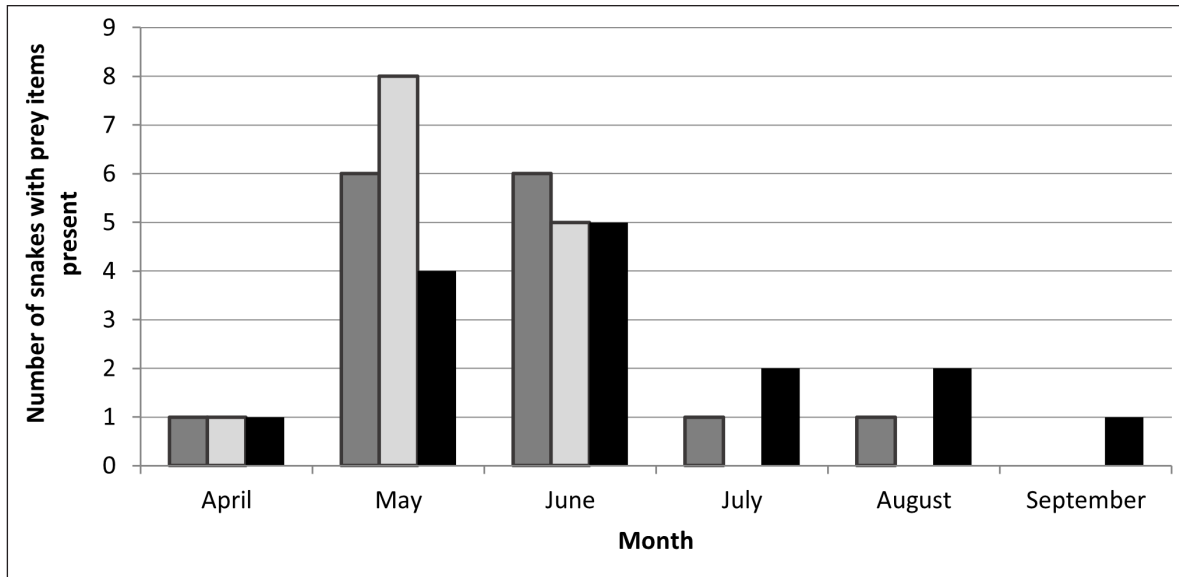| Prey item	Frequency	
	(count)	(%)
Aves	4	7.8
Reptilia		
Plestiodon obsoletus	1	1.9
Mammalia		
Rodentia		
Microtus	6	11.8
Muridae	3	5.9
Onchomys	1	1.9
Perognathus	3	5.9
Peromyscus	14	27.5
Chaetodipus	1	1.9
Spermophilus	1	1.9
Lagomorpha		
Sylvilagus	9	17.6
Insectivora		
Soricidae*	4	7.8

Figure 1. Frequency of prey items consumed by month for gravid females, non-gravid females and males in a population of Prairie Rattlesnakes (*Crotalus viridis*) near Ulysses, Kansas. Prey items were most often consumed in May and June each year. Dark gray: gravid females; light gray: non-gravid females; black: males.

Table 2. Adapted from Fitch (1998). Geographic variation in *Crotalus viridis* prey composition

Study	Origin of Sample	Main prey
Present study	SW Kansas	*Peromyscus, Sylvilagus*
Fitch (1998)	W Kansas	Ground squirrel, wood-rat
Wallace and Diller (1990)	Nez Perce Co. and Latah Co., N Idaho	Ad: vole, deer mouse Yg: shrew
McCartney (1989)	Okanagan Valley, S British Columbia	Ad: vole, gopher, mouse Yg: vole, shrew
McCartney and Gregory (1988)	Okanagan Valley, S British Columbia	Ad: vole, gopher, mouse Yg: vole, shrew
Duvall, King, and Gutzwiller (1985)	Carbon Co., S-central Wyoming, 6900'	Deer mouse
Gannon and Secoy (1984)	Leader, SW Saskatchewan	Ground squirrel, cottontail
Diller and Wallace (1985);	Nez Perce Co. and Latah Co., N Idaho	Ad: vole, deer mouse Yg: shrew
Klauber (1936)	Platteville, Boulder Co., N central Colorado	Ad: deer mouse Yg: lizard (*Holbrookia*)
Klauber (1936)	South Coronados Is., Baja California	Lizards: *Uta, Eumeces, Elgaria*
Heyrend and Call (1951) Glissmeyer (1951)	Grantville, Tooele Co., NW Utah	Ad: small mammals Yg: lizards
Fitch (1949)	Madera Co., central California	Ad: ground squirrel Yg: pocket mouse, spadefoot toad
Klauber (1956)	Pierre, Hughes Co., central South Dakota	Vole, lark bunting, deer mouse

RESULTS

Of the 183 snakes examined, 47 had prey items in the stomach or intestines. Sixteen of the 47 had identifiable animals in the stomach. Four had feathers in stomach and intestines. The remaining 27 individuals had only hairs and bone fragments in the intestines. (Appendix A). The majority (90.2%) of prey items consumed were small mammals; 7.8% were birds and one lizard accounted for 1.9% of the prey items consumed (Table 1).

Sixteen males, 15 gravid females and 16 non-gravid females had consumed prey items. There were three individuals that were notably successful at prey acquisition prior to capture. One male and one gravid female each had two different prey items in different locations within the digestive tract. One non-gravid female had three individual murids in her stomach, suggesting she found a nest and consumed its occupants. In summary, seventeen snakes fed within 24 hours of capture. Seven snakes had eaten within 2-3 days of capture, suggested by the presence of hair and no identifiable bones or intact body parts (e.g., tails, feet) in the stomach. Twenty-three snakes contained only hairs in the small and large intestines, indicating they had eaten within 4-6 days prior to capture.

Many prey items, especially small mammals, were consumed in spring between emergence from hibernac-

ula and mating in early summer (Figure 1). Four snakes (8.9%) had eaten in April; 34 snakes (75.5%) had eaten in May and June; three snakes (6.7%) had eaten in July; three snakes (6.7%) had eaten in August; and one snake (2.2%) had eaten in September. Of the 47 snakes with prey items, gravid females (33.3%), non-gravid females (33.3%) and males (33.3%) were equally represented. Two snakes with prey items – one non-gravid female and one male – had no capture date associated with them and were subsequently removed from the timeline statistics.

DISCUSSION

In this study population, a diverse composition of prey species was consumed. The majority of prey consisted of small mammals, though a small percentage consisted of birds and a single lizard species. The more frequent appearance of *Sylvilagus* spp. prey in larger adults is consistent with an expected ontogenetic shift towards larger prey. There was little overall difference in the diet composition of this population compared to other populations of Prairie Rattlesnakes and closely related species along similar latitudes (Table 2). However, the data comparison from other latitudes indicates geographic shifts in prey composition, supporting the suggestion that Prairie Rattlesnakes are generalist and opportunistic predators.

Wallace and Diller (1990) examined 106 prey remains from Northern Pacific Rattlesnakes over the course of nine years in northern Idaho. They found that first-year juveniles consumed shrews (Soricidae) exclusively and immature snakes fed exclusively on small mammals, including shrews, deer mice (Cricetidae), and voles (Cricetidae). Adult diets consisted of mice, voles, rabbits (Leporidae), and more rarely a bird or lizard. Several shrew species occur in Kansas that do not occur in Wyoming. Therefore, hairs from Kansas shrews are not part of the guard hair key published by Moore et al. (1974). While there are some species of Soricidae included in Moore et al. (1974), the medullary configurations and scale patterns were different enough from those present in the Kansas Prairie Rattlesnake diet samples that they could not be identified as any species included in the Wyoming key. Shrews are sympatric with Prairie Rattlesnakes in Kansas (Kays and Wilson 2009, Reid 2009) and there is no reason to believe they would not be part of the snake's prey base; thus, we feel confident that our samples were identifiable as shrews.

