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Population Surveys and Health Assessments of Captive and Free-Ranging Alligator Snapping Turtles

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POPULATION SURVEYS AND HEALTH ASSESSMENTS OF CAPTIVE AND FREE-RANGING ALLIGATOR SNAPPING TURTLES

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Master of Science, Biology

By

Samantha Louise Hannabass

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ABSTRACT

Alligator Snapping Turtles (*Macrochelys temminckii*) have experienced range-wide declines primarily due to overharvest for the meat market and habitat degradation in the form of damming and channelization of rivers. Head-start programs and reintroduction efforts have been initiated to release individuals throughout their historic range. Before releasing Alligator Snapping Turtles, sites need to be assessed to determine the suitability of habitat, if there is a robust turtle community already present, and the causes of the original extirpation have been eliminated. I assessed the turtle communities and documented anthropogenic impacts (e.g. boat traffic) at nine possible reintroduction sites in southeastern Kansas in the Caney, Verdigris, Fall, and Elk river drainages as possible reintroduction sites. Alligator Snapping Turtles were not detected at any of the nine sites in Kansas. The Verdigris River near Coffeyville, Kansas appears to be a suitable site to release Alligator Snapping Turtles due to the high aquatic turtle species diversity. A population of reintroduced Alligator Snapping Turtles exists on the Caney River between Hulah Lake and the Oklahoma-Kansas state border. I assessed the health of this reintroduced population in addition to a wild population and two captive populations. No individuals or populations were obviously unhealthy, but I found some hematological and plasma biochemical differences among populations—primarily due to dietary and ontogenetic factors. Further sampling efforts would be beneficial to fully understand the extent of the range of Alligator Snapping Turtles in Kansas and for identifying additional suitable release sites. The lack of negative differences between wild and reintroduced Alligator Snapping Turtle health further supports that this is a suitable species to reintroduce into its historical range.

KEYWORDS: Alligator Snapping Turtle, aquatic turtle community, health assessment, hematology, plasma biochemistry, reintroduction ecology

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OVERVIEW

Reptiles have experienced worldwide declines in recent years due to obvious factors—habitat degradation and loss, unsustainable rates of harvest, and introduction of invasive species—as well as less obvious factors such as disease, pollution, and climate change (Gibbon et al. 2000). Among the affected taxa, turtle populations have been observed exhibiting drastic declines because they are a major contributor to the exotic meat market and pet trade (Thorbjarnarson et al. 2000; Rhodin et al. 2018). In the United States, Softshell Turtles (*Apalone* spp.), Map Turtles (*Graptemys* spp.), Diamondback Terrapins (*Malaclemys terrapin*), Common Snapping Turtles (*Chelydra serpentina*), and Alligator Snapping Turtles (*Macrochelys temminckii*) are the most targeted species for the meat market, which has been a contributor to population declines (Thorbjarnarson et al. 2000; Pritchard 2006; Nickerson and Pitt 2012). Habitat loss and degradation has also played a major part in the decline of many turtle species (Moll and Moll 2000; Throbjarnarson et al. 2000). Damming and channelizing rivers can have dramatic impacts on aquatic turtle populations by changing habitat diversity, fragmenting populations, and altering natural flow cycles (Moll and Moll 2000).

Alligator Snapping Turtles typically inhabit a single river drainage for the span of their lives and are only found on land during a nesting event (Reed et al. 2002) which makes them particularly susceptible to habitat modifications such as dams, spillways, and dredging (Riedle et al. 2008a). Alligator Snapping Turtle populations have declined throughout their range—particularly along the periphery of the range—due to overharvest and habitat degradation or modification (Shipman et al. 1995; Reed et al. 2002; Riedle et al. 2005; Shipman and Riedle 2008; East et al. 2013a; Lescher et al. 2013; Baxley et al. 2014). This species has been listed in Appendix III of the Convention on International Trade in Endangered Species (CITES) to

prevent overharvest for the international meat market and pet trade (U.S. Fish and Wildlife Service and Department of the Interior 2005).

In Oklahoma and Kansas, surveys have been conducted to document the current distribution of Alligator Snapping Turtles. In Oklahoma these studies found many populations have been reduced to levels that are unlikely to recover naturally or that have been extirpated from parts of their historical range, which covers the eastern one-third of the state (Riedle et al. 2005; Riedle et al. 2008b). No individuals of this species have been found in Kansas since 1991 when an individual was captured by fishermen in a tributary of the Verdigris River, which likely indicates extirpation from this portion of its range (Shipman et al. 1995). Oklahoma has listed Alligator Snapping Turtles as a Tier I Species of Greatest Conservation Need and placed a closed-season restriction on them to eliminate pressures caused by overharvest (Riedle et al. 2005; Oklahoma Comprehensive Wildlife Conservation Strategy 2016). Alligator Snapping Turtles have been on Kansas' Species in Need of Conservation list since 1987 (KDWP 1986), after being downgraded from a Threatened status in the state due to lack of evidence of reproducing populations (Shipman et al. 1995).

Head-start programs are assumed to be beneficial for some turtle species by increasing recruitment and survival rates (Moll and Moll 2000; Dreslik et al. 2017). Head-start programs begin by collecting and incubating eggs from either captive or wild populations. Hatchlings are reared until they reach a size perceived to increase survival over that of hatchlings in the wild (Flanagan 2000; Moll and Moll 2000). Head-started juveniles are then released at sites selected based on the following factors: being within the turtles' historical range; absence of any of the factors that led to the original extirpation; and, in the case of Alligator Snapping Turtles, the

presence of a robust turtle community to provide evidence that the habitat is generally suitable to support aquatic turtles (Riedle et al. 2008b; IUCN/SSC 2013).

An Alligator Snapping Turtle head-start program was initiated at Tishomingo National Fish Hatchery (TNFH) in 1999 when it became evident there were state-wide population declines in Oklahoma (Riedle et al. 2008b). Brood stock adults were collected from Sequoyah National Wildlife Refuge (Riedle et al. 2008b; Dreslik et al. 2017) and maintained in outdoor ponds at TNFH. Their eggs were collected each year, with the first clutches produced in 2002, and incubated to reduce predation risk. Hatchlings were then reared in captivity for at least two years until they were deemed to be large enough to reduce predation risk.

While surveys have been conducted in southeastern Kansas to determine the current distribution of Alligator Snapping Turtles (Shipman et al. 1995; Riedle et al. 2008b), preliminary studies show at least 100 net nights are needed to detect this species at low densities (Voves, *in preparation*). The goal of Chapter 1 is to report the results of additional sampling to improve the understanding of the present distribution of Alligator Snapping Turtles in Kansas and to provide the groundwork for identifying suitable reintroduction sites for this species.

The Caney River in northeastern Oklahoma was identified as a suitable reintroduction site based on extensive suitable habitat, its robust turtle community and the fact that this species occurred historically in this system (Glass 1949; Riedle et al. 2008a). Additionally, this site had reduced anthropogenic stressors than many other nearby rivers and reservoirs (Hollender et al. 2018). Between 2008 and 2010, 246 juvenile Alligator Snapping Turtles, ranging 3–7 years old, were released into the Caney River (Anthony et al. 2015). Since their release, this population of Alligator Snapping Turtles has been monitored to track growth and survival rates of the reintroduced turtles (Anthony et al. 2015; Dreslik et al. 2017).

Outcomes of wildlife reintroduction initiatives were understudied until the early 2000s (Seddon et al. 2007). Until that point, most reintroduction efforts were not well planned and research relating to the establishment of the population and the outcome was lacking (Seddon et al. 2007). There is no universally applicable definition of success in reintroductions, but several endpoints have been proposed: high rates of survival of released animals, reproductive success of released animals and their offspring, and the overall persistence and trajectory of the population (Seddon 1999).

Key to monitoring threatened and endangered species—as well as reintroduced populations of such species—are health assessments. Health assessments allow researchers to determine the overall condition of individuals in a population, which can influence population dynamics (Flanagan 2000) and indicate the level of environmental stress the population is exposed to (Milton and Lutz 2003). Additionally, health assessments can provide early warnings of threats to the condition of individuals in a population, giving conservationists time to correct or ameliorate stressors that threaten the persistence of a population.

Many studies have established hematological reference ranges of either wild or captive groups of a species (e.g. Anderson et al. 1997; Christopher et al. 1999; Dickinson et al. 2002; Chaffin et al. 2008; Perpiñán et al. 2008; Rose and Allender 2011; Andreani et al. 2014), but relatively few studies have compared values among populations (Brenner et al. 2002; Rangel-Mendoza et al. 2009). Comparisons among populations can be challenging since many factors influence biochemical and hematological values (e.g. sex, habitat quality, season, food availability and food type) (Yu et al. 2013, Yang et al. 2014).

The goal of Chapter 2 is to compare hematologic and plasma biochemical values between the reintroduced population of Alligator Snapping Turtles on the Caney River, TNFH indoor and

outdoor populations, and a wild population on the Poteau River to observe any potential impacts the habitat or rearing method are inflicting on the reintroduced population. Baseline hematological values have been established only in the extreme southeastern portion of the species' range (Chaffin et al. 2008). I sought to compliment this previous work with data from populations inhabiting the northern extents of the species' range where abiotic conditions differ.

SURVEYS OF FRESHWATER TURTLE COMMUNITIES TO DETERMINE PRESENCE OF ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*) AND IDENTIFY POTENTIAL REINTRODUCTION SITES IN KANSAS

Abstract

Range-wide declines in Alligator Snapping Turtle (*Macrochelys temminckii*) populations have led to the initiation of head-start programs to reintroduce this species back into its historical range as well as spurring sampling efforts to further explore the current extent of their range. In this study the potential extent of this species' distribution was explored in southeastern Kansas. Alligator Snapping Turtles were not found at any of the nine sites sampled in southeastern Kansas, but robust aquatic turtle communities were found at numerous sites. Additional trapping effort at previously sampled locations and more sample locations need to be examined to fully understand the extent of Alligator Snapping Turtle presence in Kansas. The Verdigris River near Coffeyville, Kansas, appears suitable for reintroducing Alligator Snapping Turtles; the Caney River near the town of Elgin would be a good additional release location to extend the distribution of the population already present on this river south of the Kansas-Oklahoma border.

Introduction

The Alligator Snapping Turtle (*Macrochelys temminckii*) is the largest freshwater turtle in North America, reaching sizes over 113 kg, and occurs in rivers draining into the Gulf of Mexico (Pritchard 2006). Due to its large size and aquatic nature, Alligator Snapping Turtle populations have experienced declines resulting from multiple anthropogenic stressors including commercial harvest, fragmentations of rivers by dams, and degraded water quality

(Thorbjarnarson et al. 2000; Reed, Congdon and Gibbons 2002; Moll and Moll 2004; Pritchard 2006; Riedle et al. 2008; Ernst and Lovich 2009).

These historic declines have spurred interest in the species' status and subsequent recovery. The Alligator Snapping Turtle was originally listed as a Category 2 (C2) species and was first petitioned for federal listing under the Endangered Species Act in 1983. The 90-day finding stated that the information presented in the petition was substantial enough to support further review for listing as Threatened or Endangered (USFWS 1983). The United States Fish and Wildlife Service (USFWS) reported in the 12-month finding for the 1983 petition that listing was not justified at that time (USFWS 1984). This decision was based upon conflicting reports on the species' status throughout its range and stated that more information was required to better resolve the species' status. The 1994 candidate review by the USFWS found the population status of the Alligator Snapping Turtle to be declining (USFWS 1994). In 1996 the USFWS eliminated the C2 category and the Alligator Snapping Turtle was removed from the list of candidate species for federal listing (USFWS 1996).

In response to the 1984 USFWS findings and the first publication of Pritchard (2006), status surveys were initiated throughout much of the range of the species. Surveys in the core of the species' distribution, including Alabama (Folt and Godwin 2013), Georgia (Jensen and Birkhead 2003), Louisiana (Boundy and Kennedy 2006), and Arkansas (Trauth, Wilhide and Holt 1998; Howey and Dinkelacker 2013), presented considerable evidence for significant population declines. This earlier survey work also revealed that some populations appear to be stable or increasing, while others have been extirpated or have declined significantly. Most notable are surveys along the northern and western periphery of the species' range. Surveys in Kentucky (Baxley, Barnard and Venter 2014) and Kansas (Shipman, Edds and Shipman 1995)

failed to detect the species, suggesting they were extirpated or only persist at very low densities. Multiple surveys in Missouri (Shipman and Riedle 2008; Lescher, Briggler and Tang-Martinez 2013) and Oklahoma (Riedle et al. 2005; East, Riedle and Ligon 2013) reported both historic and ongoing declines. In response to these findings the Center for Biological Diversity (CBD) submitted a petition to the USFWS to list the Alligator Snapping Turtle as either Threatened or Endangered under the Endangered Species Act (CBD 2012). The 90-day finding on this petition stated that substantial information was presented indicating that listing may be warranted (USFWS 2015).

In light of the legal history surrounding the status of the Alligator Snapping Turtle, conservation efforts involving multiple partners were initiated on the western edge of its distribution in Kansas and Oklahoma. Research on the population status of Alligator Snapping Turtles in Kansas and Oklahoma includes state distributional surveys (Shipman, Edds and Shipman 1995; Heck 1998; Riedle et al. 2005), habitat selection and utilization (Riedle et al. 2006; Moore et al. 2014), and demography (Riedle et al. 2008; East, Riedle and Ligon 2013). Populations in both states have experienced historic declines, localized extirpations, and fragmentation resulting from dams (Riedle, Ligon and Graves 2008). Because the species is highly aquatic and rarely travels over land (Pritchard 2006) impoundments prohibit natural movements along rivers. However, while impoundments created isolated populations that are prone to extirpation, many segments of river between dams remain prime habitat for the species. Therefore, current conservation efforts in these two states emphasize the need to conserve extant populations and re-establish the species in stream segments where it has been extirpated.

To facilitate re-establishment of extirpated populations, a captive breeding program was established in 1999 at Tishomingo National Fish Hatchery in southern Oklahoma (Riedle et al.

2008). In order to improve success of the captive breeding program, research was conducted on incubation and temperature dependent sex determination (Ligon and Lovern 2009), sex determination techniques for juveniles (Ligon et al. 2014) and feeding behavior of captive reared turtles (East, Fillmore and Ligon 2013). Experimental releases of adults were conducted in wetlands associated with the Washita River in southern Oklahoma (Moore et al. 2013; 2014). Reproduction, nest site selection, and sex ratios of wild nests were monitored in this introduced population (Miller and Ligon 2014; Miller et al. 2014). Results from these studies suggest that Alligator Snapping Turtles respond well to translocation and will reproduce.

Historically, Alligator Snapping Turtle populations in Kansas were contiguous with populations in Oklahoma, specifically within the Caney, Verdigris, and Neosho rivers. Additionally, these rivers were identified as suitable release sites for head-started Alligator Snapping Turtles in Oklahoma (Riedle, Ligon and Graves 2008). Several releases occurred 2008–2010, and extensive post-introduction monitoring has taken place on the Caney River between Hulah Lake and the Kansas border. Five years of post-release monitoring (2008–2012) revealed moderate to high survivorship rates that depended on the age of the turtle at the time of release. Turtles that did survive experienced surprisingly fast growth rates (Anthony et al. 2015). Early metrics, including survival and post-release growth rates suggest that the program has been successful, at least in the short term. Additional groups of juvenile Alligator Snapping Turtles have since been released on the Verdigris River between Oologah Lake and the Kansas border and the Neosho River upstream from Grand Lake O' the Cherokees and to within 15 river km of the Kansas border (Brian Fillmore, Tishomingo National Fish Hatchery, pers. comm.).

Populations near the periphery of the geographical range of the species represent the edge of climatic, landscape, and anthropogenically-induced changes limiting the distribution of a

species (Thompson et al. 2017). Protection of peripheral populations is important, as loss of populations at the edge of a species distribution result in core populations becoming peripheral populations, which can greatly hinder recovery (Steen and Barrett 2015). This phenomenon may be exacerbated with current taxonomic revisions to the genus, splitting *Macrochelys* into at least two distinct species (Thomas et al. 2014; Folt and Guyer 2015), eliminating some populations in portions of Georgia and Florida. Continued support for proactive conservation efforts—including monitoring of current populations and reintroduction initiatives—along the western edge of the Alligator Snapping Turtle's distribution is integral in maintaining and conserving the species throughout its entire range.

Reintroduction sites are selected based on several criteria: being within the turtles' historic range; melioration of the factors that led to the original extirpation; persistence of suitable habitat; and the presence of a robust turtle community (Riedle, Ligon and Graves 2008 ; IUCN/SSC 2013). Examination of the aquatic turtle community is a critical component to identifying potential reintroduction sites because it can indicate possible pressures that are already present in the system, such as: increased nest predation (Shipman 2019), the energy input in a system (Lawton 1999), and harvest pressures (Eisemberg et al. 2011). For example, if there are few hatchlings and small juveniles in a population, it may indicate that predation rates of nests or hatchlings are high (Shipman 2019) and could have a negative impact on the natural recruitment of a reintroduced population. Understanding the community dynamics of aquatic turtles in a system is a major step in determining the suitability of potential reintroduction sites for Alligator Snapping Turtles. Additional sampling in Kansas will provide more information on the distribution of Alligator Snapping Turtles where it has been poorly studied and provide a basis for selection of potential reintroduction sites.