In more southerly populations, lizards and amphibians tend to be more inclusive in Prairie Rattlesnake diets (Fitch and Twining 1946, Fitch 1949, Glissmeyer 1951, Klauber 1956, Sparks et al. 2015), especially in young individuals (summarized in Fitch 1998, Sparks 2015). Diet composition in populations of Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*, previously *C. viridis oreganus*) in British Columbia were similar to that of populations in California (Macartney 1989, Sparks et al. 2015) except that in California, lizards were present in the diet (Sparks et al. 2015). Lizard populations sympatric with Prairie Rattlesnakes are not as common at more northern latitudes than in more southern portions of their range (Powell et al. 2016, McGinnis and Stebbins 2018), though Wallace and Diller (1990) did find that a Western Skink (*Plestiodon skiltonianus*) was consumed by a gravid female in Idaho. In Kansas, skinks (*Plestiodon* spp.), Prairie Lizards (*Sceloporus consobrinus*), Lesser Earless Lizards (*Holbrookia maculata*), Six-lined Racerunners (*Aspidoscelis sexlineatus*) and Texas Horned Lizards (*Phrynosoma cornutum*) all are sympatric with Prairie Rattlesnakes throughout their range (Collins et al. 2010). Therefore, we would expect lizards to be prevalent as part of the diet in the Kansas population. However, only one lizard, a Great Plains Skink (*P. obsoletus*), was identified among the prey remains. The specific lizard assemblage at the site of this study population is unknown, therefore we cannot predict which species to expect in the diet, nor can we predict how prevalent they should be. Given that only one immature (< 500 mm) specimen was identified to have prey contents during this study, it is still possible that lizards are consumed more frequently by smaller and younger size classes.

Prey items from each population of Timber Rattlesnakes (*Crotalus horridus*) studied by Clark (2002) varied significantly. As expected, adults ate prey larger than sub-adults and juveniles. Large snakes did not eliminate small prey from their diets as they grew; they included them along with larger prey items, as the data we collected here also demonstrate. These observations also support the idea that Prairie Rattlesnakes are generalist and opportunistic predators and consume any suitable prey that is readily available in their locale.

Graves and Duvall (1993) discussed prey selection and stated that reproductive female Prairie Rattlesnakes did not cease eating while gravid. Instead, they captured prey items as opportunities presented themselves, rather than actively foraging. Gravid females in this study population from Kansas also did not cease eating, supporting the suggestion of opportunistic feeding and consistent with the findings of Graves and Duvall (1993). The number of gravid females with prey items was surprising, given previous assumptions that food intake notably decreases or ceases altogether during gestation (Lourdais et al. 2002). Nearly 45% of all gravid females had prey items present in their digestive systems. Of these, 20% had *Sylvilagus* spp. prey in their systems. These results suggest that gravid females may select gestation areas based on microhabitat preferences that align with those of an abundance of small and large prey species, thereby maximizing the number of interactions with prey items while minimizing energy consumed in typical foraging behavior.

CONCLUSIONS

In conclusion, the prey most frequently consumed by individuals in this population was *Peromyscus* spp., followed mainly by other rodents and *Sylvilagus* spp., with only a few non-mammalian prey items selected. This is supportive of the conclusions of other studies that noted small mammals are the principal prey selected across populations (Diller and Johnson 1988, Clark 2002, Glaudus et al. 2008, Dugan and Hayes 2012). Snakes in this study consumed prey throughout the season with peak occurrences in May and June, and gravid females did not cease eating.

By comparing diet data from the population studied here with those of other populations, we can observe the species feeding ecology across populations in a more comprehensive context. The data can then be used to assess feeding variations, including ontogenetic and/or geographic shifts in prey selection, prey availability in a region, and seasonal changes in predation between or within populations.

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Appendix A. Demographics and diet results from a population of *C. viridis* collected near Ulysses, Kansas

Sex	Gravid	SVL	Month	Stomach or Intestine?	Prey ID	Timeline for eating (days)
M	NA	650	April	Stomach/Intestine	*Peromyscus*	2-3
F	N	632	April	Stomach	*Peromyscus*	1
F	Y	687	April	Stomach	*Peromyscus*	1
F	N	906	April?	Intestine	*Peromyscus*	4-6
F	Y	697	May	Intestine	*Sylvilagus*	4-6
M	NA	689	May	Intestine	*Reithrodontomys*	4-6
F	N	766	May	Intestine	*Sylvilagus*	4-6
F	Y	682	May	Intestine	Aves	4-6
F	N	763	May	Intestine	Soricidae	4-6
M	NA	1032	May	Intestine	*Peromyscus*	4-6
M	NA	744	May	Stomach	Aves	2-3
M	NA	650	May	Stomach	Aves	2-3
F	N	745	May	Stomach	*Sylvilagus*	1
F	N	749	May	Stomach	*Perognathus*	1
F	Y	810	May	Stomach	*Microtus*	2-3
F	N	730	May	Intestine	*Sylvilagus*	4-6
F	Y	739	May	Intestine	*Peromyscus*	4-6
F	Y	727	May	Intestine	*Sylvilagus*	4-6
F	N	720	May	Intestine	*Perognathus*	4-6
F	Y	840	May	Intestine	*Perognathus*	4-6
F	N	709	May	Intestine	Soricidae	4-6
F	N	673	May	Intestine	*Sylvilagus*	4-6
M	NA	694	June	Intestine	*Peromyscus*	4-6
F	Y	687	June	Intestine	*Sylvilagus*	4-6
F	N	742	June	Stomach	3-Muridae	1
F	Y	705	June	Stomach	*Microtus*	1
F	N	685	June	Stomach	*Peromyscus*	1
F	Y	724	June	Stomach	*Reithrodontomys*	1
M	NA	490	June	Intestine	*Onchomys*	4-6
F	N	713	June	Intestine	Soricidae	4-6
F	Y	715	June	Stomach AND Intestine	*Reithrodontomys* hair in stomach. *Sylvilagus* hair in intestine	R-2-3; S-4-6
M	NA	903	June	Stomach	*Plestiodon obsoletus*	1

Appendix A, continued. Demographics and diet results from a population of *C. viridis* collected near Ulysses, Kansas

Sex	Gravid	SVL	Month	Stomach or Intestine?	Prey ID	Timeline for eating (days)
F	Y	777	June	Stomach	*Microtus*	1
M	NA	904	June	Stomach	*Spermophilus*	2-3
F	N	696	June	Stomach	*Peromyscus*	1
F	Y	697	June	Stomach	Aves	1
F	N	570	June	Intestine	*Microtus*	4-6
M	NA	540	June	Stomach	*Peromyscus*	1
F	Y	685	July	Stomach	*Peromyscus*	2-3
M	NA	725	July	Intestine	Soricidae	4-6
M	NA	664	July	Animal in stomach; Hair in Intestine	*Peromyscus* in stomach *Chaetodipus* hair in intestine	P-1; C-4-6
F	Y	658	August	Stomach	*Peromyscus*	1
M	NA	550	August	Stomach	*Peromyscus*	1
M	NA	707	August	Stomach	*Reithrodontomys*	1
M	NA	562	September	Intestine	*Sylvilagus*	4-6

BODY SIZE AND AERIAL BASKING DYNAMICS OF THE SPINY SOFTSHELL (*APALONE SPINIFERA*) IN A HUMAN-MODIFIED LANDSCAPE IN TENNESSEE, USA

CHRISTOPHER J. O'BRYAN[1,2,*], M. KEVIN HAMED[3], THOMAS F. LAUGHLIN[4], AND J. WALLACE COFFEY[5,†]

[1]*School of Earth and Environmental Sciences, The University of Queensland, Brisbane QLD 4072, Australia*
[2]*Centre for Biodiversity and Conservation Science, The University of Queensland, Brisbane, QLD 4072, Australia*
[3]*Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA, 24061, USA*
[4]*Department of Biological Sciences, East Tennessee State University, Johnson City, TN, 37614, USA*
[5]*100 Bellebrook Road, Bristol, TN, 37620, USA*

Corresponding author: c.obryan@uqconnect.edu.au
†*Deceased September 27th, 2016.*

ABSTRACT: Spiny Softshells (*Apalone spinifera*) are found in aquatic environments throughout much of the central-eastern USA. Although this species is widespread throughout much of Tennessee, little is known about Spiny Softshells in the state's northeastern counties. Further, little work has investigated the role of Spiny Softshell body size on resource use, and the morphometrics of the species in a human-modified ecosystem. Here we present results of a four-month capture and basking observation study conducted in 2004. We investigated whether larger body size was positively associated with presence at limited aerial basking resources that are potentially important for thermoregulation. We found that hoop trap captures positioned next to basking sites, a proxy for aerial basking resource use, were not associated with sex or body size measurements. Opportunistic basking observations revealed most individuals basked in the afternoon. Our study, while short in duration and of low sample size, builds understanding on the body size and intraspecific effects of resource use by Spiny Softshells in a human-modified ecosystem.