The goal of this chapter is to document the current distribution of Alligator Snapping Turtles in southeastern Kansas and use aquatic turtle community structure as one component of assessing the suitability of potential reintroduction sites.

Methods

All procedures involving the handling or manipulation of animals were approved by the Missouri State University Institutional Animal Care and Use Committee (protocol 19-015.0-A; Appendix A).

I sampled nine sites along the Verdigris, Caney, Elk, and Fall rivers from 2017–2019 (Table 1). I used 0.9-meter hoop nets with 2.5-cm mesh that were baited with frozen fish and left overnight to capture turtles. In 2017, species and sex of every individual were recorded. In 2018, carapace length, plastron length, mass, sex, and species of each individual were recorded, and a shallow notch was applied to a posterior marginal scute to signify it was captured previously. In 2019, carapace length, mass, sex, and species of each individual were recorded at all sites. Plastron length was not recorded at the Elk River site in 2019. The same trapping methods were used at the Caney River Alligator Snapping Turtle reintroduction site in Oklahoma 2017–2019 and all species were recorded (Appendix B).

I calculated species richness and catch per unit effort (CPUE calculated as total number of captures divided by number of net nights) for each site in Kansas as well as for each year at the Caney River in Oklahoma. Aspects of community membership were quantified at each site by calculating the species richness, Simpson's Index (D) and species evenness (E), and Bray-Curtis similarity indices were used to compare composition among sites. Simpson's index accounts for the number of individuals of each species at a site, with larger numbers indicating

greater diversity. Similarly, large evenness values indicate that the number of individuals of each species at a site is even. The Bray-Curtis similarity ranges from 0 to 1, whereby two sites approaching 1 are more similar and two sites approaching 0 are dissimilar. Non-metric multidimensional scaling (NMDS) analyses were used to visualize similarity of species composition at each site. Bray-Curtis index was used to calculate distance matrices in two dimensions using 20 random starts. Size classes of each species was also examined at each site from 2018–19. All statistical tests and graphics were executed using RStudio (R version 3.5.2 "Eggshell Igloo").

Results

Five sites were sampled in 2017, for a total of 480 captures over 656 net nights (Table 2). These sites included the Caney River near Elgin, Kansas, and a tributary—Cedar Creek—as well as the Verdigris River near Coffeyville, Kansas, and two tributaries—Pumpkin Creek and Big Hill Creek. Two sites were sampled in 2018, for a total of 113 captures over 62 net nights (Table 3). These sites included the Verdigris River near Sycamore, Kansas, and the Elk River upstream of Elk City Lake. I observed heavy boat traffic during my sample effort at the Elk River site in 2018 which corresponded with the destruction of several traps. I decided to shorten the trapping period at the Elk River site in 2018 and add additional trap nights at this site in 2019. I also sampled two new sites in 2019—Verdigris River near Toronto Lake and Fall River near Fall River Lake (Table 3).

I did not detect Alligator Snapping Turtles at any site, but I did detect a suite of other aquatic turtle species depending upon site. Reproductively mature *Trachemys scripta* made up the majority of individuals captured at the Verdigris River at near Sycamore, the Elk River,

Verdigris River at Toronto, and Fall River (Figure 1; Ernst and Lovich 2009). The *Graptemys ouachitensis* population at the Elk River was mostly composed of large females and reproductively mature males (Figures 2 and 3; Lindeman 2013) while the populations at the Verdigris River at Toronto and the Fall River supported individuals of a range of sizes from juvenile to adult. With the exception of four individuals, all *Apalone spinifera* were above the minimum size threshold for sexual maturity (Figures 4 and 5; Ernst and Lovich 2009). There were not enough individuals captured from the Verdigris near Sycamore to characterize size distributions for species other than *T. scripta*. There were also insufficient captures of *G. pseudogeographica*, *Chelydra serpentina*, *Pseudemys concinna*, or *Chrysemys picta* to characterize size distributions at any site (Table 3).

The highest CPUEs occurred during fall sampling in 2019 on the Verdigris River at Toronto, Fall River, and Elk River (Table 4). The lowest catch rate (CPUE = 0.5390) was in the summer of 2017 which was characterized by extensive flooding. The smaller tributaries that were sampled (Cedar Creek, Pumpkin Creek, Big Hill Creek) yielded slightly higher catch rates (CPUE = 0.8300–1.0417) compared to the larger rivers into which they flowed (CPUE = 0.5390–0.7770; Table 4). The lowest Simpson's index scores were derived from the smaller tributaries and the small section of the Verdigris River I could access (Table 4). The longer sections of rivers sampled—with the exception of the Verdigris River at Toronto—yielded higher Simpson's index scores (Table 4). Species evenness was moderately low at all of the sampled sites (E = 0.3056–0.4879), probably due to high capture rates of *T. scripta* (Tables 3 and 4).

The three sites on the Verdigris River had moderate to high similarity in community structure to each other (Table 5; Figure 6). The Verdigris River near Coffeyville was moderately

similar in community structure to its tributary Pumpkin Creek based on the Bray-Curtis similarity index but appeared distant on the NMDS plot (Table 5; Figure 6). Pumpkin Creek also had low similarity to another sampled Verdigris River tributary, Big Hill Creek (Table 5). The Caney River near Elgin and its tributary Cedar Creek were also moderately similar in community structure (Table 5; Figure 6. All the sites sampled in Kansas were highly similar in community structure to the Caney River in Oklahoma where Alligator Snapping Turtles have been reintroduced based on the Bray-Curtis similarity index (Table 5) and this community was also moderately central on the NMDS plot (Figure 6).

Discussion

Turtle trapping in southeastern Kansas revealed no remnant populations of Alligator Snapping Turtles at the sites sampled. However, preliminary analyses suggest at least 100 net nights are needed to detect low density populations of Alligator Snapping Turtles (Voves, *in preparation*) and only four of my sites had sufficient effort to meet this threshold. Confounding factors such as flooding, trap vandalism and theft, and a variety of other unforeseen events prevented us from reaching my minimum goal of 100 net nights at each site. Additional surveys would be necessary to confidently say Alligator Snapping Turtles are not present in the waterways sampled. Additionally, to fully assess the current distribution of Alligator Snapping Turtles in southeastern Kansas, more sites need to be sampled. The same confounding factors that prevented us from accomplishing 100 net nights at each site prevented us from sampling sites on the Neosho, Spring, and Arkansas Rivers. Gaining access to private lands would also improve my ability to assess the current distribution in Kansas. Establishing the extent of this species' range is critical to conservation decision making because populations at the margins of

species' ranges are typically at a higher risk of extirpation that are populations occupying the core of a geographic range (Steen and Barrett 2015).

Of the sites we have sampled so far, the Verdigris River near Coffeyville appears to be the most suitable site to consider should reintroductions in Kansas become desirable. While CPUE at this site was low relative to other sites, the species diversity and evenness was high. This site and its two tributaries also showed moderately high similarity to the turtle community at the Caney River reintroduction site in Oklahoma.

The Caney River at Elgin could be used as a reintroduction site to extend the range of the population that presently exists downstream in Oklahoma. Without intervention, however, I predict that animals that were introduced in Oklahoma will naturally migrate across the state line, and it is important to note that CPUE at the Caney River at Elgin was low; however, this sampling occurred during a period of extensive flooding, a condition that is known to reduce catch rates (Munscher et al. 2020).

The Fall River site had high CPUE and a diverse turtle assemblage; however, due to a preponderance of *T. scripta*, the species evenness was low compared with other sites sampled. The turtle community was moderately similar to the Caney River reintroduction site as well. The Fall River and Verdigris River at Toronto were sampled in the fall as opposed to the summer season when trapping occurred at other sites which may be influencing the extremely high CPUE at these sites. This is supported by differences in CPUE between years at the Elk River—summer sampling in 2018 versus fall sampling in 2019. Higher fall catch rates than summer or spring catch rates have been observed in aquatic turtle sampling in Missouri as well (Wallace, Fratto and Barko 2007). Therefore, the CPUE at this site may not be representative of the turtle community that is typically sampled in the summer. Additionally, the Fall River and Verdigris

River at Toronto sites lie just inside the historical range of the Alligator Snapping Turtle (Figure 7). If turtles were released at these sites, they would only be capable of migrating upstream—and into areas they were not historically present—due to the presence of impoundments downstream of survey sites.

The Elk River had relatively high CPUE, species richness, and evenness, but I am hesitant to recommend this site for reintroduction. Over the course of one weekend in summer 2018 I encountered 14 motor boats on the Elk River and 11 more in a similar amount of time in fall 2019. High rates of boat traffic lead to increased injury in turtles (Cecala, Gibbons and Dorcas 2009; Bulté, Carriére and Blouin-Demers 2010; Bennett and Litzgus 2014; Hollender, Anthony and Ligon 2018) and it also means an increase in the potential for poaching and incidental bycatch. For a reintroduction to be successful, the population must produce enough offspring that survive to adulthood to replace individuals that die. Long-lived species are particularly sensitive to loss of adults and juveniles (Congdon, Dunham and Van Loben Sels 1993) and an increased risk of injury or death could potentially prevent a reintroduced population from reaching a self-sufficient level.

Literature Cited

Anthony, T., Riedle, J.D., East, M.B., Fillmore, B. and Ligon, D.B. 2015. Monitoring of reintroduced population of juvenile Alligator Snapping Turtles. Chelonian Conservation and Biology 14:43–48.

Baxley, D.L., Barnard, J.O. and Venter, H. 2014. A survey for the Alligator Snapping Turtle (*Macrochelys temminckii*) in western Kentucky. Southeastern Naturalist 13:337–346.

Bennett, A.M. and Litzgus, J.D. 2014. Injury rates of freshwater turtles on a recreational waterway in Ontario, Canada. Journal of Herpetology 48:262–266.

Boundy, J. and Kennedy, C. 2006. Trapping survey results for the Alligator Snapping Turtle (*Macrochelys temminckii*) in southeastern Louisiana, with comments on exploitation. Chelonian Conservation and Biology 5:3–9.

Bulté, G., Carriére, M.A. and Blouin-Demers, G. 2010. Impact of recreational power boating on two populations of Northern Map Turtles (*Graptemys geographica*). Aquatic Conservation: Marine and Freshwater Ecosystems 20:31–38.

Cecala, K.K., Gibbons, J.W. and Dorcas, M.E. 2009. Ecological effects of major injuries in Diamondback Terrapins: implications for conservation and management. Aquatic Conservation: Marine and Freshwater Ecosystems 19:421–427.

Center for Biological Diversity. 2012. Petition to list 53 amphibians and reptiles in the United States as threatened or endangered species under the Endangered Species Act. Submitted to the United States Fish and Wildlife Service, Washington D.C.

Congdon, J.D., Dunham, A.E. and Van Loben Sels, R.C. 1993. Delayed sexual maturity and demographics of Blanding's Turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conservation Biology 7:826–833.

East, M.B., Riedle, J.D. and Ligon, D.B. 2013. Temporal changes in an Alligator Snapping Turtle (*Macrochelys temminckii*) population. Journal of Wildlife Research 40:77–81.

East, M.B., Fillmore, B.M. and Ligon, D.B. 2013. Feeding behavior of captive-reared juvenile Alligator Snapping Turtles (*Macrochelys temminckii*). Southeastern Naturalist 12:692–702.

Eisemberg, C.C., Rose, M., Yaru, B. and Georges, A. 2011. Demonstrating decline of an iconic species under sustained indigenous harvest—The Pig-nosed Turtle (*Carettochelys insculpta*) in Papua New Guinea. Biological Conservation 144:2282–2288.

Ernst, C.H. and Lovich, J.E. 2009. Turtles of the United States and Canada. Johns Hopkins University Press, Baltimore, Maryland.

Folt, B. and Godwin, J.C. 2013. Status of the Alligator Snapping Turtle (*Macrochelys temminckii*) in south Alabama with comments on its distribution. Chelonian Conservation and Biology 12:211–217.

Folt, B. and Guyer, C. 2015. Evaluating recent taxonomic changes for Alligator Snapping Turtles (Testudines: Chelydridae). Zootaxa 3:447–450.

Heck, B.A. 1998. The Alligator Snapping Turtle (*Macroclemys temminckii*) in southeast Oklahoma. Proceedings of the Oklahoma Academy of Science 7853–58.

Hollender, E.C., Anthony, T.L. and Ligon, D.B. 2018. Motorboat injury rates and patterns in aquatic turtle communities. Chelonian Conservation and Biology 17:298–302.

Howey, A.F. and Dinkelacker, S.A. 2013. Characteristics of a historically harvested Alligator Snapping Turtle (*Macrochelys temminckii*) population. Copeia 2013:58–63.

Jensen, J.B. and Birkhead, W.S. 2003. Distribution and status of the Alligator Snapping Turtle (*Macrochelys temminckii*) in Georgia. Southeastern Naturalist 2:25–34.

Lawton, J.H. 1999. Are there general laws in ecology? Oikos 84:177–192.

Lescher, T.C., Briggler, J.T. and Tang-Martinez, Z. 2013. Abundance, population structure, and conservation of Alligator Snapping Turtles (*Macrochelys temminckii*) in Missouri between 1993–1994 and 2009. Chelonian Conservation and Biology 12:1653–168.

Ligon, D.B., Backues, K., Fillmore, B.M. and Thompson, D.M. 2014. Ontogeny of gonad and genital morphology in juvenile Alligator Snapping Turtles (*Macrochelys temminckii*). Herpetological Conservation and Biology 9:146–155.

Ligon, D.B. and Lovern, M.B. 2009. Temperature effects during early life stages of the Alligator Snapping Turtle (*Macrochelys temminckii*). Chelonian Conservation and Biology 8:74–83.

Lindeman, P.V. 2013. The Map Turtle and Sawback Atlas: Ecology, Evolution, Distribution, and Conservation. V.H. Hutchinson (ed.). University of Oklahoma Press, Norman, Oklahoma, pp. 229–254.

Miller, J.L. and Ligon, D.B. 2014. Sex ratios in naturally incubating *Macrochelys temminckii* nests, a species with type II temperature-dependent sex determination. Journal of Thermal Biology 39:6–11.

Miller, J.L., Thompson, D.M., Heywood, J. and Ligon, D.B. 2014. Nest-site selection among reintroduced *Macrochelys temminckii*. The Southwestern Naturalist 59:188–192.

Moll, D. and Moll, E.O. 2004. The ecology, exploitation, and conservation of river turtles. Oxford University Press, New York, New York.

Moore, D.B., Ligon, D.B., Fillmore, B.M. and Fox, S.F. 2014. Spatial use and selection of habitat in a reintroduced population of Alligator Snapping Turtles (*Macrochelys temminckii*). The Southwestern Naturalist 59:30–37.

Moore, D.B., Ligon, D.B., Fillmore, B.M. and Fox, S.F. 2013. Growth and viability of a translocated population of Alligator Snapping Turtles (*Macrochelys temminckii*). Herpetological Conservation and Biology 8:141–148.

Munscher, E., Gray, J., Tuggle, A., Ligon, D.B., Gladkaya, V., Franklin, C., Drake, C., Ricardez, V., Butterfield, B.P., Norrid, K. and Walde, A. 2020. Discovery of an Alligator Snapping Turtle (*Macrochelys temminckii*) population in metropolitan Houston, Harris County, Texas. Urban Naturalist 32:1–13.

Pritchard, P.C.H. 2006. The Alligator Snapping Turtle: Biology and Conservation. Milwaukee Krieger Publishing Company, Malabar, Florida.

Reed, R.N., Congdon, J. and Gibbons, J.W. 2002. The Alligator Snapping Turtle [*Macrochelys* (*Macroclemys*) *temminckii*]: a review of ecology, life history, and conservation, with demographic analyses of the sustainability of take from wild populations. Report to Division of Scientific Authority, U.S. Fish and Wildlife Service.

Riedle, J.D., Shipman, P.A., Fox, S.F., Hackler, J.C. and Leslie, D.M. 2008. Population structure of the Alligator Snapping Turtle on the western edge of its distribution. Chelonian Conservation and Biology 7:100–104.

Riedle, J.D., Ligon, D.B. and Graves, K. 2008. Distribution and management of Alligator Snapping Turtles, *Macrochelys temminckii*, in Kansas and Oklahoma. Transactions of the Kansas Academy of Science 111:21–28.

Riedle, J.D., Shipman, P.A., Fox, S.F. and Leslie, D.M. 2006. Microhabitat use, home range, and movements of the Alligator Snapping Turtle, *Macrochelys temminckii*, in Oklahoma. Southwestern Naturalist 51:35–40.

Riedle, J.D., Shipman, P.A., Fox, S.F. and Leslie, D.M. 2005. Status and distribution of the Alligator Snapping Turtle, *Macrochelys temminckii*, in Oklahoma. Southwestern Naturalist 50:79–84.

Shipman, A.M. 2019. Investigation of nest predation as a cause of turtle population declines on the Sequoyah National Wildlife Refuge, Oklahoma. Master's thesis. Rochester Institute of Technology, pp. 51.

Shipman, P.A. and Riedle, J.D. 2008. Status and distribution of the Alligator Snapping Turtle (*Macrochelys temminckii*) in southeastern Missouri. Southeastern Naturalist. 7:331–338.

Shipman, P.A., Edds, D.R. and Shipman, L.E. 1995. Distribution of the Alligator Snapping Turtle (*Macroclemys temminckii*) in Kansas. Transactions of the Kansas Academy of Science 98:83–91.