Key Words: exploitative competition; thermoregulation; conservation; chelonian; human pressure; reptile; habitat use; morphometrics; ontogenetic shift; ontogeny

INTRODUCTION

The Spiny Softshell (*Apalone spinifera*; Figure 1) is a wide-ranging semi-aquatic turtle found throughout much of the central-eastern USA (Powell et al. 2016). In Tennessee, Spiny Softshells are found in freshwater streams, rivers, and ponds, and are thought to occur statewide; however, verified records are sparse for much of the state's northeastern counties (Scott and Redmond 2008, O'Bryan et al. 2015), with little published information on their natural history and morphometrics from the region (Rowell 1970, Jackson 1971).

Spiny Softshells are sexually dimorphic, with females being significantly larger than males (Webb 1962, Graham 1991), and they exhibit a wide range of body sizes that may result in larger individuals having a high demand for or competitive advantage over resources. Because of their larger size, females may exhibit social dominance and outcompete smaller individuals for food and aerial basking sites through direct and indirect competitive interactions (Lindeman 2000). Although exploitative competition has been observed interspecifically in other turtle species such as between the invasive Pond Slider (*Trachemys scripta*) and native turtles (Cadi and Joly 2003), little is known about the effects of body size on resource use within Spiny Softshell populations (see Lindeman 2000). Furthermore, while they are found in most aquatic ecosystem types across their range, Spiny Softshells may be susceptible to human-mediated environmental disturbances (e.g., Brown et al. 2012) such as surface water runoff and chemical pollution because of their ability to exchange respiratory gases cutaneously (Marchand 1942, Dunson 1960, Stone et al. 1992, Ultsch 2006). In light of this, an improved understanding of their presence and population dynamics in human-modi-
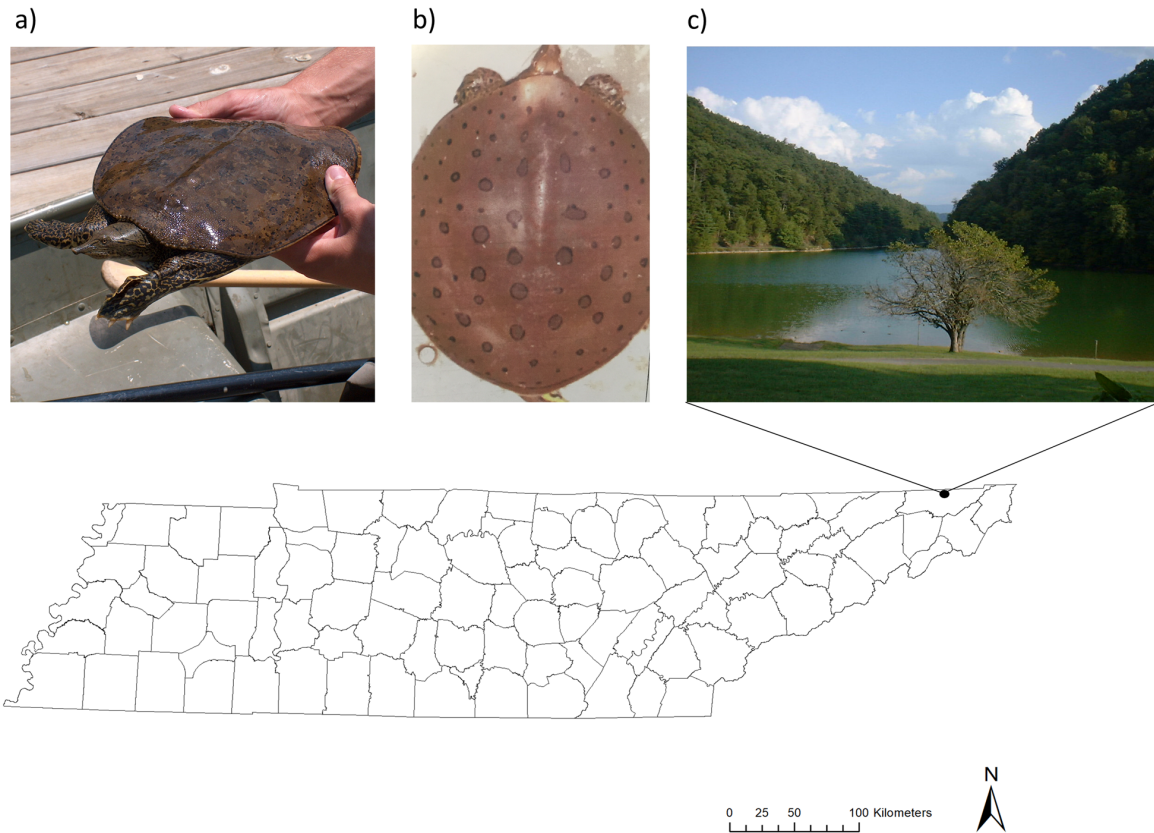
Fig. 1. a) Spiny Softshell (*Apalone spinifera*) female and b) male individuals captured during the project. Photo credit: Corey Brown (2004). c) The study site, Steele Creek Park Lake, City of Bristol, Sullivan County, Tennessee, USA. Photo credit: Christopher O'Bryan (2004).

fied landscapes, as well as the effects of intraspecific dynamics on resource use is a research direction of interest (see Plummer et al. 2008).

Here we provide results from a short-term capture and aerial basking observation study of Spiny Softshells in a reservoir in northeast Tennessee (Figures 1a and 1b). We hypothesized that: (1) Spiny Softshell body size measurements – that is, carapace, plastron, right-hind foot, and body mass estimates – would be considerably larger at trapping sites positioned next to aerial basking resources (i.e., sites that may be important for thermoregulation) than at non-basking sites; and (2) that female Spiny Softshells would be associated with aerial basking sites more than males. In addition to testing our hypotheses, we also provide morphometric and capture data, including metrics for additional turtle species captured during the study. We also provide information on opportunistically observed Spiny Softshell aerial basking events, including time of basking and basking structures used. Our results help build understanding of the body size and basking dynamics of Spiny Softshells in a human-modified landscape in Tennessee.

METHODS
Study site

We conducted the study in Steele Creek Park Lake in Bristol, Tennessee (36.573147, -82.233734; Figure 1c), which is in the Holston River drainage. Opened in 1964 by the City of Bristol, Tennessee, Steele Creek Park is the state's third largest municipal park, containing a 21.9 ha human-made impoundment with a spillway and ~890 ha of undeveloped forest (Rowell 1970, Jackson 1971). While the park has conducted a series of natural history inventories (including turtle surveys in the reservoir; Rowell 1970; Jackson 1971), little is known about the size distribution of Spiny Softshells in the Steele Creek Park Lake, their use of basking structures, and the inter- and intraspecific dynamics of the turtle community.

Captures

All captures were made from 4 May – 27 September, 2004 using finger-throated hoop traps (Memphis Net & Twine Co., Memphis, TN) across 20 trapping locations in Steele Creek Park Lake (four of the 20 sites were randomly selected on the day of trap placement) with total trap time of approximately 938 hours. Ten of the sites were considered 'basking' sites, such that the traps were positioned directly next to a basking structure, and ten were 'non-basking' sites. Basking sites were determined based on known aerial basking locations, which included areas with fallen trees, logs/snags, or gradual embankments. Non-basking sites were comparable in condition to basking sites but did not contain known aerial basking structures. We used an aluminum jon boat for deploying traps, securing each trap with poly-braided rope to tree branches or concrete blocks underneath the surface to hold the entrance in place, and traps were baited with ocean perch and chicken gizzards, or beef liver. For each individual turtle captured, we recorded carapace length, carapace width, plastron length, and straight right hind

foot length using calipers or metric tape measurer in addition to mass, using a PESOLA spring scale (PESOLA Präzisionswaagen AG, Schindellegi, Switzerland). We also documented patterns for individual identification. Since Spiny Softshells exhibit sexual dimorphism (Graham 1991) females were identified based on a distinct blotchy pattern on the carapace (see Figure 1a; Graham 1991); however, males retain juvenile morphology (see Figure 1b; Graham 1991). We determined turtles were adult males if the pre-cloacal portion of the tail extended beyond the posterior edge of the carapace (Webb 1962, Robinson and Murphy 1978, Berry and Shine 1980). When other species were captured, we noted the species and sex (if possible), and we measured carapace length and plastron length.

Basking observations

We visually searched for aerial basking Spiny Softshells opportunistically during hoop trap deployment, trap checks, and trap retrieval throughout the project. We used binoculars to scan possible aerial basking structures from the boat, including woody vegetation, lakeside structures such as embankments, and floating vegetation. We scanned the same basking structures during every opportunistic basking survey. These opportunistic surveys were only conducted during weather conditions conducive for aerial basking (e.g., little or no rain). We noted the time, location, and basking material used. We searched between 0900 – 1700 hours, depending on trap checks and deployments.

Analysis

To test our hypothesis of Spiny Softshell sex association with basking site captures, we conducted a Pearson's chi-square test with Yates' continuity correction that tests for dependence in count data. To determine if there was evidence for an association between Spiny Softshell morphometrics and sex, and between morphometrics and basking and non-basking site captures, we conducted a Welch two-sample t-test for comparing differences of the means. For all tests, we used R Statistical Software (R Core Team 2019) and applied a statistical evidence level of $p < 0.05$.

RESULTS

Capture results

We captured 17 unique Spiny Softshells and five recaptures ($n = 22$). All captured turtles were considered adults. Females ($n = 11$) were nearly twice as common as males ($n = 6$). All recaptures were female. We captured 59% of Spiny Softshells at basking sites ($n = 13$), the majority being female ($n = 11$). The remaining 41% of captures were from non-basking sites ($n = 9$), with females also ($n = 5$) outnumbering males ($n = 4$).

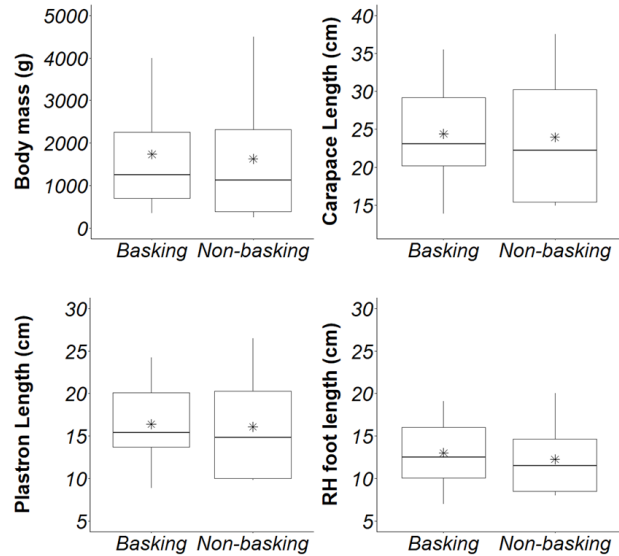We found no evidence that Spiny Softshell sex was associated with capture presence at traps positioned next

Figure 2. Boxplots of body mass, carapace length, plastron length, and right-hind foot length for basking ($n = 13$) and non-basking ($n = 9$) site captures of Spiny Softshells (*Apalone spinifera*) from May – September 2004 in Steele Creek Park Lake, Bristol, Tennessee, USA. The top and bottom of each boxplot represents the 3rd and 1st quartiles, respectively, the middle line of the boxplot is the median, the stars represent the mean, and the whiskers represent the range (min and max). There was no evidence that these four morphometrics' means were associated with basking or non-basking sites (p > 0.05).

to basking and non-basking sites ($\chi^2 = 0.47$; df = 1; p = 0.492). Further, there was no evidence that Spiny Softshell morphometrics were positively associated with these sites (see Figure 2). For example, mean body mass was not associated with basking site captures ($n = 13$) compared to non-basking site captures ($n = 9$; t = 0.15; df = 14.06; p = 0.883). Similar lack of evidence was found for carapace length (t = 0.11; df = 13.45; p = 0.916), carapace width (t = 0.04; df = 9.01; p = 0.970), plastron length (t = 0.10; df = 13.53; p = 0.92), and right-hind foot length measures (t = 0.36; df = 14.60; p = 0.727).

Captured female Spiny Softshells ($n = 11$) were significantly larger than males ($n = 6$) (Table 1), with female measurements being nearly twice as large as males for most morphometrics (carapace length: 29.09 cm vs 15.23 cm, t = 7.35; df = 10.72; p < 0.001; carapace width: 24.50 cm vs 13.36 cm, t = 6.92; df = 8.94; p < 0.001; plastron length: 19.67 cm vs 9.98 cm, t = 7.19; df = 10.92; p < 0.001; right-hind foot length: 15.04 cm vs 8.30 cm, t = 6.52; df = 11.71; p < 0.001). Furthermore, mean female body mass was over sixfold great-

Table 1. Morphometrics of individual male ($n = 6$) and female ($n = 11$) Spiny Softshells (*Apalone spinifera*) captured from May – September 2004 in Steele Creek Park Lake, Bristol, Tennessee, USA. Asterisks denote a statistically significant difference between males and females (p < 0.5).

Sex	Carapace length (cm)*	Carapace width (cm)*	Plastron length (cm)*	Right-hind foot length (cm)*	Body mass (g)*
Females (N = 11)	29.09 (20.20 - 37.50; SD 6.14)	24.50 (17.30 - 31.55; SD 4.69)	19.67 (13.70 - 26.5; SD 4.37)	15.04 (10.06 - 20.00; SD 3.29)	2,386.36 (700 – 4,500; SD 1,305.77)
Males (N = 6)	15.23 (13.90 - 16.50; SD 0.87)	13.36 (11.90 - 14.20; SD 0.86)	9.98 (8.90 - 11.10; SD 0.70)	8.30 (7.00 - 9.00; SD 0.73)	391.67 (250 – 500; SD 96.21)

Table 2. Morphometrics of additional semi-aquatic turtle species captured from May – September 2004 in Steele Creek Park Lake, Bristol, Tennessee, USA.

Species	Carapace length (cm)	Plastron length (cm)	Number captured
Snapping Turtle (*Chelydra serpentina*)	23.28 (range 14.05 - 31.5; SD 8.77)	16.73 (range 11.7 - 20.5; SD 4.53)	3
Pond Slider (*Trachemys scripta*)	14.83 (range 9.1 - 29.2; SD 5.58)	12.54 (range 7.6 - 22.1; SD 4.34)	17
Painted Turtle (*Chrysemys picta*)	10.84 (range 7.5 - 14.2; SD 3.74)	9.15 (range 6.2 - 12.1; SD 4.17)	2

er than males (2,386.36 g vs 391.67 g, t = 5.04; df = 10.20; p <0.001). For morphometrics of additional turtle species captured during the study, see Table 2.

Opportunistic basking survey results

We opportunistically observed 41 basking Spiny Softshells. Nearly a quarter were observed basking in the morning between 0953 and 1200 (*n* = 9) and the majority were observed in the afternoon between 1201 and 1640 (*n* = 32). Over half of the observed basking individuals were recorded on coarse woody debris (*n* = 24; on downed trees and branches) that were touching the bank (*n* = 21) or floating in open water (*n* = 3). The rest of the recorded basking individuals were observed basking on the banks of the reservoir (*n* = 17) either on rocks (*n* = 14) or on coconut logs used for erosion control (*n* = 3).

DISCUSSION

We provide trap capture, visual observation, and morphometric data of Spiny Softshells from a four-month study in a reservoir in northeast Tennessee. Our results of Spiny Softshell morphometrics are consistent with previous studies in human-modified systems. For instance, Plummer and Mills (2015) studied Spiny Softshell growth and longevity in an urban stream in Arkansas, and their mean plastron lengths for females (22.4 cm) and males (10.5 cm) were similar to our results (females: 19.7 cm; males: 10.0 cm).