Steen, D.A. and Barrett, K. 2015. Should states in the USA value species at the edge of their geographic range? The Journal of Wildlife Management 79:872–876.

Taggart, T.W. 2020. Kansas Herpetofaunal Atlas: An On-line Reference. Sternberg Museum of Natural History, Fort Hays State University. <https://webapps.fhsu.edu/ksherp>.

Thomas, T.M., Granatosky, M.C., Bourque, J.R., Krysko, K.L., Moler, P.E., Gamble, T., Suarez, E., Leone, E., Enge, K.M. and Roman, J. 2014. Taxonomic assessment of Alligator Snapping Turtles (Chelydridae: *Macrochelys*), with the description of two new species from the southeastern United States. Zootaxa 3786:141–165.

Thompson, D.M., Ligon, D.B., Patton, J.C. and Papes, M. 2017. Effects of life-history requirements on the distribution of a threatened reptile. Conservation Biology 31:427–436.

Thorbjarnarson, J., Lagueux, C.J., Bolze, D., Klemens, M.W. and Meylan, A.B. 2000. Human use of turtles: A worldwide perspective. *In* Turtle conservation. M.W. Klemens (ed.). Smithsonian Institution Press, Washington, D.C., pp. 33–84.

Trauth, S.E., Wilhide, J.D. and Holt, A. Population structure and movement patterns of Alligator Snapping Turtles (*Macroclemys temminckii*) in northeastern Arkansas. Chelonian Conservation and Biology 3:64–70.

United States Fish and Wildlife Service. 1983. Endangered and threatened wildlife and plants: findings on certain petitions. Federal Register 48:27273–27274.

United States Fish and Wildlife Service. 1984. Endangered and threatened wildlife and plants: finding on a petition to list the Alligator Snapping Turtle as a threatened species. Federal Register 49:7416–7415.

United States Fish and Wildlife Service. 1994. Endangered and threatened wildlife and plants: animal candidate review for listing as endangered or threatened species. Federal Register 59:58982–59028.

United States Fish and Wildlife Service. 1996. Endangered and threatened wildlife and plants: Notice of final decision on identification of candidates for listing as endangered or threatened. Federal Register 61:64481–64485.

United States Fish and Wildlife Service. 2015. Endangered and threatened wildlife and plants: 90-day findings on 31 petitions. Federal Register 80:37568–37579.

United States Geological Survey. 2017. Alligator Snapping Turtle (*Macrochelys temminckii*) rASTUx_CONUS_2001v1 habitat map. Gap Analysis Project. DOI: 10.5066/F7PK0DK8

Wallace, J.E., Fratto, Z.W. and Barko, V.A. 2007. A comparison of three sampling gears for capturing aquatic turtles in Missouri: the environmental variables related to species richness and diversity. Transactions of the Missouri Academy of Science 41:7–13.

Table 1. GPS locations of midpoints or boat ramps of sample sites in Kansas 2017–2019. Locations were defined using the WGS84 geodetic datum.

Site	Northing	Easting
Caney Elgin	37.002483	-96.291530
Cedar Creek	37.009771	-96.255164
Verdigris Coffeyville	37.044393	-95.591668
Big Hill Creek	37.065244	-95.606810
Pumpkin Creek	37.035369	-95.577263
Verdigris Sycamore	37.350114	-95.686521
Verdigris Toronto	37.828934	-95.963335
Elk River	37.258029	-95.849624
Fall River	37.707282	-96.140453

Table 2. Summary of turtles captured at five sites in Kansas in summer, 2017. Numbers represent the number of individual captures of a species of a given sex at each site.

Species*	Sex**	Caney Elgin	Cedar Creek	Pumpkin Creek	Big Hill Creek	Verdigris Coffeyville
TRSC	M	31	14	28	29	18
	F	13	9	26	5	13
GROU	M	1	2	14	9	35
	F	5	0	7	19	29
	U	5	0	3	3	3
GRPS	M	4	3	0	0	0
	F	5	2	0	0	1
	U	5	0	0	0	3
APSP	M	5	0	0	15	3
	F	29	18	1	4	17
	U	5	1	1	9	15
STOD	U	0	1	2	1	0
PSCO	U	0	0	0	0	8
CHSE	F	0	0	1	0	0

*TRSC = *Trachemys scripta*; GROU = *Graptemys ouachitensis*; GRPS = *G. pseudogeographica*; APSP = *Apalone spinifera*; STOD = *Sternotherus odoratus*; CHSE = *Chelydra serpentina*; PSCO = *Pseudemys concinna*

**M = Male; F = Female; U = Unknown

Table 3. Straight-line carapace length (SCL) and mass of each species by sex for each site sampled in 2018 and 2019. Both Elk River sampling events are combined. Values reported are mean±s.d.

Site	Species*	Sex**	Number	Mean SCL (mm)	Mean mass (g)
Verdigris Sycamore	TRSC	M	18	178.89±34.39	908.56±440.74
		F	4	213.55±5.14	1412.50±186.44
	GROU	M	2	86.65±20.58	96.50±40.31
		F	0	-	-
	APSP	M	3	161.47±12.62	440.33±82.05
		F	2	272.45±0.21	1905.00±63.64
Elk River	TRSC	M	91	180.45±29.40	876.23±355.47
		F	32	196.62±36.39	1247.19±542.47
		J	9	-	-
	GROU	M	24	97.01±8.71	132.53±34.48
		F	16	157.88±29.12	590.94±244.50
		J	1	-	-
	GRPS	M	4	99.15±7.33	134.50±14.73
		F	3	200.80±32.24	1191.67±470.59
	APSP	M	21	176.14±14.13	558.81±130.45
		F	23	283.50±76.80	2492.09±1888.79
	CHSE	M	3	266.70±59.87	5293.33±2907.67
		F	1	260.50	4800.00
Verdigris Toronto	TRSC	M	77	173.41±29.13	792.88±388.46
		F	39	189.66±33.47	1089.05±526.41
		J	2	-	-
	GROU	M	14	100.19±10.87	143.04±45.86
		F	13	144.72±41.05	508.59±342.45
		J	1	-	-
	GRPS	M	4	108.55±10.87	178.25±45.86
		F	13	153.22±56.90	698.77±586.48
		J	1	-	-

*TRSC = *Trachemys scripta*; GROU = *Graptemys ouachitensis*; GRPS = *G. pseudogeographica*; APSP = *Apalone spinifera*; STOD = *Sternotherus odoratus*; CHSE = *Chelydra serpentina*; PSCO = *Pseudemys concinna*

**M = Male; F = Female; J = Juvenile

Table 3 continued. Straight-line carapace length (SCL) and mass of each species by sex for each site sampled in 2018 and 2019. Both Elk River sampling events are combined. Values reported are mean±s.d.

Site	Species*	Sex**	Number	Mean SCL (mm)	Mean mass (g)
Verdigris Toronto (cont.)	APSP	M	4	158.50±55.21	474.38±320.32
		F	8	287.63±60.11	2260.38±1298.54
	PSCO	F	1	110.10	220.00
Fall River	TRSC	M	87	174.95±29.01	795.06±371.22
		F	27	212.43±27.25	1455.31±513.54
		J	19	-	-
	GROU	M	23	99.85±7.75	118.13±21.82
		F	19	131.18±46.37	426.82±459.59
		J	3	-	-
	GRPS	M	9	96.14±11.21	111.61±28.10
		F	13	113.34±48.83	322.27±419.40
		J	9	-	-
	APSP	M	13	179.83±15.92	557.54±161.80
		F	8	312.98±99.65	3629.44±1942.84
		J	2	-	-
	CHSE	J	1	-	-
	PSCO	M	0	-	-
		F	1	95.20	135.00
		J	3	-	-
	CHPI	M	1	119.00	205.00
		F	0	-	-
		J	1	-	-

*TRSC = *Trachemys scripta*; GROU = *Graptemys ouachitensis*; GRPS = *G. pseudogeographica*; APSP = *Apalone spinifera*; STOD = *Sternotherus odoratus*; CHSE = *Chelydra serpentina*; PSCO = *Pseudemys concinna*; CHPI = *Chrysemys picta*

**M = Male; F = Female; J = Juvenile

Table 4. Number of species (# Species), number of captures (# Captures), number of net nights (Effort), catch per unit effort (CPUE), Simpson's Index (D), Evenness (E), and sampling dates of each river site surveyed in Kansas. Higher Simpson's Index values indicate a higher relative diversity. Evenness values close to 1 indicate an equal abundance of each species. The Elk River and Caney River sites were sampled in summer and fall months and are represented with all captures combined as well as with captures split in groups depending on the year/season sampled.

Site	# Species	# Captures	Effort	CPUE	D	E	Sampling Dates
Caney Elgin	4	108	139	0.7770	0.6764	0.4879	5/21/17–6/29/17
Cedar Creek	5	50	48	1.0417	0.6320	0.3927	6/22/17–6/29/17
Verdigris Coffeyville	5	145	269	0.5390	0.6787	0.4217	6/13/17–6/23/17
Pumpkin Creek	5	83	100	0.8300	0.4918	0.3056	6/9/17–6/13/17
Big Hill Creek	4	94	100	0.9400	0.6716	0.4844	5/22/17–6/17/17
Verdigris Sycamore	3	29	18	1.6111	0.3900	0.3550	7/4/18–7/5/18
Elk River	5	228	68*	3.3529	0.5940	0.3691	2018–2019
Elk River 2018	5	84	44*	1.9091	0.6278	0.3901	7/6/18–7/7/18
Elk River 2019	4	144	24	6.0000	0.5722	0.4128	9/14/19
Fall River	7	239	25	9.5600	0.6284	0.3229	9/7/19
Verdigris Toronto	5	179	22*	8.1364	0.5115	0.3178	8/24/19
Caney River	7	2141	866	2.4723	0.5700	0.2929	2017–2019

*One net removed from each count because the net had a large hole and no turtles were captured.

Table 5. Bray-Curtis similarity index. Values close to 1 indicate a similar community structure among sites.

	Caney Elgin	Cedar Creek	Verdigris Coffeyville	Pumpkin Creek	Big Hill Creek	Verdigris Sycamore	Elk River	Fall River	Verdigris Toronto
Cedar Creek	0.3797								
Verdigris Coffeyville	0.3597	0.5077							
Pumpkin Creek	0.4031	0.5789	0.5000						
Big Hill Creek	0.2772	0.3750	0.2469	0.3107					
Verdigris Sycamore	0.5766	0.2658	0.6667	0.5357	0.5284				
Elk River	0.3988	0.6475	0.4048	0.4791	0.4224	0.7743			
Fall River	0.4697	0.6609	0.4427	0.4969	0.4715	0.7836	0.1263		
Verdigris Toronto	0.4355	0.6332	0.5309	0.3893	0.4579	0.7211	0.1794	0.1435	
Caney Oklahoma	0.9040	0.9544	0.8731	0.9253	0.9159	0.9733	0.8075	0.8008	0.8457

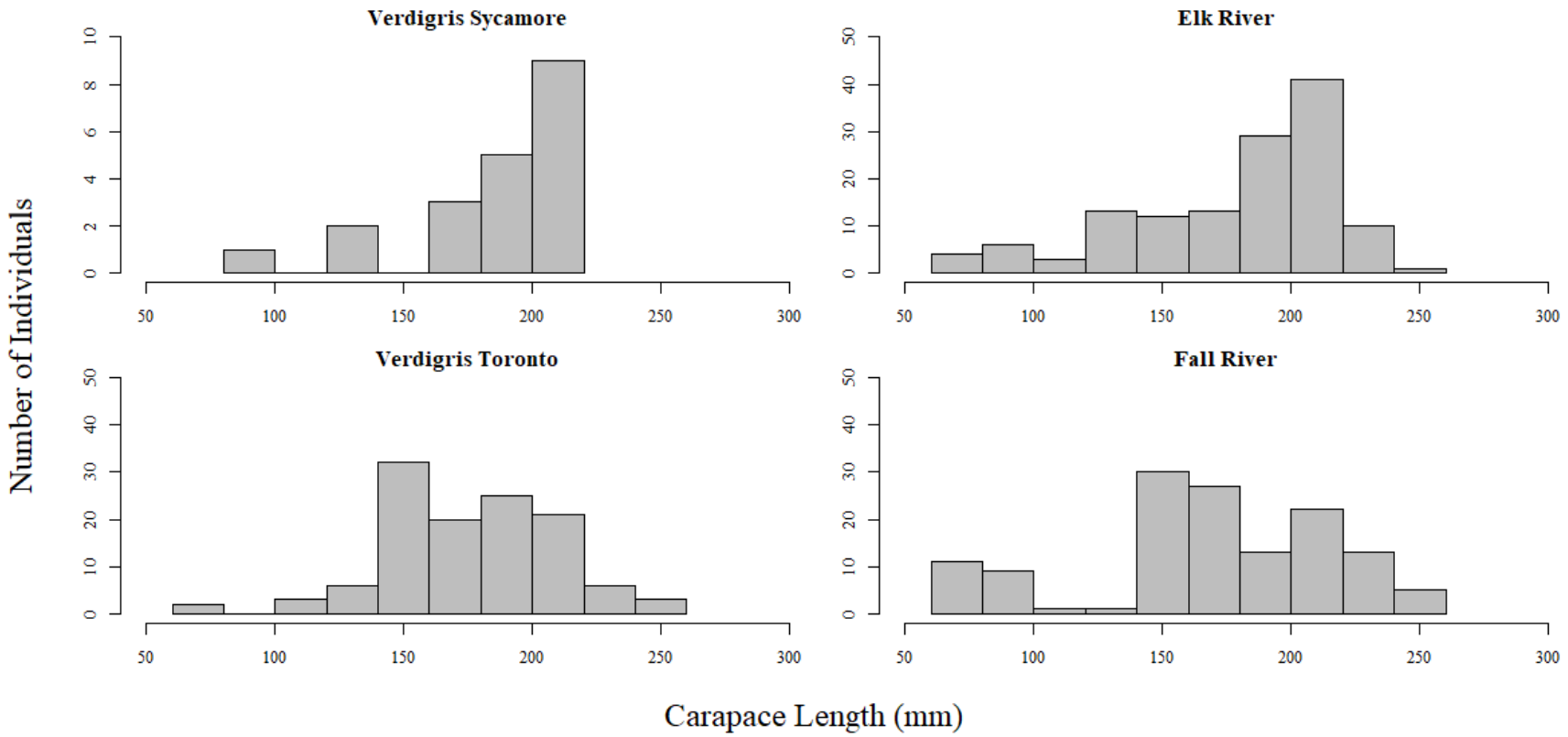

Figure 1. Morphometric distribution of both sexes of *Trachemys scripta* at each site from 2018–2019.

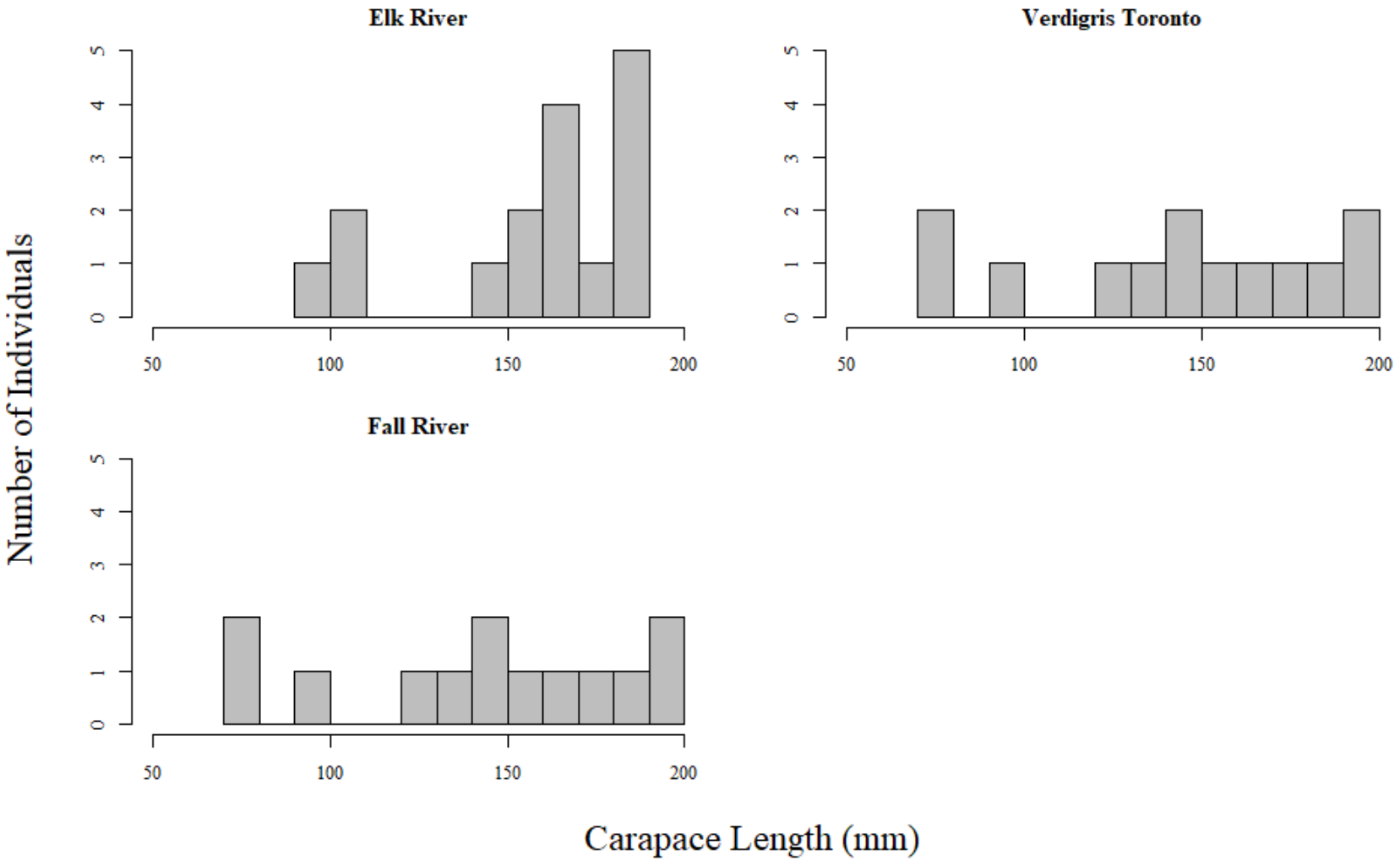

Figure 2. Morphometric distribution of female *Graptemys ouachitensis* at each site from 2018–2019.