We found that females were considerably larger than males across nearly all metrics, but sex was not statistically associated with basking site captures. Similarly, we found no evidence that morphometrics were associated with basking site captures. However, our results should be interpreted with caution because we had low sample sizes, and we were not able to ascertain population size distribution. There is also a possibility that turtles were missed due to sampling biases (Mali et al. 2013, Tesche and Hodges 2015). For instance, we used hoop traps positioned next to aerial basking structures as a proxy for thermoregulatory resource use, and a more thorough investigation that accounts for direct observations of basking events (e.g., camera trap surveys and/or basking traps) may reveal different results. While our results do not provide evidence to suggest that larger individuals were associated with basking site captures, similar studies have found that larger Spiny Softshells use basking sites more than smaller ones (Schneider et al. 2019). The reasons behind these findings are unclear. One plausible explanation is that larger individuals have an intraspecific competitive advantage over smaller individuals for resources such as basking sites and basking times. For example, Lindeman (1999) found that, across four freshwater turtle species, larger individuals caused or resisted displacement from basking locations in 70% of all interactions, and that larger turtles elicited avoid-

ance behaviors from smaller turtles, but not vice-versa, suggesting that larger individuals act as a barrier to basking sites for smaller individuals. Similarly, larger body size of the invasive Red-eared Slider (*Trachemys scripta elegans*) was an important predictor of basking site activity over other species (Polo-Cavia et al. 2010), indicating the prominence of body size for thermoregulatory resource competition within and among freshwater turtles.

Larger individuals may have different thermoregulatory requirements compared to smaller individuals. For example, Bulté and Blouin-Demers (2010) found that large female Northern Map Turtles (*Graptemys geographica*) were not able to thermoregulate as optimally as smaller individuals, and that they had much lower maximum body temperatures and a narrower daily range of body temperatures compared to smaller turtles. Similar findings were discovered for other reptile species, where the large Lutz's Tree Iguanas (*Liolaemus lutzae*) of southeast Brazil selected microhabitats with lower temperatures compared to juveniles, possibly to avoid risk of overheating (Maia-Carneiro and Rocha 2013). Likewise, juvenile Diamond-backed Watersnakes (*Nerodia rhombifer*) showed higher thermal tolerance than adults (Winne and Keck 2005), indicating they may be able to tolerate basking resources during hotter time-periods. Other studies on turtle basking dynamics have shown that the duration of basking times between male and female turtles did not differ significantly, even though the investigators predicted that females would bask longer in order to encourage rapid egg production (Lefevre and Brooks 1995; Millar et al. 2012). We suggest that future research focus on the interface between intraspecific basking resource competition and thermoregulatory requirements of Spiny Softshells.

Although we conducted this study in Tennessee's third largest municipal park, an area with considerable undeveloped land, it is at increasing risk of habitat loss and pollution due to sustained urban development and infrastructure expansion in and around its boundaries. The aquatic herpetofauna in this reservoir may therefore be at heightened risk of decline (Brown et al. 2012, but see Plummer et al. 2008). The human pressures already present within and around the park may be exacerbated by climate change (Gibbons 2013), which can alter turtle habitat selection and hatchling survivability (Butler 2019, Parren et al. 2021). While our study cannot elucidate population health and resilience of the Spiny Softshell in this modified system, future research should incorporate estimates of population growth and survival over time using multiple sampling methods (Tesche and Hodges 2015, Butler 2019). Such investigations will be crucial for a better understanding of the downstream effects of human land-use on aquatic turtles, and their resilience under environmental change.

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DIURNAL CHORUSING IN NINE SPECIES OF NORTH AMERICAN ANURANS

MALCOLM L. MCCALLUM[1] AND JAMIE L. MCCALLUM[2]

[1]Aquatic Resources Center, School of Agriculture & Applied Sciences, Cooperative Research and Extension, Langston University, P.O. Box 1730, Langston, Oklahoma 73050, USA, e-mail: malcolm.mccallum.tamut@gmail.com
[2]Northern Oklahoma College, Department of Biology and Agriculture, Tonkawa, Oklahoma 73702

ABSTRACT: A year-long survey of diurnal frog calling behavior was conducted at the Red River Research and Education Park in Shreveport (Caddo Parish), Louisiana to investigate the prevalence of daytime breeding choruses in the species present at the park. We determined that 60% (9/15) of species known from the area participated in daytime breeding choruses. Four of these were new species reports for the behavior. One species was identified entirely based on a daytime chorus from outside the normal breeding season. Although we believe daytime chorusing is widespread in frogs, several species did not diurnally chorus during our study. Diurnal calling may be an important indicator of the peak breeding season for a species and it may also be a useful tool at times when nocturnal surveys are impossible.

Key Words: breeding chorus, diurnal calling behavior, frog calls, Anura, anurans, frogs, toads

INTRODUCTION

Much research has focused on calling behavior in frogs (Gerhardt 1994), but these studies are typically performed at night. In fact, virtually all descriptions of anuran calling behavior are based on nocturnal surveys and observations. However, many species continue calling for mates throughout daytime hours (pers. obs.), despite the lack of attention to this behavior. Although there are mentions of diurnal breeding choruses in the literature (e.g., Mesoamerican Cane Toad (*Rhinella marina*) (Krakauer 1968; Meshaka 2011), the Coqui (*Eleutherodactylus coqui*) (Meshaka 2011), Greenhouse Frog (*Eleutherodactylus planirostris*) (Goin 1947; Meshaka et al. 2004), and the Cuban Treefrog (*Osteopilus septentrionalis*) (Meshaka 2001, 2011)), few studies specifically investigate the prevalence of diurnal breeding choruses in frogs. The only known study specifically approaching diurnal calling in North America was conducted at the U.S. Department of Energy's Savannah River Site in South Carolina and used automated recording systems to continuously record calling behavior (Bridges and Dorcas 2000). It found that Southern Cricket Frogs (*Acris gryllus*), American Bullfrogs (*Lithobates catesbieanus*), Green Frogs (*L. clamitans*) and Eastern Narrow-mouthed Toads (*Gastrophryne carolinensis*) regularly called during the day, albeit less frequently than during the night. Southern Leopard Frogs (*Lithobates sphenocephalus*), Green Treefrogs (*Hyla cinerea*), Pinewoods Treefrogs (*H. femoris*), and Cope's Gray Treefrogs (*H. chrysoscelis*) day-called sporadically, and Barking Treefrogs (*H. gratiosa*) did not call during the day. Further, Spring Peepers (*Pseudacris crucifer*) and Blanchard's Cricket Frogs (*Acris blanchardi*) oviposit during the day and night in New York (Wright 1914) and diurnally chorus early in the breeding season (Kenney and Stearns 2015).

Meshaka and Layne (2015) note diurnal observations of calling among several species in South Florida. Here, rainfall the previous night induced Oak Toads (*Anaxyrus quercicus*), Southern Toads (*Anaxyrus terrestris*), Squirrel Treefrogs (*Hyla squirella*), Southern Chorus Frogs (*Pseudacris nigrita*), Little Grass Frogs (*Pseudacris ocularis*), Eastern Narrow-mouthed Toads and Green Treefrogs to call during the day (Duellman and Schwartz 1958, Meshaka and Layne 2015). Male Squirrel Treefrogs appear to be fertile year-round in South Florida (Meshaka and Layne 2015). Eastern Narrow-mouthed Toads from south-central Florida are known to diurnally call in March (Meshaka and Layne 2015). Southern Cricket Frogs diurnally call throughout the year (Meshaka and Layne 2015). Groups of Pinewoods Treefrogs also sometimes call on sunny days in upland sites in Florida (Meshaka and Layne 2015), but are probably not breeding calls.

Eastern Narrow-mouthed Toads may also day-call in Georgia (Wright 1931), coastal Texas (Pope 1919), and the Grand Cayman Islands (Meshaka and Layne 2015). Pig Frogs (*Lithobates grylio*) and Florida Gopher Frogs (*Rana capita*) occasionally perform breeding calls during the day in Florida (Meshaka and Layne 2015). Reports of

Figure 1. Historical aerial imagery of the Red River Research and Education Park (Shreveport (Caddo Parish), Louisiana) in 2003-2004. Yellow markers indicate each of nine observation stations (Source: Google Earth). Labels are sampling sites pictured in Fig. 2.

Figure 2. Habitats at nine listening stations at the Red River Research and Education Park, Shreveport, Caddo Parish, Louisiana, during 2003–2004. Labels refer to sampling points in Fig. 1. (Photos by Jamie McCallum).

day-calling Pinewoods Tree Frogs also exist for Georgia (Wright 1931) and in the Carolinas (Martof et al. 1980). Of note, Dundee and Rossman (1989) reported large choruses of Eastern Spadefoots (*Scaphiopus holbrooki*) in Louisiana that were sometimes active during the day.

Most studies of frog calling ignore daylight hours, and many guidelines (e.g. North American Amphibian Monitoring Program (NAAMP), FrogWatch) recommend surveys in the early evening hours. There has been much discussion about the importance of natural history in the face of conservation needs (Bury 2006, McCallum and McCallum 2006), and diurnal chorusing data are certainly lacking. Herein, we provide observations demonstrating diurnal calling in multiple species of anurans.

MATERIALS AND METHODS

The study site was an oxbow lake and wetland at the Red River Research and Education Park (a.k.a. C. Bickham-Dickson Park) in Shreveport, Caddo Parish, Louisiana (Population ~ 400,000). This 249 ha urban wetland (Fig. 1) surrounded an oxbow lake that was connected by a small channel to the Red River during most of the year. During the winter, the Red River frequently inundated the park. The vegetation in the park was a mix of native and exotic species (MacRoberts et al. 2008).

We visited the Red River Watershed Research and Education Park between 1100 and 1300 hrs 249 times from 20 September 2003 through 4 January 2005, totaling 249 visits. Some stations could not be visited during floods. Each visit lasted 60–120 min. We drove the perimeter road with the windows down and stopped at nine watch stations (Fig. 1, 2). Whenever frogs were heard calling, we stopped, left the vehicle, listened quietly, then recorded the location and species heard. All sessions were recorded with a hand-held digital audio recorder for later review and verification. For the purpose of this study, isolated single calling males were excluded because these were more characteristic of a rain-induced call than calling for mates. We also recorded ambient temperature and wind speed using a Kestrel® hand-held weather unit and then noted any precipitation during each stop. We could not survey frog calling at night because of city ordinance, locking of the park gate outside of working hours, and a lack of funds for automated recorders. The minimum number of stops during each visit was one per each of the nine stations selected at the beginning of the study. Results were statistically analyzed with regressions using MiniTab 13.0.

RESULTS

Nine species of anurans called diurnally during our study (Table 1). Among those, only the American Toad (*Anaxyrus americanus*) had not been physically observed at the park or in the immediate surrounding area. The Pickerel Frog (*Lithobates palustris*) and Cope's Gray Treefrog were known from the surrounding area and were not previously observed at the site, but were detected via daytime choruses. The Green Treefrog, Gray Treefrog (*H. versicolor*), and Cajun Chorus Frog (*Pseudacris fouquettei*) were previously recorded at the park, but were not detected during our daily visits.

Based on our observations, diurnal and nocturnal chorusing strongly overlapped for Blanchard's Cricket Frog. For this species, diurnal calling surveys may be as useful as nocturnal ones. Six species had daytime detection windows that were shorter than the known nocturnal choruses (Table 1). For example, Cope's Gray Tree Frog breeds from March – July but was only observed chorusing diurnally in October (Table 1). Surveys for these species would be more efficient if performed at night.

There was sufficient data to assess interactions among ambient temperature, wind speed and chorusing in five of the nine species observed calling diurnally (Table 2). Among these five species, only Blanchard's Cricket Frog responded to ambient temperature or wind speed (r^2 = 0.307). Depending on the date, temperature and wind speed influenced whether this species diurnal chorused (Table 3).

DISCUSSION

Previous observations suggest that using a limited listening window in the evening may cause some species to go undetected (Bridges and Dorcas 2000). In fact, our data support this concern. Cope's Gray Treefrog was not previously known at the park; however, we detected diurnal choruses in the fall. This species would have gone undetected had we not surveyed the entire year. Whether fall diurnal chorusing was errant or typical behavior for the region is unknown. However, there have been observations of overwintering Cope's Gray Treefrog tadpoles in Shreveport, (McCallum and McCallum 2004) of a size suggesting fall oviposition. If this species breeds in the fall, its tadpoles would need to overwinter before metamorphosing.

Blanchard's Cricket Frog breeding choruses take place between March and October in the Arkansas Ozarks (McCallum 2003, Trauth et al. 2004). Females with large vitellogenic ova are present from April to August and males have sperm present throughout the year in most of Arkansas (McCallum et al. 2011). Day-calling is prominent from March to September in northwestern Louisiana. By September, females with yolked egg clutches are rare in Arkansas, and the population has largely turned over to young-of-the-year (McCallum 2003, McCallum et al. 2011). Considering the latitudinal differences between northwestern Louisiana and most of Arkansas, diurnal calling closely overlaps the presence of ripe females in the population. We pose that diurnal calling may indicate the peak breeding activity and potentially reflect testosterone levels in male frogs. However, more in-depth studies are needed to validate these two hypotheses.

Our study suggests that diurnal chorusing by anurans might be more widespread than previously known and that failure to consider this may result in undetected but present species in status surveys and inventories. We suspect strongly that this behavior is much more common across species than previous reports would suggest. We found four species that had not previously been reported in the peer-reviewed literature to chorus during the day: American Toad, Fowler's Toad (*Anaxyrus fowleri*), Blanchard's Cricket Frog, and the Pickerel Frog. This may constitute an important tool and consideration for both applied and theoretically-focused herpetologists. Because no animals were directly handled in this study, IACUC approval was not necessary.

Post-script: While this paper was in peer review and an unpublished version posted to BioarXiv (McCallum and McCallum 2018), a continental assessment of diurnal calling was performed in Australia (Callaghan and Rowley 2020) confirming further the widespread nature of diurnal calling.

Table 1. Observations of Anuran species at the Red River Research and Education Park, Shreveport, Louisiana and records of diurnal breeding choruses.

Species	Present?*	Calling Season (NAAMP)	Detection Window		Detectability	
			Earliest Diurnal chorusing	Latest Diurnal chorusing	No. of visits from first to last calling day N/T (%)	Total Visits N/T (%)
Blanchard's Cricket Frog (*Acris blanchardi*)	Yes	March – Oct.**	15 March 2004	3 September 2004	72/87 (83%)	72/248 (29%)
Eastern Narrow-mouthed Toad (*Gastrophryne carolinensis*)	Yes	May – July	4 June 2004	11 July 2004	9/19 (47%) (28 June – 1 July = 4/9 (44%) of calling days)	9/248 (3.6%)
Fowler's Toad (*Anaxyrus fowleri*)	Yes	April – July	4 June 2004	1 July 2004	8/15 (53%) (27 June – 1 July = 5/8 (63%) of calling days)	8/248 (3.2%)
American Bullfrog (*Lithobates catesbeianus*)	Yes	April – July	28 March 2004	27 June 2004	8/49(16%)	8/248 (3.2%)
Southern Leopard Frog (*Lithobates sphenocephalus*)	Yes	January – July	25 January 2004	1 March 2004	5/30 (17%)	5/248 (2%)
American Toad (*Anaxyrus americanus*)	No	Est. Mar – June***	27 June 2004	19 July 2004	2/12 (17%)	2/248 (0.8%)
Bronze Frog (*Lithobates clamitans*)	Yes	March – July	27 June 2004	11 July 2004	2/8 (25%)	2/248 (0.8%)
Pickerel Frog (*Lithobates palustris*)	Maybe	March	1 February 2004	1 February 2004	--	1/248 (0.4%)
Cope's Gray Treefrog (*Hyla chrysoscelis*)	Maybe	March – July	17 October 2004	17 October 2004	--	1/248 (0.4%)
Bird-voiced Treefrog (*Hyla avivoca*)	Maybe	April – July	--	--	--	0/248 (0%)
Green Treefrog (*Hyla cinerea*)	Yes	March – July	--	--	--	0/248 (0%)
Gray Treefrog (*Hyla versicolor*)	Yes	April – June	--	--	--	0/248 (0%)
Squirrel Treefrog (*Hyla squirrella*)	Maybe	June	--	--	--	0/248 (0%)
Spring Peeper (*Pseudacris crucifer*)	Maybe	January – May	--	--	--	0/248 (0%)
Cajun Chorus Frog (*Pseudacris fouquettei*)	Yes	December – May	--	--	--	0/248 (0%)
Rio Grande Chirping Frog (*Eleutherodactylus cystignathoides*)	Maybe	?	--	--	--	0/248 (0%)

*Yes = physically observed in park, No = not physically observed at park or in area, Maybe = not physically observed in park but present nearby.
** NAAMP surveys suggest March –July, but our personal observations in the area suggest this frog calls through October at night.
***No NAAMP records in northern Louisiana, southern Arkansas or northeastern Texas. These dates based on the closest NAAMP route at Vicksburg National Battlefield, Mississippi (510610).