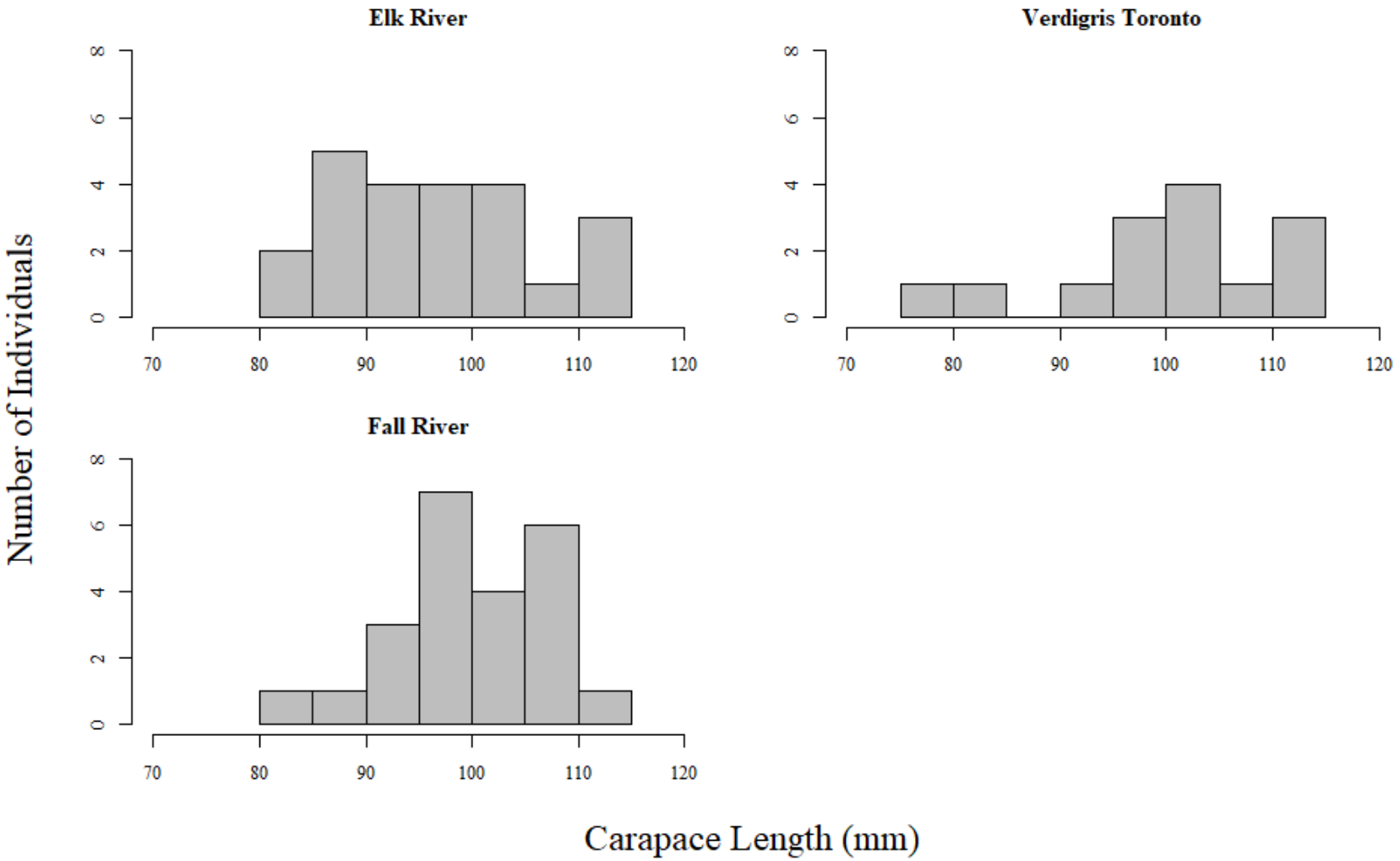

Figure 3. Morphometric distribution of male *Graptemys ouachitensis* at each site from 2018–2019.

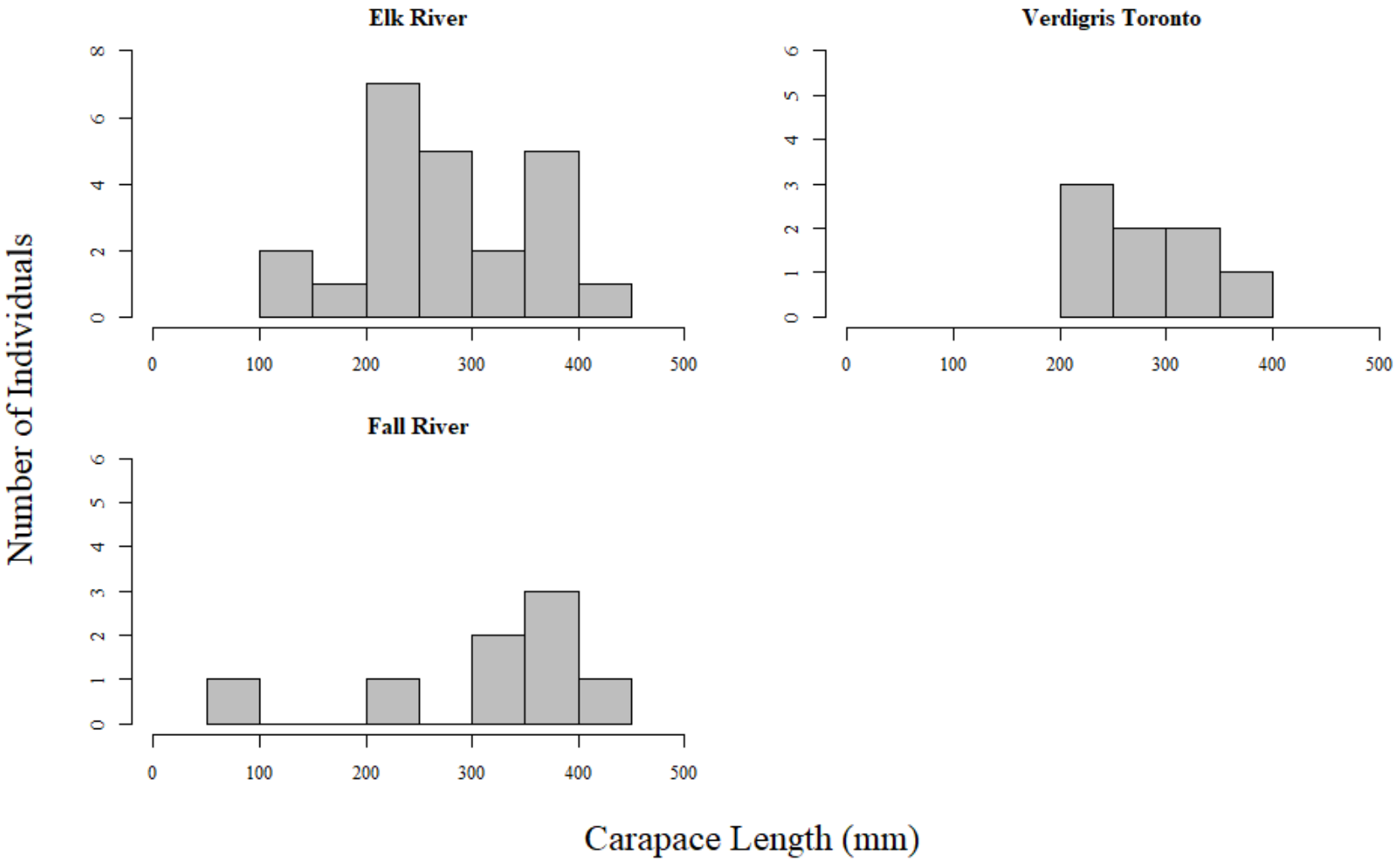

Figure 4. Morphometric distribution of female *Apalone spinifera* at each site from 2018–2019.

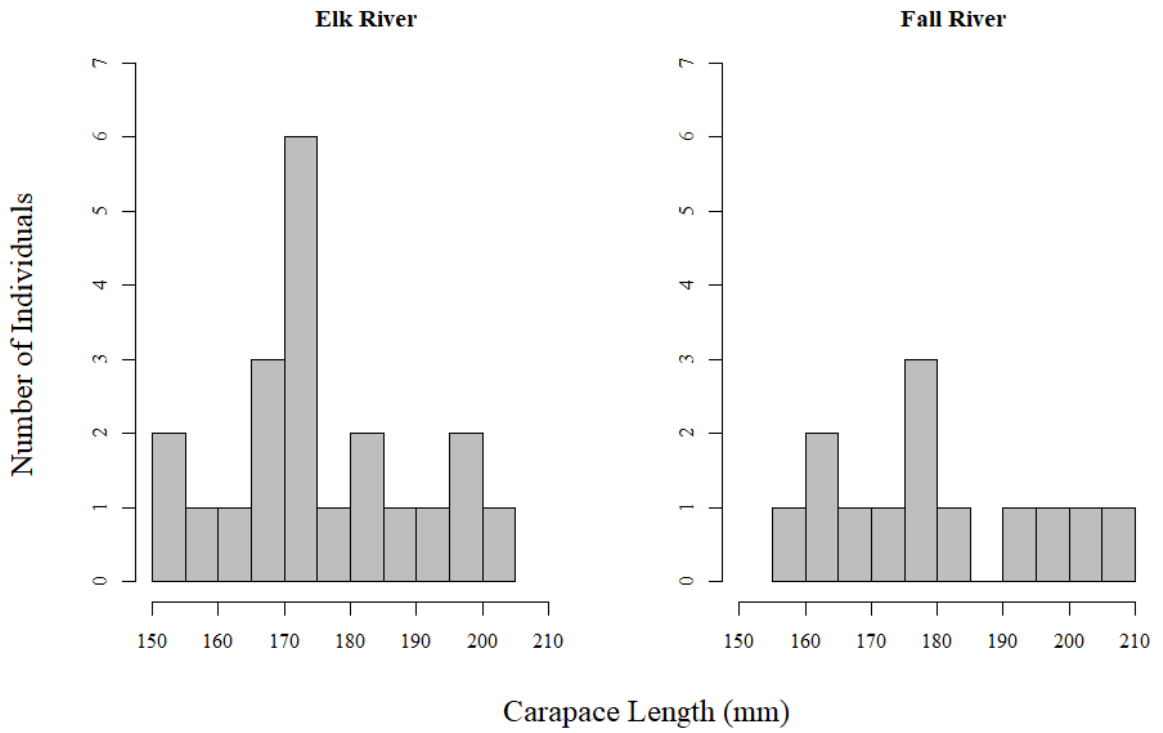

Figure 5. Morphometric distribution of male *Apalone spinifera* at each site from 2018–2019.

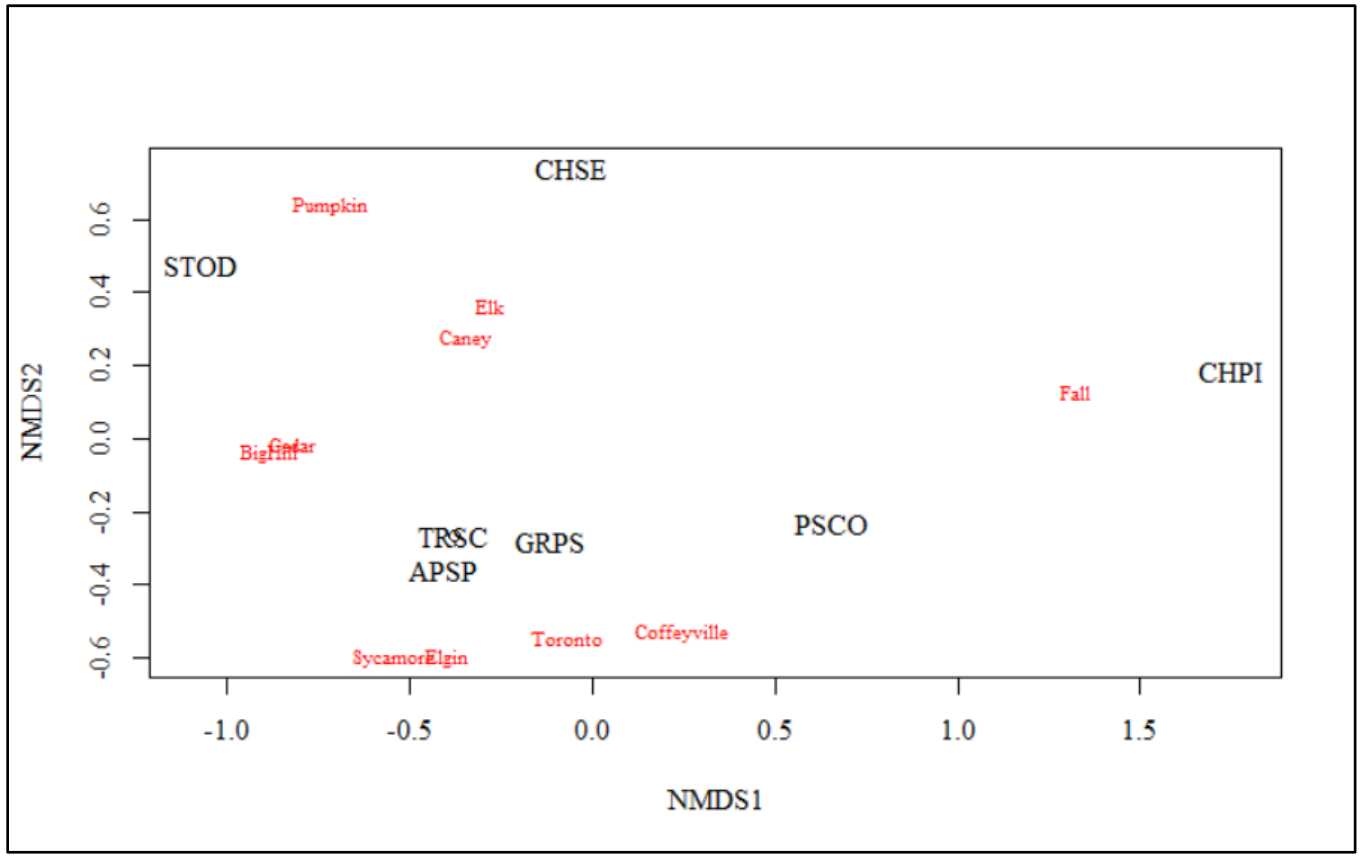

Figure 6. Non-metric multidimensional scaling (NMDS) visualization of similarity of sample site community composition. The Bray-Curtis index was used to calculate distance matrices from species and site using the function "metaMDS" with two dimensions and 20 random starts. Convergence was reached and stress = 0.0127. Red text is used to indicate site abbreviations and black text is species abbreviations. Distance on this plot is a representation of the level of similarity in the community structure among sites.