Table 2. Best Subsets Regression results for the possible interaction between the date, temperature, and wind speed on expression of diurnal calling in fives species of anurans.

Blanchard's Cricket Frog (*Acris blanchardi*)

Vars	R²	R²$_{adj}$	C-P	S	Date	Temp	Wind
1	24.1	23.7	21.8	0.4054		X	
1	18.8	18.4	39.1	0.4193	X		
2	29.4	28.7	6.5	0.3919	X	X	
2	26.0	25.3	17.5	0.4011		X	X
3	30.7	29.8	4.0	0.3889	X	X	X

American Bullfrog (*Lithobates catesbeianus*)

Vars	R²	R²$_{adj}$	C-P	S	Date	Temp	Wind
1	4.1	3.6	1.0	0.17987			X
1	0.9	0.5	8.5	0.18282		X	
2	4.5	3.6	2.0	0.17988		X	X
2	4.2	3.3	2.8	0.18018	X		X
3	4.5	3.2	4.0	0.18027	X	X	X

Eastern Narrow-mouthed Toad (*Gastrophryne carolinensis*)

Vars	R²	R²$_{adj}$	C-P	S	Date	Temp	Wind
1	4.8	4.3	4.8	0.18967		X	
1	2.7	2.3	9.8	0.19172	X		
2	6.0	5.2	3.6	0.18880	X	X	
2	5.2	4.4	5.6	0.18960		X	X
3	6.7	5.5	4.0	0.18854	X	X	X

Fowler's Toad (*Anaxyrus fowleri*)

Vars	R²	R²$_{adj}$	C-P	S	Date	Temp	Wind
1	3.6	3.2	5.0	0.18027		X	
1	2.4	2.0	8.0	0.18144	X		
2	5.0	4.1	3.8	0.17940		X	X
2	4.2	3.3	5.7	0.18016	X	X	
3	5.7	4.5	4.0	0.17910	X	X	X

Southern Leopard Frog (*Lithobates sphenocephalus*)

Vars	R²	R²$_{adj}$	C-P	S	Date	Temp	Wind
1	0.6	0.2	0.6	0.14568			X
1	0.3	0.0	1.4	0.14592		X	
2	0.8	0.0	2.2	0.14586		X	X
2	0.8	0.0	2.2	0.14587	X		X
3	0.9	0.0	4.0	0.14613	X	X	X

Table 3. Results from Binomial logistic regression for the influence of the date, wind speed and temperature on the occurrence of calling by male Blanchard's Cricket Frogs (*Acris blanchardi*).

Response Information

Variable	Value	Count
Calling	1	72
No calling	0	159
Missing values	--	17

Logistic Regression Table

Predictor	Coefficient	SE Coefficient	Z	P	Odds Ratio	95% CI Lower	95% CI Upper
Constant	-191.41	62.32	-3.20	0.001			
Date	0.00509	0.001641	3.10	0.002	1.01	1.00	1.01
Temperature	0.16651	0.03313	5.03	< 0.001	1.18	1.11	1.26
Wind Speed	0.3466	0.1440	2.41	0.016	1.41	1.07	1.88
Log-Likelihood	-101.431						

Test that all slopes are zero

G	df	P
83.784	3	< 0.001

Goodness-of-Fit Tests

Method	Chi Square	df	P
Pearson	181.425	227	0.988
Deviance	202.862	227	0.874
Hosmer-Lemeshow	26.494	8	0.001

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NOTES ON THE OPERATION OF TWO TYPES OF AQUATIC REMOTELY OPERATED VEHICLES USED DURING A MOCK FRESHWATER TURTLE SURVEY

ERIC H. DIEFENBACHER*, MARGARET FALL, SEAN MCPHERSON, JASON ROBERTS, AUSTIN LAFORCE, THOMAS TRACY, MATHEW VON DAUBER, CAMRYN O'DONNELL, MADELYN CURRIER, PETER WILSON, JOSHUA CRANDALL

Division of Environmental & Renewable Resources, School of Agriculture, Business & Technology, SUNY Morrisville, P.O. Box 901, 80 Eaton St., Morrisville, NY, USA 13815

Correspondence: Prof. Eric H. Diefenbacher, diefeneh@morrisville.edu

ABSTRACT: Aquatic remotely operated vehicles (ROV) show merit in providing *in situ* observations of sea turtles and freshwater turtles. However, turtles must be spotted above the water surface first then an ROV deployed for underwater observation. None have been used as a tool to survey for turtles solely under the water surface without direct observation first. Here we report on observations of two types of aquatic ROV used during a mock turtle survey to determine the potential of freshwater turtles being found under the water surface without being directly observed first and if accurate species identification could be done.

Key Words: ROV, Freshwater turtle

INTRODUCTION

Freshwater turtles play critical roles in their respective environments (Congdon et al. 1986; Mitchell 1988; Shine and Iverson 1995; Ernst and Lovich 2009; Mitchell and Buhlmann 2009; Lovich et al. 2018) and studying them *in situ* can be inherently difficult given their natural history. Most field study methods consist of snorkeling, sounding pole surveys, visual encounter with or without binoculars from a shore or boat, or trapping with baited and un-baited hoop nets or basking traps (MacCulloch and Gordon 1978; Vogt 1980; Sterrett et al. 2010). Traps can be difficult to transport and time consuming to set up and check frequently, with most studies involving several trap days. Observations of aquatic and semi-aquatic turtles *in situ* are often limited to surface activity, such as basking or foraging, making underwater behaviors in an environment largely underrepresented. Recent advancements with aquatic remotely operated vehicle (ROV) technology, also known as aquatic drones, are becoming useful tools in aquatic systems monitoring (Pedrosa de Lima et al. 2020), and show some merit in observing turtle behavior in marine and freshwater studies (Smolowitz et al. 2015; Karcher 2019). In these situations where aquatic ROVs have been used to view turtle behavior, the aquatic ROV was deployed after a turtle was spotted by direct observation from a boat or shore, by an unmanned aerial vehicle (UAV), or by tracking a satellite tag signal.

None of these techniques have been used to fully survey for turtles in freshwater environments where individual turtles can remain submerged and initially out of sight of the operator. Presumably, an aquatic ROV used to survey submerged turtles without the surveyor spotting the turtle at the surface first can also allow the operator to find and make accurate species identifications while operating in real time and later during image or video review. Here, we report on observations gained from a mock turtle survey meant to determine if accurate species identifications could be made in freshwater environments under optimal operating conditions for two types of aquatic ROVs.

MATERIALS AND METHODS

Aquatic ROVs: Power Vision Power Ray and Power Vision Power Dolphin (PowerVision Inc., Beijing, China) aquatic ROVs were chosen based on their midlevel price range comparable to other consumer level aquatic ROVs (Figure 1). Both devices required a remote control and either a cell phone or tablet with an installed software program to operate the drone. For this study a Samsung Galaxy A50 cell phone with Android version 11 operating system was used with the Power Dolphin along with the installed software program Vision+2 required for operation. An Apple iPAD mini with iOS 9.3.6 operating system was used along with the installed software Vision+ required for operation with the Power Ray. Communication

Fig. 1. Power Ray and remote control (left) and Power Dolphin and remote control (right).

between the drone, remote control, and digital device occurred through a built in wifi signal.

The Power Ray had the capability to submerge in the water column as well as to propel across the water surface. Two propellers were oriented horizontally in the back for forward, reverse, and pivoting maneuvers. One propeller was situated vertically to allow ascension or descension in the water column. The camera was fixed on the front of the drone, offered a 95° field of view, and had no ability to pan in any direction independently of the ROV body. An external hard drive was attached to a communication cable 50m long that screwed into the top of the drone. During operation the hard drive stayed with the operator, while the cable and ROV were placed in the water.

The Power Dolphin floated on top of the water and was propelled by two rear propellers that were oriented horizontally. The camera was mounted on the front of the ROV with a user-adjustable tilt mechanism that could be oriented up and down for initial positioning, and operated remotely to pan up and down in real time using a remote control independent of the direction the ROV was moving along the surface. Field of view for the camera was 132°. For this study the camera was angled at 45° under the surface.