Pumpkin = Pumpkin Creek; Elk = Elk River; Caney = Caney River in Oklahoma; Cedar = Cedar Creek; BigHill = Big Hill Creek; Fall = Fall River; Sycamore = Verdigris at Sycamore; Elgin = Caney River at Elgin; Toronto = Verdigris River at Toronto; Coffeyville = Verdigris River at Coffeyville

TRSC = *Trachemys scripta*; GROU = *Graptemys ouachitensis*; GRPS = *G. pseudogeographica*; APSP = *Apalone spinifera*; STOD = *Sternotherus odoratus*; CHSE = *Chelydra serpentina*; PSCO = *Pseudemys concinna*; CHPI = *Chrysemys picta*

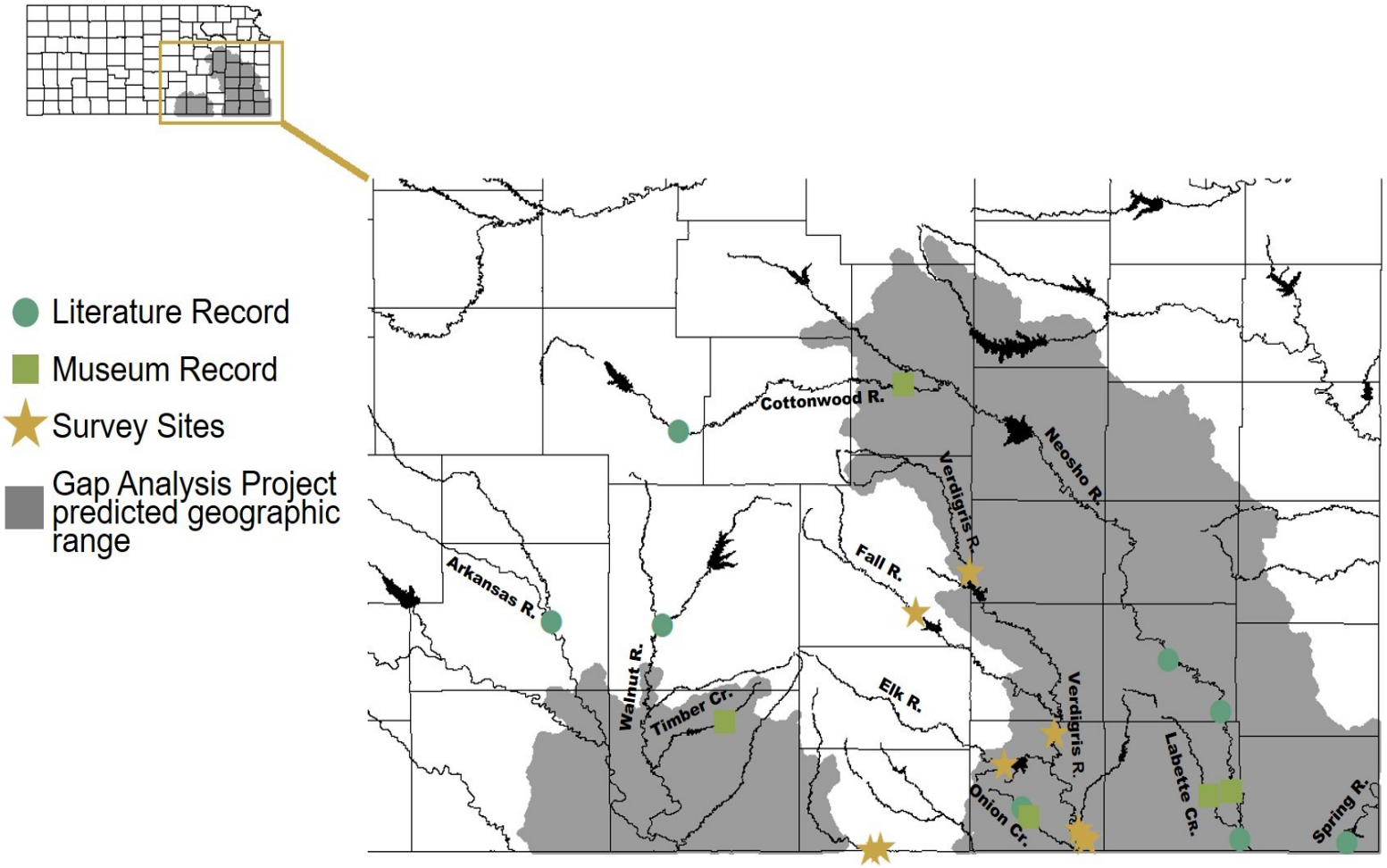

Figure 7. Historical distribution of Alligator Snapping Turtles in Kansas with historical record locations and sample site locations from 2017–2019 (USGS 2017; Taggart 2020). Map created by K. Voves.

A COMPARISON OF HEMATOLOGY AND PLASMA BIOCHEMISTRY OF CAPTIVE, REINTRODUCED, AND WILD ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*)

Abstract

Assessing the health of reintroduced populations is critical to effective post-reintroduction monitoring of species of conservation concern. In this study, I compared assessments of health among four groups: indoor- and outdoor-housed captive Alligator Snapping Turtles, a free-ranging reintroduced population, and a wild population. These comparisons were made to inform husbandry practices and identify differences between reintroduced and wild populations to aid in the post-reintroduction management of the species. Twenty-five indoor captive, 25 outdoor captive, 28 reintroduced, and 17 wild Alligator Snapping Turtles were inspected over a 40-day period in 2018. The indoor and outdoor captive populations differed significantly in plasma concentrations of sodium, potassium, and phosphorus, which likely result from dietary differences between the two groups. In comparison to free-ranging reintroduced turtles, those in the wild population had significantly higher concentrations of solutes that are generally indicative of a high protein diet, including total protein, uric acid, and globulins. This may suggest that, following release, reintroduced Alligator Snapping Turtles undergo a period of learning to acquire novel prey that their wild conspecifics acquire at an earlier age. The captive outdoor population showed the lowest levels of several stress indicators, and both the indoor and wild populations had high concentrations of AST and CK, two solutes that have been shown in other taxa to correlate with high levels of conspecific aggression. There were no individuals from any of the four groups surveyed that returned health screening results

that indicated the presence of serious health complications—such as organ failure—and while the average biochemical concentrations differed slightly from previously published reference ranges obtained in Georgia and Florida, no group or individual was found to exhibit markedly compromised health.

Introduction

Chelonians world-wide are declining, primarily due to overharvest for meat and the pet trade, climate change, introduction of invasive species and pathogens, and wide-scale habitat degradation (Moll and Moll 2004; Throbjarnarson et al. 2000; Rhodin et al. 2018). Detecting and quantifying the declines of populations requires time- and labor-intensive monitoring, and oftentimes the specific causes of a population's decline are difficult to identify (Stickel 1978; Hall et al. 1999). Appropriate conservation measures vary, but in cases where causes of a decline can be identified and mitigated, head-start and reintroduction programs have been implemented to reestablish or augment populations that otherwise would be unable to recover (Reed et al. 2004; Riedle et al. 2008; Tuberville et al. 2015).

Evidence for the success of efforts to reestablish populations using reintroduction can only be derived from well-designed monitoring efforts, both during captive rearing and after reintroduction. Such evidence is key to identifying and correcting deficiencies in the management program and ascertaining the extent to which reintroductions are necessary to establish a stable, self-sustaining population. Important endpoints include population-level variables such as birth rate, death rate, and the resulting intrinsic rate of increase, as well as ontogenetic growth patterns, age at which males and females achieve sexual maturity, and physical condition.

The Alligator Snapping Turtle (*Macrochelys temminckii*) has experienced population declines throughout its range in the southeastern United States, due primarily to unsustainable harvest rates and habitat modifications that include channelization of natural waterways, point-source pollution, and—perhaps most importantly—river impoundments that fragment populations and disrupt natural movement patterns (Reed et al. 2002; Pritchard 2006; Riedle et al. 2008). The species has been listed in Appendix III of the Conservation on International Trade in Endangered Species (CITES) to prevent over-harvest for the international meat market and pet trade (U.S. Fish and Wildlife Service and Department of the Interior 2005). The Alligator Snapping Turtle is designated as an at-risk species in all of the states in its range, and in Oklahoma it is one of just two reptiles that are listed as Tier I Species of Greatest Conservation Need (Oklahoma Comprehensive Wildlife Conservation Strategy 2016).

Alligator Snapping Turtles historically occurred in the Caney River in northern Oklahoma and southern Kansas, but the last record was reported in 1942 (Glass 1949). Targeted surveys were conducted in 1997–98, and no evidence of the species' recent occurrence was found (Riedle et al. 2005). Because the habitat remained apparently suitable for Alligator Snapping Turtles, but natural recolonization was impeded by a downstream river impoundment, reintroductions of head-started juveniles and post-release monitoring efforts were initiate in 2008 (Anthony et al. 2015; Dreslik et al. 2017). These efforts primarily involved monitoring growth and survival rates, both of which serve as useful benchmarks for assessing the effectiveness of a reintroduction effort. Because delayed maturity and long generation times are characteristics of Alligator Snapping Turtles, more definitive benchmarks of success—such as reproduction rates and population growth patterns—cannot be applied for short-term assessments (Seigel and Dodd 2000; Moll and Moll 2004; Nickerson and Pitt 2012).

Health assessments play a crucial role in both the head-start and post-release phases of reintroduction programs, as they are useful for identifying physiological ailments whose long-term ramifications may be serious but may otherwise be difficult to identify at early stages. When health assessments are conducted for species for which physiologically normal ranges are not well-established, comparisons of health assessments among populations can be useful for identifying and interpreting deviant results. Patterns of variation can be used to identify factors affecting entire populations, such as habitat quality, food availability, and seasonal shifts in meteorological conditions. Consequently, health assessments of individuals can be used to both detect stressors affecting individual animals, as well as identify ecological and physiological factors that may affect entire populations.

Hematological and biochemical reference ranges have been established for wild populations of Alligator Snapping Turtles in Florida and Georgia (Chaffin et al. 2008), but there have been no studies of health parameters in northern populations where seasonal patterns are likely markedly different. Comparisons of health parameters among widely disparate populations are challenging because of the likelihood of confounding factors (e.g., habitat characteristics, day length, water temperature, food availability and type), but comparisons among populations that inhabit similar conditions can highlight important ecological differences among populations and usefully inform management decisions (Brenner et al. 2002; Rangel-Mendoza et al. 2009; Yu et al. 2013; Yang et al. 2014).

The objective of this study was to compare hematological and biochemical parameters among populations of captive, reintroduced, and wild Alligator Snapping Turtles to inform husbandry practices in captivity and assess the physiological condition of reintroduced Alligator Snapping Turtles relative to wild conspecifics.

Methods

Sample Populations. I surveyed four populations of Alligator Snapping Turtles in summer 2018. Two populations were captive and part of a propagation and head-start program at Tishomingo National Fish Hatchery in southeastern Oklahoma. One of these two captive populations was housed indoors in plastic tanks and raceways and maintained in flow-through water systems during summer and static water during winter. Neither the water nor the building the turtles were housed in were temperature controlled and exhibited daily and seasonal fluctuations. These turtles were fed a commercially available pelleted diet *ad libitum*. The second populations of captive animals were maintained in two adjacent outdoor ponds at the same hatchery. Both ponds were a maximum of 2 m in depth and included forage consisting of crayfish (Virile Crayfish, *Faxonius virilis*), small fish (predominantly Bluegill Sunfish, *Lepomis macrochirus*, and Mosquitofish, *Gambusia affinis*) and submerged vegetation, including Watermilfoil (*Myriophyllum* sp.) and Coontail (*Ceratophyllum demersum*). A nearby spring-fed creek served as the water source for both indoor and outdoor captive populations.

The remaining two populations were free-ranging, but one was a naturally occurring wild population inhabiting the Poteau River in eastern Oklahoma and the other was composed of reintroduced Alligator Snapping Turtles inhabiting the Caney River in northern Oklahoma. The reintroduced stock originated from the head-start program at Tishomingo National Fish Hatchery, but all turtles that were sampled had been released 2–10 years prior to this study.

Both indoor and outdoor captive turtles were captured by hand and processed within 24 hours of capture. In contrast, free-ranging turtles in both the wild and reintroduced populations were captured using 0.9-meter hoop nets with 2.5-cm mesh that were baited with frozen fish, set in the afternoon, and then checked the following morning.

Superficial Assessment. Upon capture each turtle was measured, and all visible physical abnormalities were documented (Berry and Christopher 2001; Herbst and Jacobson 2003). Carapace length, plastron length, pre-cloacal tail length, total tail length, and mass were measured. Ears, nose, mouth, shell, and limbs were thoroughly inspected and abnormalities (e.g. scarring, abrasions, abscesses, parasites) were documented.

Sample Collection and Processing. I drew blood from the dorsal coccygeal vein using a 21-g, heparinized needle and 3-mL syringe (Campbell 1996). If a sample contained visible lymph contamination, a new sample was obtained (Crawshaw and Holz 1996). Whole blood was deposited into lithium heparin microtainer tubes (BD Microtainer®, Becton Dickinson, Franklin Lakes, New Jersey 07417, USA) immediately after collection. Three microhematocrit tubes were filled and centrifuged at 12,700 G for five minutes. These samples were then used to measure packed cell volume and total plasma solids.

Two blood smears were made, air-dried, and stained using DipQuick® (JorVet Dip Quick Stain Kit, Jorgensen Laboratories, Loveland, Colorado 80538, USA). White blood cell differential counts were performed by counting 100 individual white blood cells and differentiating between heterophils, basophils, eosinophils, azurophils, monocytes, and lymphocytes. Complete white blood cell counts were performed using an Eopette kit (Eopette Eosinophil Staining Kit for Avian Leukocyte Manual Counting Method, Exotic Animal Solutions LLC, Rockledge, Florida 32955, USA). Heterophil to lymphocyte ratios (H:L) were calculated.

The remainder of the whole blood was centrifuged at 12,700 G for five minutes and the plasma fraction was then aspirated and transferred to a clean microcentrifuge tube. Plasma samples were frozen for later analysis. All hematological analyses and plasma separation were

completed within 2–3 h of collecting blood to minimize changes in hematological values or plasma biochemical concentrations (Jacobson et al. 1992). Plasma samples were analyzed using a VetScan VS2 (Abaxis Inc., Union City, California 94587, USA) with Avian/Reptilian Profile Plus Abaxis rotors which determined concentrations of glucose, albumin, aspartate aminotransferase (AST), creatinine kinase (CK), uric acid, globulin, total protein, calcium, phosphorus, potassium, and sodium. Bile acid concentrations are also evaluated with these rotors; however, concentrations were too low to be detected in most samples in this study and were not included.

Statistical Analyses. I compared each hematological and plasma biochemical parameter among populations using analysis of variance (ANOVA) when data met assumptions of normality and homogeneity of variance, and non-parametric Kruskal-Wallis when they did not. I used Tukey's and Dunn's post-hoc tests to conduct pair-wise comparisons among populations when differences were detected. Carapace length and site—among wild and reintroduced populations—were treated as covariates on the concentrations of total protein, globulins, uric acid, and calcium were assessed using analysis of covariance (ANCOVA). Analytes that exhibited significant interaction of the covariates carapace length and site were then examined using a linear regression model, with each site analyzed individually. Statistical significance was set at $\alpha < 0.05$, and all statistical tests were executed using RStudio (R version 3.5.2 "Eggshell Igloo").

All procedures involving the handling or manipulation of animals were approved by the Missouri State University Institutional Animal Care and Use Committee (protocol 19-015.0-A; Appendix A).

Results

Ninety-five Alligator Snapping Turtles were sampled, including 25 captives that were maintained indoors, 25 captives maintained in outdoor ponds, 17 wild turtles from the Poteau River in eastern Oklahoma, and 28 reintroduced turtles from the Caney River in northern Oklahoma. All assessments were conducted within a narrow time frame to minimize potentially confounded effects of season. The Caney River was sampled 25 May–15 June, the two captive populations were sampled 19–24 June, and the Poteau River was sampled 27 June–1 July 2018.

Visual assessment of physical condition. Among the four populations, there was a significant difference in size distribution of turtles sampled ($P < 0.001$, $F_{3,89} = 31.47$; Figure 1). Post-hoc Tukey tests revealed significant differences in carapace length between each population, except between the wild and reintroduced populations. Deviations from optimal physical condition included abrasions, healed scars (ranging from minor to extensive), congenital shell deformities, skin sloughing, missing toenails, missing tail tips, and presence of leeches (Table 1). One specimen from the wild population had survived an injury that had resulted in loss of its tail and a portion of the posterior section of the carapace (Appendix C). However, these injuries appeared to be mended.

Dietary components.

Protein and Metabolites. There was a significant difference among populations for three protein analytes—uric acid ($F_{3,84} = 2.77$, $P = 0.04$), total protein ($F_{3,89} = 5.25$, $P = 0.002$), and globulins ($F_{3,86} = 7.58$, $P = 0.0002$). The wild population had higher concentrations than the reintroduced population for all three of these protein analytes and maintained this trend with the highest average concentrations of protein analytes of the four populations (Table 2; Figure 2). The relationship between carapace length and total protein concentration significantly depended

on site ($F_{1,36} = 16.42$; $P < 0.001$; Figure 6a). Both the reintroduced and wild populations had total protein concentrations that increased significantly with carapace length (respectively, $F_{1,23} = 43.96$, $P < 0.001$, $R^2 = 0.6416$; $F_{1,13} = 24.79$, $P < 0.001$, $R^2 = 0.6295$) , but the slope of the relationship was greater and the y-intercept was lower for the reintroduced population (Figure 6a). The relationship between carapace length and uric acid concentration was not significantly dependent on site ($F_{1,36} = 2.32$; $P = 0.1364$). For the wild population, uric acid concentrations decreased significantly with carapace length ($F_{1.36} = 5.438$; $P = 0.0364$; $R^2 = 0.2407$; Figure 6b). The relationship between carapace length and globulin concentration also significantly depended on site ($F_{1,36} = 11.325$; $P = 0.002$). Both the reintroduced and wild populations had globulin concentrations that increased significantly with carapace length (respectively, $F_{1,23} = 35.65$, $P < 0.001$, $R^2 = 0.5908$; $F_{1,13} = 5.587$, $P = 0.034$, $R^2 = 0.2468$; Figure 6c).

Ions. There was a significant difference among populations for all four ionic concentrations—sodium ($F_{3,86} = 20.88$, $P < 0.001$), phosphorus ($F_{3,85} = 4.77$, $P = 0.004$), potassium ($F_{3,88} = 16.43$, $P < 0.001$), and calcium ($F_{3,86} = 4.00$, $P = 0.02$; Figure 3). The outdoor population of captive turtles exhibited significantly lower sodium than the other three populations in addition to significantly lower phosphorus than the indoor population (Table 2). The indoor population had significantly lower potassium concentrations than the other three populations and significantly lower calcium than the wild population (Table 2). There was not a significant interaction between the covariates carapace length and site for calcium concentration ($F_{1,36} = 0.3467$; $P = 0.5597$). However, calcium concentration increased significantly with carapace length ($F_{1,38} = 46.07$; $P < 0.001$; $R^2 = 0.5361$; Figure 6d).

Stress. Three variables that have been shown to correlate with stress were measured, including eosinophil concentrations, heterophil-to-lymphocyte ratios (H:L), and plasma glucose

concentrations and populations differed significantly in each of these ($P < 0.001$, $P = 0.003$, $P < 0.001$, respectively). The outdoor population had indicators of lower stress than both the wild and reintroduced populations including eosinophil concentration, H:L, and glucose concentration (Table 2; Figure 4). The outdoor captive population also had significantly lower glucose concentrations than the indoor captive population (Table 2).

Physical exertion. Aspartate aminotransferase (AST) and creatinine (CK) concentrations were used to infer metabolic activity and were observed to differ among populations ($F_{3,88} = 9.91$, $P < 0.001$; $H = 10.51$, d.f. = 3, $P = 0.01$, respectively). The indoor captive population had significantly higher AST concentrations than the reintroduced and outdoor captive populations and significantly lower CK concentrations than the reintroduced and wild populations (Table 2). The wild population had significantly higher AST than the reintroduced population and significantly higher CK than the outdoor population (Table 2; Figure 5).

Discussion

Plasma chemistry analyses are typically used to study the health of individuals; however, distributions of these same variables (e.g., means and ranges) may also be evaluated within and among groups or populations to draw group-level inferences about broader-scale environmental and physiological challenges. Previous studies have indicated there are underlying differences in the ecology and physiology of populations within a species that drive many of the population-level differences seen in the health assessments of those animals, which is consistent with this study (Brenner et al. 2002; Innis et al. 2007; Chaffin et al. 2008; Rangel-Mendoza et al. 2009; Keller et al. 2012). For example, this study had multiple analyte mean values for each population that were outside the established reference ranges for Alligator Snapping Turtles in Georgia and

Florida (Chaffin et al. 2008). However, none of the populations exhibited mean analyte values that deviated substantially from these established reference ranges, which could be interpreted to indicate that population- or perhaps regional-level variation accounts for the differences, and not necessarily that any of the populations reported here included individuals that were generally unhealthy.

Before drawing conclusions regarding population or regional level differences within this study, consideration of environmental factors that may influence hematology or plasma biochemistry—such as seasonality and water temperature—was necessary.

All samples were collected within 40 days and were distributed across relatively small latitudinal and elevation gradients (34.4–36.0°; 125–267 m); therefore, any effects of seasonality or geography were likely minimal and therefore unlikely to have substantially influenced the population level differences that I observed. Furthermore, if seasonality was the primary factor behind interpopulation differences in analyte concentrations, then the greatest differences would be expected between the reintroduced and wild populations as they were sampled at the beginning and end of the study, respectively—about a month apart. Chelonians tend to exhibit differences in some ions and white blood cell counts post-hibernation compared to mid-summer. Post-hibernation individuals exhibit lower phosphorus and higher sodium concentrations along with higher eosinophils and lower heterophils and lymphocytes than individuals sampled mid-summer (Campbell 2004a; Wilkinson 2004; Eatwell et al. 2014). There were no significant differences in eosinophil and lymphocyte counts between the reintroduced and wild populations and heterophil counts were higher in the reintroduced population—a pattern that is precisely opposite of what would be expected of seasonal effects. If seasonal effects were influential despite the short time span over which samples were collected, the effects would not match

previously published patterns; therefore, I believe that the most parsimonious conclusion is that season was not a driving factor in observed interpopulation differences.

Water temperature was not controlled for in this study; however, all four populations lived in water that fluctuated daily and seasonally with the ambient temperature. Albumin, CK, potassium, and phosphorus are all typically found in higher concentration at low temperatures while glucose is found in higher concentrations at high temperatures (Anderson et al. 1997). None of the four populations had significant differences in more than one of these analytes, suggesting that water temperature was likely not a primary driver of population-level differences in physiology.

Often-times, discerning the cause of elevated plasma chemistry and hematology values proves challenging in reptiles. Slightly elevated values can be indicative of such factors as seasonality and habitat differences, while extreme concentrations can indicate organ failure and disease, specifically renal and hepatic failure and heart damage. Because they are strictly aquatic, Alligator Snapping Turtles have uninterrupted access to fresh water and hydration issues are therefore not expected (Dantzler and Schmidt-Nielsen 1966; Campbell 2004a). However, extremely high levels of uric acid and extremely low levels of potassium and sodium typically indicate renal failure (Campbell 2004a). No individual presented a combination of these extreme analyte concentrations, so renal failure was likely not a factor across any population, or for that matter, for any individual in the study. Additionally, individuals with compromised renal systems would likely also exhibit abnormal concentrations of other electrolytes, which was not observed in this study. Aspartate aminotransferase (AST) tends to be a non-tissue-specific enzyme that is found in the liver, kidneys, heart, and muscles (Wilkinson 2004; Eatwell et al. 2014). Elevated levels of AST are indicative of compromised hepatic tissue when they occur in

the absence of concurrently elevated CK—a combination that would be consistent with muscle damage (Campbell 2004a). No individual had AST concentrations indicative of hepatic failure, as all elevated AST concentrations were associated with elevated CK. Heart damage can be identified with a combination of high AST and high CK concentrations (Wilkinson 2004). However, all elevated AST and CK concentrations were either within or lower than the established reference ranges for Alligator Snapping Turtles (Chaffin et al. 2008) whereas heart damage may be identified by AST and CK concentrations that are much higher than the reference range.

There is little evidence to indicate that seasonality, water temperature, or organ failure account for differences among populations; therefore, by elimination it is likely that the observed differences among populations indicate differences in habitat, diet, and ontogeny.

Dietary Components. Protein. Uric acid, total protein, and globulin concentrations are all useful indicators for dietary protein intake (Figueres 1997; Wilkinson 2004). All three analytes were higher in the wild population than the reintroduced population. Alligator Snapping Turtles are opportunistic consumers (Sloan et al. 1996; Harrel and Stringer 1997; Elsey 2006), so this could result from a multitude of factors related to high protein diets in the wild population—differential preferences for higher protein food items, higher availability of protein in the environment, or greater success at capturing live prey that may contain more protein than plants or partially-decomposed detritus.

Based on the positive linear relationship of carapace length with total protein and globulin concentrations in the reintroduced population, it can be inferred that larger individuals consumed higher protein diets. This makes intuitive sense, as larger individuals are likely to have more experience hunting live prey and have fewer gape limitations placed on their ability to

successfully consume prey. The relationship was also present in the wild population for total protein but not for globulin concentrations, and the relationship was not as strong as with the reintroduced population. Though the positive relationship was not present in the wild population for globulin or uric acid concentrations, the y-intercepts for all three protein analytes were higher than for the reintroduced population. This may indicate that wild Alligator Snapping Turtles have an advantage at a smaller size because of their exposure to natural forage items as hatchlings and young juveniles, whereas reintroduced turtles likely acquire their foraging skills following years in captivity where food availability is likely much higher and less stochastic. In a study that compared the diets of reintroduced and wild juvenile Alligator Snapping Turtles, the wild population consumed a diet that was proportionally higher in protein-rich forage than the reintroduced population (East and Ligon 2013). The need for captive-reared animals to develop foraging skills upon release has been reported in other taxa, including Black-footed Ferrets (*Mustela nigripes*) and Atlantic Salmon (*Salmo salar*) (Vargas and Anderson 1999; Brown et al. 2003), and therefore may represent a fertile avenue for improving the quality of head-started hatchlings prior to reintroduction by introducing mechanisms for learning active forage behavior prior to release.

Globulins and total protein concentrations were higher in the wild population than the outdoor captive population, again suggesting that there may be a learning curve to acquiring prey in a natural setting. Yet, there were no significant differences in any of the protein analytes between the indoor captive and wild populations, likely because the commercial feed diet supplied to them had similar in protein as the natural diet of wild Alligator Snapping Turtles in the populations surveyed.

Finally, uric acid concentrations had a negative relationship with carapace length in the wild population, a pattern that contradicts the positive relationship of carapace length with total protein and globulin concentrations. However, the relationship was weak, and additional research to ascertain its biological significance may be warranted.

Ions. The greatest difference in ion concentrations were between the two groups of captive Alligator Snapping Turtles that were housed indoors and outdoors. These two populations differed significantly in phosphorus, potassium, and sodium. Previous studies have demonstrated that plasma sodium and potassium concentrations of Spiny Softshell Turtles (*Apalone spinifera*) and Painted Turtles (*Chrysemys picta*) are influenced by concentrations in the surrounding aqueous environment; therefore, these concentrations may be indicative of differences in the water in which the two captive populations lived (Dunson and Weymouth 1965; Trobec and Stanley 1971). However, both the indoor and outdoor captive populations were housed in water derived from the same spring-fed canal system, so sodium and potassium concentrations should not be different between the two populations based on water chemistry. These three ionic concentrations, however, can be influenced by differences in diet (Chaffin et al. 2008; Kimble et al. 2012; Yu et al. 2013; Lloyd et al. 2016) and ontogenetic stage (Anderson et al. 1997; Dennis et al. 2001; Brenner et al. 2002; Innis et al. 2007; Rose and Allender 2011), which are both consistent with the population-level patterns observed in this study.

The diet consumed by the indoor captive population is known, and the range of forage items consumed by those in the outdoor ponds can be reasonably inferred. The indoor population was fed a commercially produced pelleted diet *ad libitum*, while the turtles reared in outdoor ponds were able to freely and selectively forage on available vegetation, fish, crayfish and other macroinvertebrates, and the soil substrate. The indoor population had higher plasma phosphorus

and sodium concentrations than the outdoor population, likely indicating that these nutrients were more readily obtained in the commercial diet than from naturally occurring forage items. However, the outdoor captive population had higher plasma potassium concentrations. This nutrient occurs in high concentrations in many plants (Ward 1966; Carlson et al. 1985; King 1996)—which were not available to the indoor population.

In addition to the influence of diet, plasma phosphorus concentrations also tend to be positively correlated with age (Dennis et al. 2001; Innis et al. 2007; Rose and Allender 2011). Phosphorus was lower in the outdoor captive population than the larger reintroduced and wild populations. However, the smaller indoor population did not follow this trend. Therefore, I infer that the phosphorus concentration in the commercial feed this population consumes was likely high enough to overcome any size-related effects on plasma concentrations.

Calcium concentration tends to be highly influenced by reproductive condition with mature females typically exhibiting higher calcium concentrations during vitellogenesis than males or juveniles (Anderson et al. 1997; Brenner et al. 2002). The wild population included several large, reproductively mature females and the reintroduced population included at least one female that was approaching reproductive maturity. However, no other reintroduced turtles that were sampled were sexually mature, and the two captive populations were composed entirely of sexually immature animals. Therefore, the wild population was expected to have higher calcium concentrations than the two captive populations; however, the only significant difference was between the wild and indoor captive populations. A lack of difference between the wild and reintroduced populations may be due to the reintroduced population approaching reproductive maturity, as is likely the case for several of the animals sampled that exceeded the minimum size for sexual maturity (Dobie 1971). Alternatively, due to the timing of sampling,

females collected in the wild population may have been previtellogenic. Serial samples collected throughout the year would be helpful in identifying influences of reproductive phase on plasma calcium concentrations (Callard et al. 1978; Lutz and Dunbar-Cooper 1987; Rostal et al. 1994; Rostal et al. 1998a; Rostal et al. 1998b).

Upon pooling data from the reintroduced and wild populations—the two groups that included mature and nearly mature individuals—there was a significant and positive relationship between carapace length and calcium concentration. Thus, plasma calcium may be useful for identifying sexual maturity of female Alligator Snapping Turtles. The indoor population exhibited lower calcium concentrations compared to the wild population, whereas, the outdoor captive population did not. The mean calcium concentration among animals housed indoors was lower than that for animals housed outdoors, but the difference was not statistically significant. The indoor captive population also lacked exposure to natural levels of UVB lighting, and although vitamin D can be obtained from diet, UVB deficiency often leads to hypocalcemia (Boyer 1996; McArthur et al. 2004; McWilliams 2005; Ferguson et al. 2009; Innis and Knotek 2020). The combination of small size and lack of UVB lighting may be the cause of dissimilarity between the indoor captive population and the wild population. The relative importance of these factors deserves further study.

Stress. It is often challenging to assess stress levels in vertebrates because capture and restraint can trigger an acute response that results in elevated glucocorticoid hormone concentrations within minutes (Muir and Pfister 1987; Langkilde and Shine 2006; Davis et al. 2008). However, other endpoints, including white blood cell counts and plasma glucose concentrations may be used in place of or in conjunction with measurements of stress hormones to infer stress levels because they typically take several hours or days to exhibit a response

(Davis et al. 2008). Elevated plasma glucose is often a product of an acute stress response and can be used to indirectly measure stress levels (Wilkinson 2004; Eatwell et al. 2014). When chronic stressors are present, heterophils typically increase and lymphocytes decrease with elevated stress, so the heterophil to lymphocyte ration (H:L) can be used as an indicator of chronic stress (Aguirre et al. 1995; Campbell 2004b; Wilkinson 2004; Zhang et al. 2011). Additionally, eosinophils have been shown to decrease in the presence of stress hormones (Jain 1993; Davis et al. 2008).

The outdoor captive population had lower H:L and glucose concentrations than the wild and reintroduced populations and lower glucose than the indoor captive population, from which I infer that outdoor turtles in the captive population may have experienced fewer or lower intensity stressors than the other populations studied. This population previously experienced the same living conditions as the indoor captive population and was moved to the more natural outdoor ponds to acclimate before being released at reintroduction sites. Previous studies with this head-start population have revealed that hatchery-reared animals grow fastest when they are in low density tanks (Sardina 2018) and after they have been released into natural environments (Anthony et al. 2015). Increased growth rates and lower stress indicators have also been seen in captive Chinese Softshell Turtles (*Pelodiscus sinensis*) when housed in lower densities (Chen et al. 2007) and Eastern Box Turtles (*Terrapene carolina carolina*) when housed in a more natural captive habitat setting (Case et al. 2005). The difference in enclosure and habitat was enough to produce a pattern of lower stress indicators in the outdoor captive population in comparison to the indoor captive population. The outdoor population also had significant indicators of lower stress compared to the wild and reintroduced populations. The Caney River and the Poteau River turtles had higher stress indicators (i.e. glucose and H:L) than either captive population, which is

consistent with stress-induced low eosinophil counts. Overall, the outdoor population may be inferred to have fewer stressors than the indoor population, including lower density accommodations and likely better access to submerged structure that can serve as cover objects.

Physical Exertion. Moderately elevated AST can be indicative of metabolically costly aggressive interactions, high activity levels, captivity, and growth (Dickinson et al. 2002; Rousselet et al. 2013; Andreani et al. 2014 López et al. 2017; Mumm et al. 2019). High CK can be associated with muscle damage, particularly in aggressive males during breeding and intrasexual aggressive encounters (O'Connor et al. 1994; Dickinson et al. 2002; Andreani et al. 2014; Mumm et al. 2019).

The indoor captive population had a significantly higher AST concentration than the outdoor captive and reintroduced populations, as well as a higher average concentration than the wild population. The indoor captive population had the smallest carapace length and were housed in the most crowded conditions. This high density often caused high levels of aggression among individuals (D.B. Ligon, pers. obs.) which was evident in the physical assessments conducted in this study. Sixty percent of this population had evidence of injury either at the time of examination or from previous encounters in the form of abrasions, abscesses, scars, and shortened tails (Table 1). The captive outdoor population had a high proportion of individuals with scarring—but fewer individuals with abrasions and abscesses than the indoor population—while the reintroduced turtles had just four individuals with abrasions and only two with visible scarring. High AST concentrations in the indoor captive population could reasonably be attributed to high levels of conspecific aggression, however, this population also had the lowest CK concentration which may also result from aggression and muscle damage.

High AST concentrations combined with low CK concentrations suggests growth was the primary influencing factor of high AST in the indoor population. The indoor populations had a smaller average carapace length than the other three populations, and most animals, including other chelonians, tend to exhibit faster size-specific growth rates at small sizes (Rocha 1995; Onorato 1996). However, if growth was the primary factor for high AST, the outdoor population would also be expected to have elevated AST concentrations and low CK concentrations because they were also smaller in size compared with the reintroduced and wild populations and grow at comparable rates to the indoor captive turtles (D.B. Ligon, unpublished data). Because the outdoor population showed similar trends in CK concentrations to the indoor population, but had lower AST concentrations compared to the wild population than would be expected based on the concentration of the indoor population, it is likely that—in addition to growth—conspecific aggression resulting from higher densities in living quarters was likely at least partially responsible for the high AST concentration of the indoor populations.

Finally, the wild population had a relatively high AST concentration compared with the reintroduced and outdoor captive populations along with the highest average CK concentration that was significantly greater than both captive populations. The wild population was the only population that contained samples from reproductively mature individuals that would exhibit breeding aggression. High AST combined with high CK concentrations support the hypothesis of muscle damage incurred during breeding and territorial encounters within the wild population (Campbell 2004a).