Study Site: Trials were conducted as transect surveys at Rogers Environmental Education Center in Sherburne, Chenango County, New York in an ~0.5-hectare pond. Submergent aquatic vegetation was nearly absent, and the water column was clear enough to view the bottom of the pond (maximum depth 5 m). Bottom sediments consisted of silt, sand, small boulders, and low growing vegetation. This site was chosen to simulate optimal operating conditions recommended by the manufacturer.

Mock Survey Design: Three turtle shells were submerged in random locations on a transect line 25m long at a depth of 1m and at a distance ~2m from the shoreline. Shells of a Snapping Turtle (*Chelydra serpentina*) 287mm carapace length (CL), Red-eared Slider (*Trachemys scripta elegans*) 208mm CL, and Eastern Musk Turtle (*Sternotherus odoratus*) 88.6mm CL were designated as large, medium, and small-sized turtles, respectively.

Aquatic ROVs were driven along a transect line by five different operators each time recording video and capturing still images. The operator did not know where the turtle shells were placed on the transect line. For video, each operator positioned the ROV at the beginning of the transect and initiated recording before the ROV was driven down the transect line. At the end of the transect line, video recording was turned off. For still images, this procedure was repeated, however, the operator would take a picture for each presumed turtle shell on the screen of the digital receiver device being used. Images and video were later reviewed in the lab for clarity and quality in identifying species of turtle shells.

RESULTS

Both the underwater drone (Power Ray) and surface drone (Power Dolphin) provided clear images and video of the turtle shells along the transect (Figure 2). Identification of species based on the shell could be done in the field in real time with five out of five operators, 100%, visually confirming *C. serpentina* and *T. s. elegans* during their respective trial run, while *S. odoratus* was confirmed by only two of the five operators, 40%. Image and video review in the lab by the operators showed complimentary results to *in situ* observations with 100% of the operators identifying *C. serpentina* and *T. s. elegans* during the first video playback. However, only three out of five operators, 60%, were able to identify *S. odoratus* during the first video playback. Two additional playbacks were needed in order for all operators to identify *S. odoratus*. The difficulty in confirming *S. odoratus* was attributed
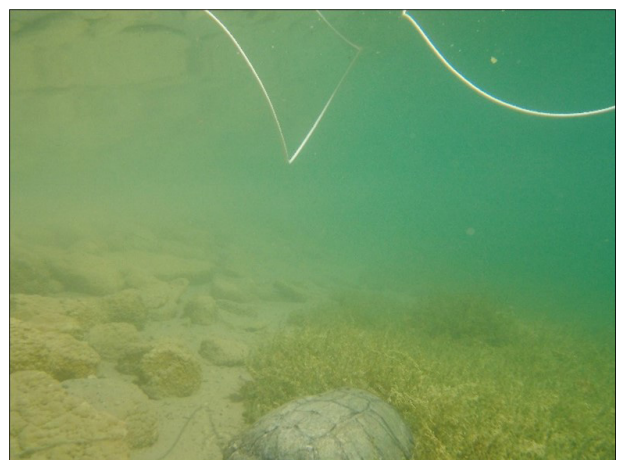

Fig. 2. Images of a Snapping Turtle (*Chelydra serpentina*) shell taken from Power Dolphin surface drone (left) and Power Ray underwater drone (right) during transect surveys while drone was moving over the transect.
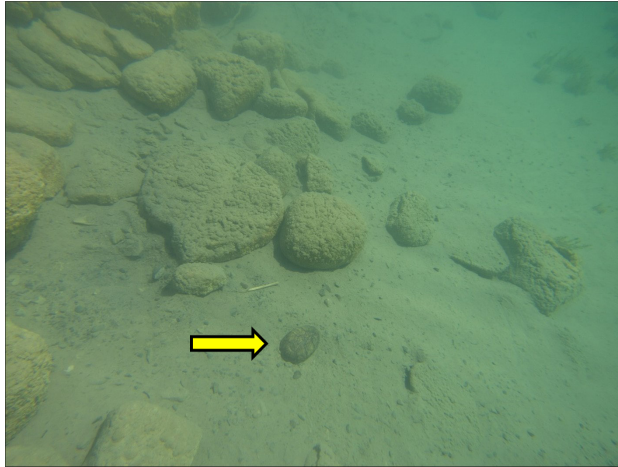
Fig. 3. Image taken from Power Dolphin surface drone showing shell of an Eastern Musk Turtle (*Sternotherus odoratus*)- (arrow).

to its superficial resemblance to rocks on the pond bottom (Figure 3). Viewing still images yielded better results with all five operators able to identify all shells upon first image review in the lab.

DISCUSSION

The objective of this exercise was to determine if aquatic ROVs allowed for accurate species identification without first having spotted the turtle. Testing under optimal conditions yielded accurate species identifications both in the field and in the lab for medium and large sized turtle shells. However, it was noted that small-sized turtles may be harder to spot if they are stationary in the water column. Further testing is also needed to determine if species with similar shell morphologies can be distinguished between one another, something we did not test in this study.

There were some operational difficulties worth noting. For the Power Ray, the operators had difficulty keeping the ROV on a straight path at a constant depth when it was submerged, even with data such as tilt, depth, and yaw displayed on the tablet screen. This difficulty resulted in more difficult image acquisition as the camera on the Power Ray was fixed and offered only a 95° field of view. Therefore, the camera angle and field of view offered to the operator was limited to the direction the operator was driving the ROV. At times, the bottom of the pond could easily drift out of view during operation requiring the operator to drive the ROV very close to the bottom of the pond to view any potential turtle shells on the transect. This also increased the instances of times when large rocks and other obstructions had to be avoided. If the ROV drove too close to the bottom sediment, silt would plume up from the bottom and obscure the camera image temporarily. Although the images and video were clear, it was obvious that organisms on the bottom of the pond were going to be difficult to find. The communication cable of the Power Ray often got caught on itself and vegetation, effectively limiting the range and depth at which the ROV could be used. We found that while using this ROV, a two-person operation was best whereby one person operated the ROV while the other made sure the communication cable remained untangled.

The Power Dolphin, by comparison, was always visible at the surface making driving in a straight line, potential course corrections, and avoiding obstacles very easy. While this ROV could navigate a preprogrammed path, this feature was not used in this exercise due to the inability of the GPS signal to locate the correct area;

however, this feature could prove useful in future surveys and should be investigated. The camera could easily be panned up and down remotely from the bank, but this feature was not necessary for this exercise and, therefore, remained fixed. This feature may also be useful in future surveys whereby turtles could be spotted at the surface first then followed, and the camera can be panned down to watch the turtle as it descends through the water column. The 132° field of view of the camera offered a wide view of the submerged environment with resulting images and video very clear, making it easy to spot turtle shells. Video from the video recording surveys appeared fast-moving. This may have been attributed to the speed, depth of shells, and 45° camera angle at which the ROVs was set, thereby requiring video to be reviewed at least twice to determine if a turtle shell was accurately spotted. We did not view video at slower playback speed nor frame-by-frame. Future studies could use these playback features which may help identify turtle shells.

The manufacturer recommends ROVs be used in optimal conditions to function appropriately. Vegetation and other obstructions could get caught in the propellers during surveys, presumably resulting in wasted survey time as the ROV is dislodged from vegetation which could also damage the propellers as well as disrupt turtle behavior. Conditions such as weather, water clarity, visibility, underwater terrain, and flow could make surveys using ROVs challenging for searches where freshwater turtles are not spotted from a bank or boat first. The trials described here were conducted on clear days with no cloud cover, which lead to the sun's glare obstructing underwater views depicted on tablet and cell phone screens. In such instances, having a sun shade surround the screen would have been useful and is highly recommended.

To our knowledge, no published literature exists regarding the potential for using aquatic ROV's to survey turtles under the water surface without first spotting them above the water. Results from this study indicate that with some limitation, aquatic ROVs can be useful tools in finding fresh-water turtles *in situ*. Despite some operational difficulties, aquatic ROVs have the ability to investigate the water column in a unique way. With practice, efficient operators could make accurate species identifications and conduct thorough underwater surveys, thereby in part closing the gap in *in situ* observations of aquatic turtle biology.

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