Conclusion. Differences among populations in this study can be attributed to differences in habitat, differential access to a wide range of forage items, and captive husbandry and environmental conditions. My results suggest that captive-produced Alligator Snapping Turtles

may be at a disadvantage in capturing and consuming protein-rich animal prey. Therefore, head-start efforts may benefit from expanded opportunities and training to capture mobile prey.

Myriad factors can influence biochemical and hematological variables, complicating the interpretation of results. Nonetheless, making population-level comparisons, particularly between wild and captive contexts, can be a valuable tool for assessing—and potentially addressing—underlying differences that may have long-term consequences.

Literature Cited

Aguirre, A.A., G.H. Balazs, T.R. Spraker, and T.S. Gross. 1995. Adrenal and hematological responses to stress in juvenile Green Turtles (*Chelonia mydas*) with and without fibropapillomas. Physiol Zool, 68(5):831–854.

Andreani, G., E. Carpené, A. Cannavucciuolo, N. Di Girolamo, E. Ferlizza, G. Isani. 2014. Reference values for hematology and plasma biochemistry variables, and protein electrophoresis of healthy Hermann's Tortoises (*Testudo hermanni* ssp.). Bet Clin Pathol, 2014(43):573–583.

Anderson, N.L., R.F. Wack, and R. Hatcher. 1997. Hematology and clinical chemistry reference ranges for the clinically normal, captive New Guinea Snapping Turtle (*Elseya novaeguineae*) and the effects of temperature, sex, and sample type. J Zoo Wildl Med, 28(4):394–403.

Anthony, T., J.D. Riedle, M.B. East, B. Fillmore, and D.B. Ligon. 2015. Monitoring of a reintroduced population of juvenile Alligator Snapping Turtles. Chelonian Conserv Biol, 14(1):43–48.

Berry, K.H., and M.M. Christopher. 2001. Guidelines for the field evaluation of Desert Tortoise health and disease. J Wildl Dis, 37(3):427–451.

Boyer, T.H. 1996. Metabolic Bone Disease. *In:* Reptile Medicine and Surgery. D.R. Mader (ed.). W.B. Saunders Company, Philadelphia, Pennsylvania, pp. 385–392.

Brenner, D., G. Lewbart, M. Stebbins, and D.W. Herman. 2002. Health survey of wild and captive Bog Turtles (*Clemmys muhlenbergii*) in North Carolina and Virginia. J Zoo Wildl Med, 33(4):311–316.

Brown, C., T. Davidson, and K. Laland. 2003. Environmental enrichment and prior experience of live prey improve foraging behavior in hatchery-reared Atlantic Salmon. J Fish Biol, 2003(63):187–196.

Callard, I.P., V. Lance, A.R. Salhanick, and D. Barad. 1978. The annual ovarian cycle of *Chrysemys picta*: correlated changes in plasma steroids and parameters of vitellogenesis. Gen Comp Endocrinol, 35(3):245–257.

Campbell, T.W. 1996. Clinical Pathology. *In:* Reptile Medicine and Surgery. D.R. Mader (ed.). W.B. Saunders Company, Philadelphia, Pennsylvania, pp. 248–257.

Campbell, T.W. 2004. Clinical chemistry of reptiles. *In:* Veterinary Hematology and Clinical Chemistry. M.A. Thrall, D.C. Baker, T.W. Campbell, D. DeNicola, M.J. Fettman, E.D. Lassen, A. Rebar, and G. Weiser (eds.). Lippincott Williams & Wilkins, Baltimore, Maryland, pp. 493–498.

Campbell, T.W. 2004. Hematology of Reptiles. *In:* Veterinary Hematology and Clinical Chemistry. M.A Thrall, D.C. Baker, T.W. Campbell, D. DeNicola, M.J. Fettman, E.D.

Lassen, A. Rebar, and G. Weiser (eds.). Lippincott Williams & Wilkins, Baltimore, Maryland, pp. 493–498.

Carlson, E., M. Kipps, A. Lockie, and J. Thomson. 1985. A comparative evaluation of vegan, vegetarian, and omnivore diets. J Plant Foods, 6(2):89–100.

Case, B.C., G.A. Lewbart, and P.D. Doerr. 2005. The physiological and behavioural impacts of and preference for an enriched environment in the Eastern Box Turtle (*Terrapene carolina carolina*). Appl Anim Behav Sci, 92(2005):353–365.

Chaffin, K., T.M. Norton, K. Gilardi, R. Poppenga, J.B. Jensen, P. Moler, C. Cray, E.S. Dierenfeld, T. Chen, M. Oliva, F.C. Origgi, S. Gibbs, L. Mazzaro, and J. Mazet. 2008. Health assessment of free-ranging Alligator Snapping Turtles (*Macrochelys temminckii*) in Georgia and Florida. J of Wildl Dis, 44(3):670–686.

Chen, X., C. Niu, and L. Pu. 2007. Effects of stocking density on growth and non-specific immune responses in juvenile Soft-shelled Turtle, *Pelodiscus sinensis*). Aquac Res, 38:1380–1386.

Crawshaw, G.J., and P. Holz. 1996. Comparison of plasma biochemical values in blood and blood-lymph mixtures from Red-eared Sliders, *Trachemys scripta elegans*. Bull Assoc Reptil Amphib Vet, 6(2):7–9.

Dantzler, W.H., and B. Schmidt-Nielsen. 1966. Excretion in fresh-water turtle (*Pseudemys scripta*) and Desert Tortoise (*Gopherus agassizii*). Am J Physiol, 210:198–210.

Davis, A.K., D.L. Maney, and J.C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol, 22(5):760–772.

Dennis, P.M., R.A. Bennett, K.E. Harr, and B.A. Lock. 2001. Plasma concentration of ionized calcium in healthy iguanas. J Am Vet Med Assoc, 219(3):326–328.

Dickinson, V.M., J.L. Jarchow, and M.H. Trueblood. 2002. Hematology and plasma biochemistry reference range values for free-ranging Desert Tortoises in Arizona. J Wildl Dis, 28(1):143–153.

Dobie, J.L. 1971. Reproduction and growth in the Alligator Snapping Turtle *Macroclemys temminckii* (Troost). Copeia 1971(4):645–658.

Dreslik, M.J., J.L. Carr, D.B. Ligon, and E.J. Kessler. 2017. Recovery of the Alligator Snapping Turtle (*Macrochelys temminckii*) in the Mississippi River Valley drainages of southern Illinois, Oklahoma, and Louisiana. Illinois Natural History Survey Technical Report, 2017(28):1–59.

Dunson, W.A., and R.D. Weymouth. 1965. Active uptake of sodium by Softshell Turtles (*Trionyx spinifer*). Science, 149:67–69.

East, M.B., and D.B. Ligon. 2013. Comparison of diet among reintroduced and wild juvenile Alligator Snapping Turtles (*Macrochelys temminckii*) and adult female Ouachita Map Turtles (*Graptemys ouachitensis*). Southwest Nat, 58(4):450–458.

Eatwell, K., J. Hedley, and R. Barron. 2014. Reptile haematology and biochemistry. In Practice, 36(1):34–42.

Elsey, R.M. 2006. Food habits of *Macrochelys temminckii* (Alligator Snapping Turtle) from Arkansas and Louisiana. Southeast Nat, 5(3):443–452.

Ferguson, G.W., W.H. Gehrmann, B. Peavy, C. Painter, R. Hartdegen, T.C. Chen, M.F. Holick, J.E. Pinder. 2009. Restoring vitamin D in Monitor Lizards: exploring the efficacy of dietary and UVB sources. J Herpetol Med Surg, 19(3):81–88.

Figueres, J.M. 1997. Treatment of articular gout in a Mediterranean Pond Turtle, *Mauremys leprosa*. Bull Assoc Reptil Amphib Vet, 7(4):5–8.

Glass, B.P. 1949. Records of *Macrochelys temminckii* in Oklahoma. Copeia, 1949:138–141.

Hall, R.J., P.F.P. Henry, and C.M. Bunck. 1999. Fifty-year trends in a box turtle population in Maryland. Biol Conserv, 88(2):165–172.

Harrel, J.B., and G.L. Stringer. 1997. Feeding habits of the Alligator Snapping Turtle (*Macroclemys temminckii*) as indicated by teleostean otoliths. Herpetol Rev, 28(4):185–187.

Herbst, L.H., and E.R. Jacobson. 2003. Practical approaches for studying sea turtle health and disease. *In:* The Biology of Sea Turtles, volume II. P.L Lutz, J.A. Musick, J. Wyneken (eds.). CRC Press, Boca Raton, Florida, pp. 385–410.

Innis, C., and Z. Knotek. 2020. Tortoises and freshwater turtles. *In:* Exotic Animal Laboratory Diagnosis. J.J. Heatley and K.E. Russell (eds.). Wiley Blackwell, pp. 255–289.

Innis, C.J., M. Tlusty, and D. Wunn. 2007. Hematologic and plasma biochemical analysis of juvenile head-started Northern Red-bellied Cooters (*Pseudemys rubriventris*). J Zoo Wildl Med, 38(3):425–432.

Jacobson, E.R., J. Schumach, and M. Green. 1992. Field and clinical techniuques for sampling and handling blood for hematologic and selected biochemical determinations in the Desert Tortoise, *Xerobates agassizii*. Copeia, 1992(1):237–241.

Jain, N.C. 1993. The Eosinophils. *In:* Essentials of Veterinary Hematology. Lea & Febiger, Malvern, Pennsylvania, pp. 247–257.

Keller, K.A., D.S. Guzman, J. Paul-Murphy, B.A. Byrne, S.D. Owens, P.H. Kass, and S. Weber, III. 2012. Hematologic and plasma biochemical values of free-ranging Western Pond Turtles (*Emys marmorata*) with comparison to a captive population. J Herpetol Med Surg, 22(3-4):99–106.

Kimble, S.J.A., and R.N. Williams. 2012. Temporal variance in hematologic and plasma biochemical reference intervals for free-ranging Eastern Box Turtles (*Terrapene carolina carolina*). J Wildl Dis, 48(3):799–802.

King, G. 1996. Reptiles and Herbivory. Chapman and Hall, London, pp. 29–60.

Langkilde, T., and R. Shine. 2006. How much stress do researchers inflict on their study animals? A case study using a Scincid lizard, *Eulamprus heatwolei*. J Exp Biol, 209:1035–1043.

Lloyd, T.C., MC. Allender, G. Archer, C.A. Phillips, J. Byrd, and A.R. Moore. 2016. Modeling hematologic and biochemical parameters with spatiotemporal analysis for the free-ranging Eastern Box Turtle (*Terrapene carolina carolina*) in Illinois and Tennessee, a potential biosentinel. EcoHealth, 13:467–479.

López, J., M. Waters, A. Routh, T.F. Rakotonanahary, L. Woolaver, A. Thomasson, E. Holmes, and H.W. Steinmetz. 2017. Hematology and plasma chemistry of the Ploughshare Tortoise (*Astrochelys yniphora*) in a captive breeding program. J Zoo Wildl Med, 48(1):102–115.

Lutz, P.L., and A. Dubar-Cooper. 1987. Variations in the blood chemistry of the loggerhead sea turtle, *Caretta caretta*. Fish Bull, 85(1):37–43.

McArthur, S., J. Meyer, and C. Innis. 2004. Anatomy and Physiology. *In:* Medicine and Surgery of Tortoises and Turtles. S. McArthur, R. Wilkinson, J. Meyer (eds.). Wiley-Blackwell, Ames, Iowa, pp. 35–72.

McWilliams, D.A. 2005. Nutrition research on calcium homeostasis. II. Freshwater turtles (with recommendations). Int Zoo Yearb, 2005(39):77–85.

Moll, D., and E.O. Moll. 2004. The ecology, exploitation, and conservation of river turtles. Oxford University Press, New York, New York, pp. 252.

Muir, J.J., and H.P. Pfister. 1987. Time course of the corticosterone and prolactin response following predictable and unpredictable novelty stress in *Rattus norvegicus*. Physiol Behav, 40(1):103–107.

Mumm, L.E., J.M. Winter, K.E. Andersson, G.A. Glowacki, L.A. Adamovicz, and M.C. Allender. 2019. Hematology and plasma biochemistries in the Blanding's Turtle (*Emydoidea blandingii*) in Lake County, Illinois. PLoS One, 14(11):1–15.

Nickerson, M.A., and A.L. Pitt. 2012. Historical turtle population decline and community changes in an Ozark River. Bulletin of the Florida Museum of Natural History, 51(4):257–267.

O'Connor, M.P., J.S. Grumbles, R.H. George, L.C. Zimmerman, and J.R. Spotila. 1994. Potential hematological and biochemical indicators of stress in free-ranging Desert

Tortoises and captive tortoises exposed to a hydric stress gradient. Herpetological Monographs, 1994(8):5–26.

Oklahoma Department of Wildlife Conservation. 2016. Oklahoma Comprehensive Wildlife Conservation Strategy. http://www.wildlifedepartment.com/cwcs/ApprovedOKCWCSintroduction.pdf. *Accessed on* 30 March 2020.

Onorato, D. 1996. The growth rate and age distribution of *Sternotherus minor* at Rainbow Run, Florida. J Herpetol, 30(3):301–306.

Pritchard, P.C.H. 2006. Population Status and Trends. *In:* The Alligator Snapping Turtle: Biology and Conservation. Krieger Publishing Company, Malabar, Florida, pp. 82–87.

Rangel-Mendoza, J., M. Weber, C.E. Zenteno-Ruiz, M.A. López-Luna, and E. Barba-Macías. 2009. Hematology and serum biochemistry comparison in wild and captive Central American River Turtles (*Dermatemys mawii*) in Tabasco, Mexico. Res Vet Sci, 87(2):313–318.

Reed, R.N., J. Congdon, and J.W. Gibbons. 2002. The Alligator Snapping Turtle [*Macrochelys* (*Macroclemys*) *temminckii*]: a review of ecology, life history, and conservation, with demographic analyses of the sustainability of take from wild populations. Report to Division of Scientific Authority, US Fish and Wildlife Service, pp. 1–17.

Rhodin, A.G.J., C.B. Standford, P.P. van Dijk, C. Eisemberg, L. Luiselli, R.A. Mittermeier, R. Hudson, B.D. Horne, E.V. Goode, G. Kuchling, A. Walde, E.H.W. Baard, K.H. Berry, A. Bertolero, T.E.G. Blanck, R. Bour, K.A. Buhlmann, L.J. Cayot, S. Collett, A. Currylow, I. Das, T. Diagne, J.R. Ennen, G. Forero-Medina, M.G. Frankel, U. Fritz, G. García, J.W. Gibbons, P.M. Gibbons, G. Shiping, J. Guntoro, M.D. Hofmeyer, J.B. Iverson, A.R. Kiester, M. Lau, D.P. Lawson, J.E. Lovich, E.O. Moll, V.P. Páez, R. Palomo-Ramos, K. Platt, S.G. Platt, P.C.H. Pritchard, H.R. Quinn, S.C. Rahman, S.T. Randrianjafizanaka, J. Schaffer, W. Selman, H.B. Shaffer, D.S.K. Sharma, S. Haitao, S. Singh, R. Spencer, K. Stannard, S. Sutcliffe, S. Thomson, and R.C. Vogt. 2018. Global conservation status of turtles and tortoises (Order Testudines). Chelonian Conserv Biol, 17(2) 135–161.

Riedle, J.D., D.B. Ligon, K. Graves. 2008. Distribution and management of Alligator Snapping Turtles, *Macrochelys temminckii*, in Kansas and Oklahoma. Trans Kans Acad Sci, 111:21–28.

Riedle, J.D., P.A. Shipman, S.F. Fox, and D.M. Leslie, Jr. 2005. Status and distribution of the Alligator Snapping Turtle, *Macrochelys temminckii*, in Oklahoma. Southwest Nat, 50(1):79–84.

Rocha, C.F.D. 1995. Growth of the tropical sand lizard *Liolaemus lutzae* in southeastern Brazil. Amphib Reptil, 16(3):257–264.

Rose, B.M.W., and M.C. Allender. 2011. Health assessment of wild Eastern Box Turtles (*Terrapene carolina carolina*) in east Tennessee. J Herpetol Med Surg, 21(4):107–112.

Rostal, D.C., V.A. Lance, J.S. Grumbles, and A.C. Alberts. 1994. Seasonal reproductive cycle of the Desert Tortoise (*Gopherus agassizii*) in the Eastern Mojave Desert. Herpetological Monographs, 8(1994):72–82.

Rostal, D.C., D.W. Owens, J.S. Grumbles, D.S. MacKenzie, and MS. Amoss, Jr. 1998. Seasonal reproductive cycle of the Kemp's Ridley Sea Turtle (*Lepidochelys kempi*). Gen Comp Endocrinol, 109:232–243.

Rostal, D.C., T.R. Robeck, J.S. Grumbles, P.M. Burchfield, and D.W. Owens. 1998. Seasonal reproductive cycle of the Galápagos Tortoise (*Geochelone nigra*) in captivity. Zoo Biol, 17:505–517.

Rousselet, E., N.I. Stacy, K. LaVictoire, B.M. Higgins, ME. Tocidlowski, J.P. Flanagan, and C.A.J. Godard-Codding. 2013. Hematology and plasma biochemistry analytes in five age groups of immature, captive-reared Loggerhead Sea Turtles (*Caretta caretta*). J Zoo Wildl Med, 44(4):859–874.

Sardina, K.E. 2018. Increasing Alligator Snapping Turtle head-starting success through captive housing enrichment and inoculation of hatchlings with digestive microbiota. Master's thesis. Missouri State University.

Seigel, R.A., and C.K. Dodd, Jr. 2000. Manipulation of turtle populations for conservation: Halfway technologies or viable options? *In:* turtle conservation. M.W. Klemens (ed.). Smithsonian Institution Press, Washington, D.C., pp. 218–238.

Sloan, K.N., K.A. Buhlmann, and J.E. Lovich. 1996. Stomach contents of commercially harvested adult Alligator Snapping Turtles, *Macroclemys temminckii*. Chelonian Conserv Biol, 2(1):96–99.

Stickel, L.F. 1978. Changes in a box turtle population during three decades. Copeia, 1978(2):221–225.

Thorbjarnarson, J., C.J. Lagueux, D. Bloze, M.W. Klemens, and A.B. Meylan. 2000. Human use of turtles: A worldwide perspective. *In:* Turtle conservation. M.W. Klemens (ed.). Smithsonian Institution Press, Washington, D.C., pp. 33–84.

Trobec, T.N., and J.G. Stanley. 1971. Uptake of ions and water by the Painted Turtle, *Chrysemys picta*. Copeia, 1971(3):537–542.

Tuberville, T.D., T.M. Norton, K.A. Buhlmann, and V. Greco. 2015. Head-starting as a management component for Gopher Tortoises (*Gopherus polyphemus*). Herpetol Conserv Biol, 10(1):455–471.

U.S. Fish and Wildlife Service and Department of the Interior. 2005. Inclusion of Alligator Snapping Turtle (*Macroclemys* [=*Macrochelys*] *temminckii*) and all species of map turtle (*Graptemys* spp.) in Appendix III to the Convention of International Trade in Endangered Species of Wild Flora and Fauna. Federal Register Environmental Document, pp. 74700–74703.

Vargas, A. and S.H. Anderson. 1999. Effects of experience and cage enrichment on predatory skills of Black-footed Ferrets (*Mustela nigripes*). J Mammal, 80(1):263–269.

Ward, G.M. 1966. Potassium metabolism of domestic ruminants—A review. J Dairy Sci, 49(3):268–276.

Wilkinson, R. 2004. Clinical Pathology. *In:* Medicine and Surgery of Tortoises and Turtles. S. McArthur, R. Wilkinson, J. Meyer (eds.). Wiley-Blackwell, Ames, Iowa, pp.141–186.

Yang, P.Y., P.H. Yu, S.H. Wu, and C.H. Chie. 2014. Seasonal hematology and plasma biochemistry reference range values of the Yellow-marginated Box Turtle (*Cuora flavomarginata*). J Zoo Wildl Med, 45(2):278–286.

Yu, P.H., P.Y. Yang, Y.S. Chiu, and C.H. Chie. 2013. Hematologic and plasma biochemical reference values of the Yellow Pond Turtle *Mauremys mutica* and the effects of sex and season. Zool Stud, 52(1):24–29.

Zhang, F., H. Gu, and P. Li. 2011. A review of Chelonian hematology. Asian Herpetol Res, 2(1):12–20.

Table 1. Results of the physical examination of alligator snapping turtles in terms of number of turtles presenting each abnormality for each population.

		Reintroduced (n = 30)	Indoor captive (n = 25)	Outdoor captive (n = 25)	Wild (n = 17)
Ears	Normal	30	25	25	17
Nose	Scarring	-	2	2	-
	Abrasion	-	-	3	2
	Normal	30	23	20	15
Mouth	Worn/damaged beak	-	3	5	-
	Abrasion	3	-	-	-
	Normal	25	22	19	11
Eyes	Scarring	-	-	2	-
	Abrasion	4	-	3	2
	Normal	24	25	20	12
Shell	Slight deformities or extra scutes	11	8	10	-
	Peeling	-	-	1	5
	Wear or scarring	-	10	-	5
	Abscess	-	2	-	-
	Propeller damage	-	-	-	2
	Normal	17	5	7	5
Appendages	Leeches (anywhere on body)	6	-	-	12
	Missing toenail	2	4	10	2
	Abscess	-	8	-	-
	Abrasion	-	3	-	4
	Scarring	2	5	10	-
	Skin peeling	-	6	12	-
	Normal	24	7	1	2
Completely Normal*		17	4	1	2

*These counts include turtles with extra scutes or slight carapacial deformities, but no turtles with significant shell deformity

Table 2. Mean, standard error, and sample size of each hematological and plasma biochemistry parameter for reintroduced, captive, and wild populations of alligator snapping turtles. Superscript letters denote significant differences among populations. Means in the same row with different superscripts are significantly different ($P < 0.05$).

	Reintroduced		Indoor captive		Outdoor captive		Wild	
Analyte	Mean±SE	*n*	Mean±SE	*n*	Mean±SE	*n*	Mean±SE	*n*
TWBC (cells/µl)	13948±959	24	12355±728	24	12768±882	25	12288±826	15
Heterophil (cells/µl)	7738±550^{c}	25	3149±248^{a}	23	4414±305^{ab}	23	5977±474^{b}	16
Basophil (cells/µl)	1123±176^{a}	24	2363±234^{b}	25	1763±195^{ab}	24	2236±271^{b}	16
Lymphocyte (cells/µl)	3569±358^{b}	24	2089±239^{a}	24	3302±316^{b}	24	2359±278^{ab}	15
Monocyte (cells/µl)	580±102	25	377±70	24	581±58	25	460±70	15
Azurophil (cells/µl)	469±60^{ab}	23	265±66^{a}	23	507±64^{ab}	24	710±104^{b}	15
Eosinophil (cells/µl)	485±59^{a}	24	3366±221^{b}	24	1501±151^{c}	23	653±110^{a}	14
H:L	2.27±0.23^{b}	24	1.85±0.25^{ab}	23	1.41±0.17^{a}	24	2.71±0.28^{b}	15

TWBC = Total white blood cell count, H:L = Heterophil to lymphocyte ratio, GLU = Glucose, TPS = Total plasma solids, PCV = Packed cell volume, ALB = Albumin, AST = Aspartate aminotransferase, CK = Creatinine kinase, UA = Uric acid, GLOB = Globulin, TP = Total protein, Ca^{++} = Calcium, P = Phosphorus, K^{+} = Potassium, Na^{+} = Sodium.

Table 2 continued. Mean, standard error, and sample size of each hematological and plasma biochemistry parameter for reintroduced, captive, and wild populations of alligator snapping turtles. Superscript letters denote significant differences among populations. Means in the same row with different superscripts are significantly different ($P < 0.05$).

	Reintroduced		Indoor captive		Outdoor captive		Wild	
Analyte	Mean±SE	*n*	Mean±SE	*n*	Mean±SE	*n*	Mean±SE	*n*
GLU (mg/dl)	66.57 ± 3.46^{b}	28	60.04 ± 2.48^{b}	23	46 ± 2.08^{a}	24	61.06 ± 3.66^{b}	16
TPS (mg/dl)	2.38 ± 0.21^{a}	28	3.27 ± 0.23^{b}	25	2.56 ± 0.17^{ab}	25	3.12 ± 0.18^{ab}	16
PCV (%)	22.30 ± 1.02^{a}	27	24.75 ± 0.93^{a}	24	29.22 ± 0.74^{b}	23	25.06 ± 0.94^{a}	16
ALB (g/dl)	0.88±0.063	28	0.8±0.048	24	0.86±0.047	25	0.92±0.046	16
AST (U/l)	75.96 ± 4.23^{a}	27	104.42 ± 5.00^{c}	25	83.08 ± 3.50^{ab}	24	94.19 ± 4.21^{bc}	16
CK (µmol/l)	231.8 ± 30.85^{ac}	25	154.5 ± 11.23^{b}	24	172.04 ± 9.31^{ab}	24	586.47 ± 213.51^{c}	15
UA (mg/dl)	1.04 ± 0.08^{a}	27	1.24 ± 0.14^{ab}	23	1.19 ± 0.05^{ab}	22	1.48 ± 0.13^{b}	16
GLOB (g/dl)	2.38 ± 0.12^{a}	27	2.82 ± 0.10^{bc}	23	2.59 ± 0.090^{ab}	25	3.12 ± 0.097^{c}	15
TP (g/dl)	3.20 ± 0.18^{a}	28	3.60 ± 0.14^{ab}	24	3.45 ± 0.13^{a}	25	4.11 ± 0.13^{b}	16
Ca^{++} (mg/dl)	7.47 ± 0.37^{ab}	27	6.87 ± 0.23^{a}	23	7.80 ± 0.32^{ab}	25	8.51 ± 0.42^{b}	15
P (mg/dl)	3.81 ± 0.17^{b}	27	3.85 ± 0.17^{b}	23	3.18 ± 0.11^{a}	23	3.84 ± 0.19^{b}	16
K^{+} (mmol/l)	3.83 ± 0.083^{b}	27	3.19 ± 0.099^{a}	24	4.23 ± 0.15^{c}	25	3.99 ± 0.091^{bc}	16
Na^{+} (mmol/l)	129.15 ± 0.47^{c}	26	126.28 ± 0.67^{b}	25	123.71 ± 0.44^{a}	24	128.47 ± 0.54^{bc}	15

TWBC = Total white blood cell count, H:L = Heterophil to lymphocyte ratio, GLU = Glucose, TPS = Total plasma solids, PCV = Packed cell volume, ALB = Albumin, AST = Aspartate aminotransferase, CK = Creatinine kinase, UA = Uric acid, GLOB = Globulin, TP = Total protein, Ca^{++} = Calcium, P = Phosphorus, K^{+} = Potassium, Na^{+} = Sodium.

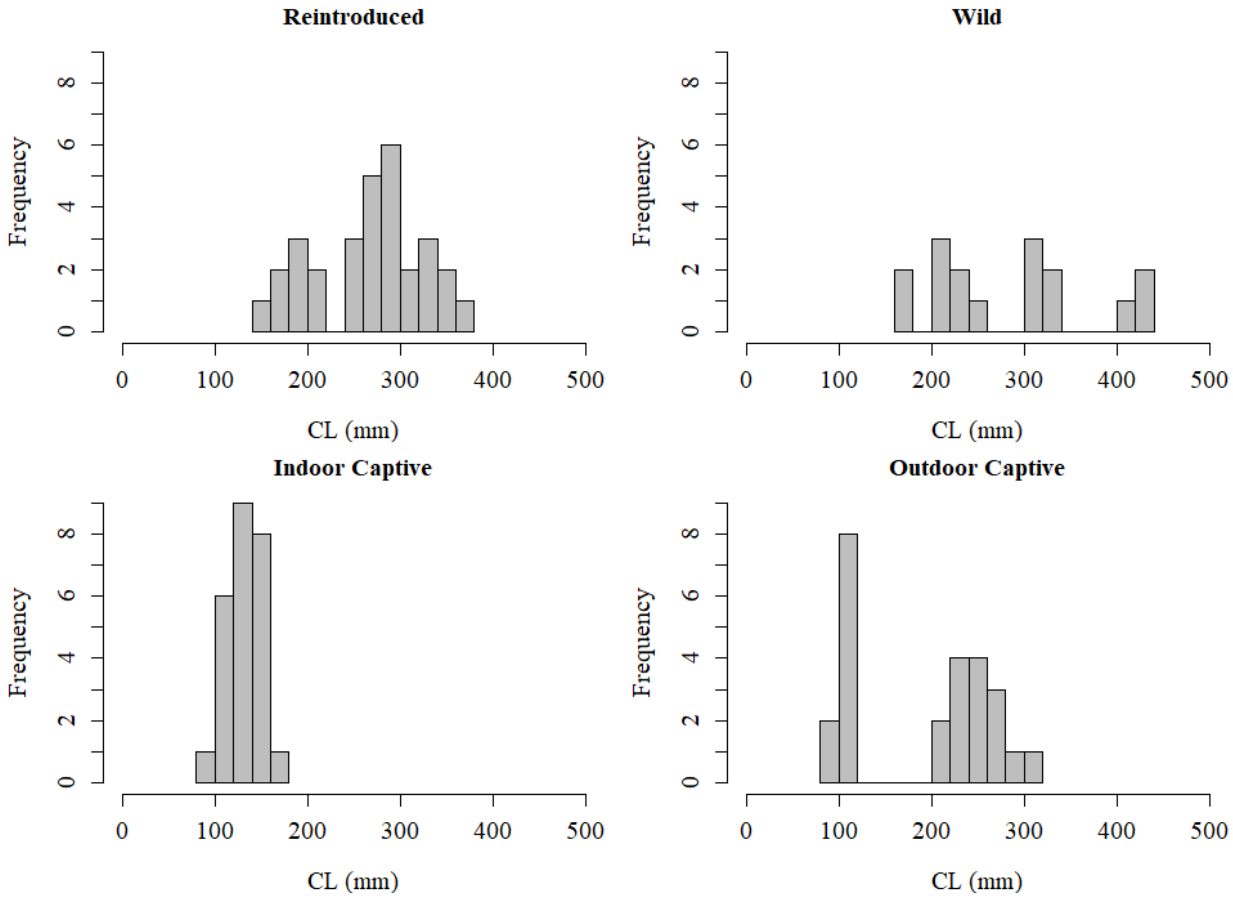

Figure 1. Size histograms of the reintroduced, wild, indoor captive, and outdoor captive populations of Alligator Snapping Turtles used in this study.

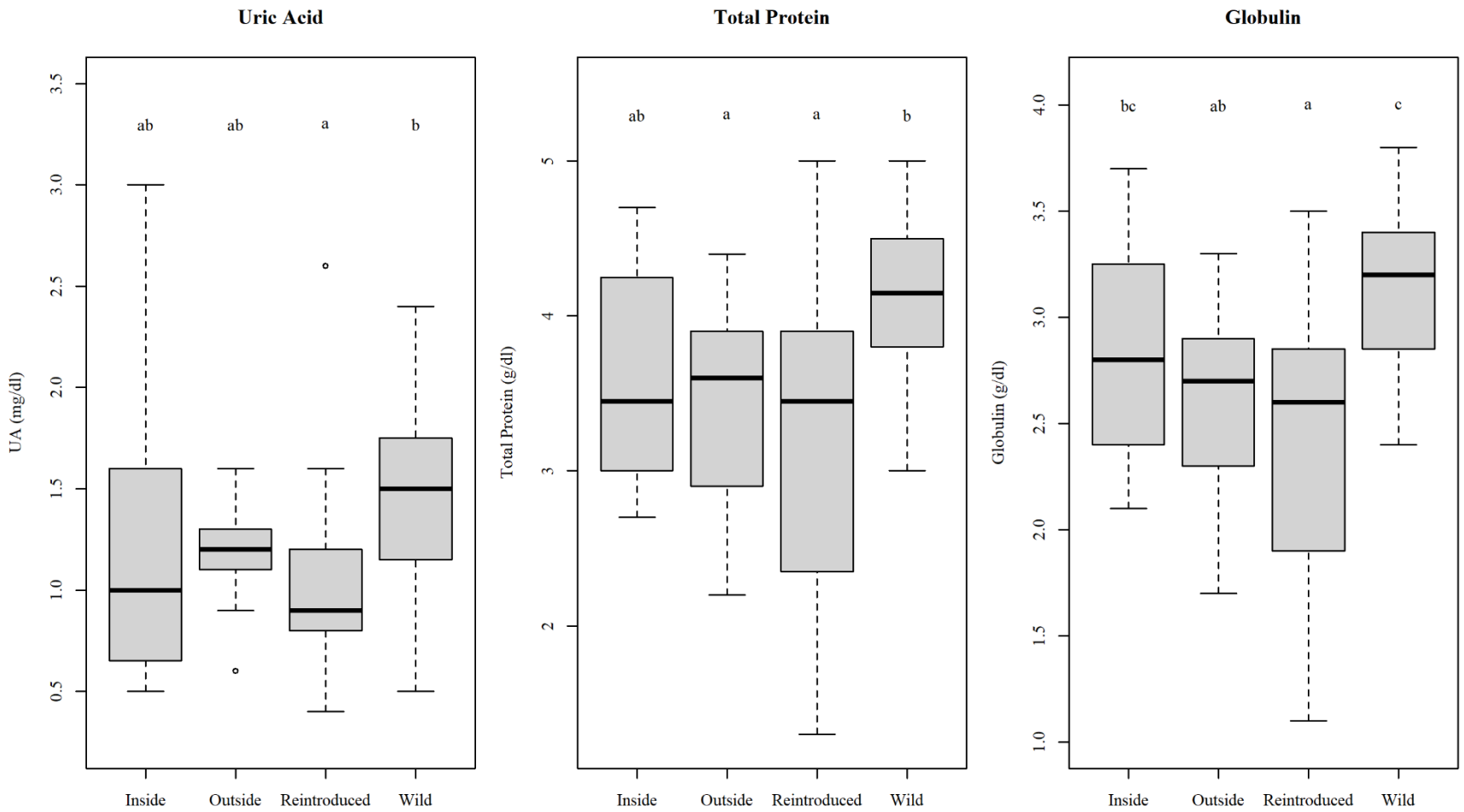

Figure 2. Box plots of ANOVA results for protein analytes: uric acid, total protein, and globulins. Letters denote significant differences between sites. The first and third quartiles are represented by the bounds of the box (respectively) with the median shown as the dark line in the center of the box.

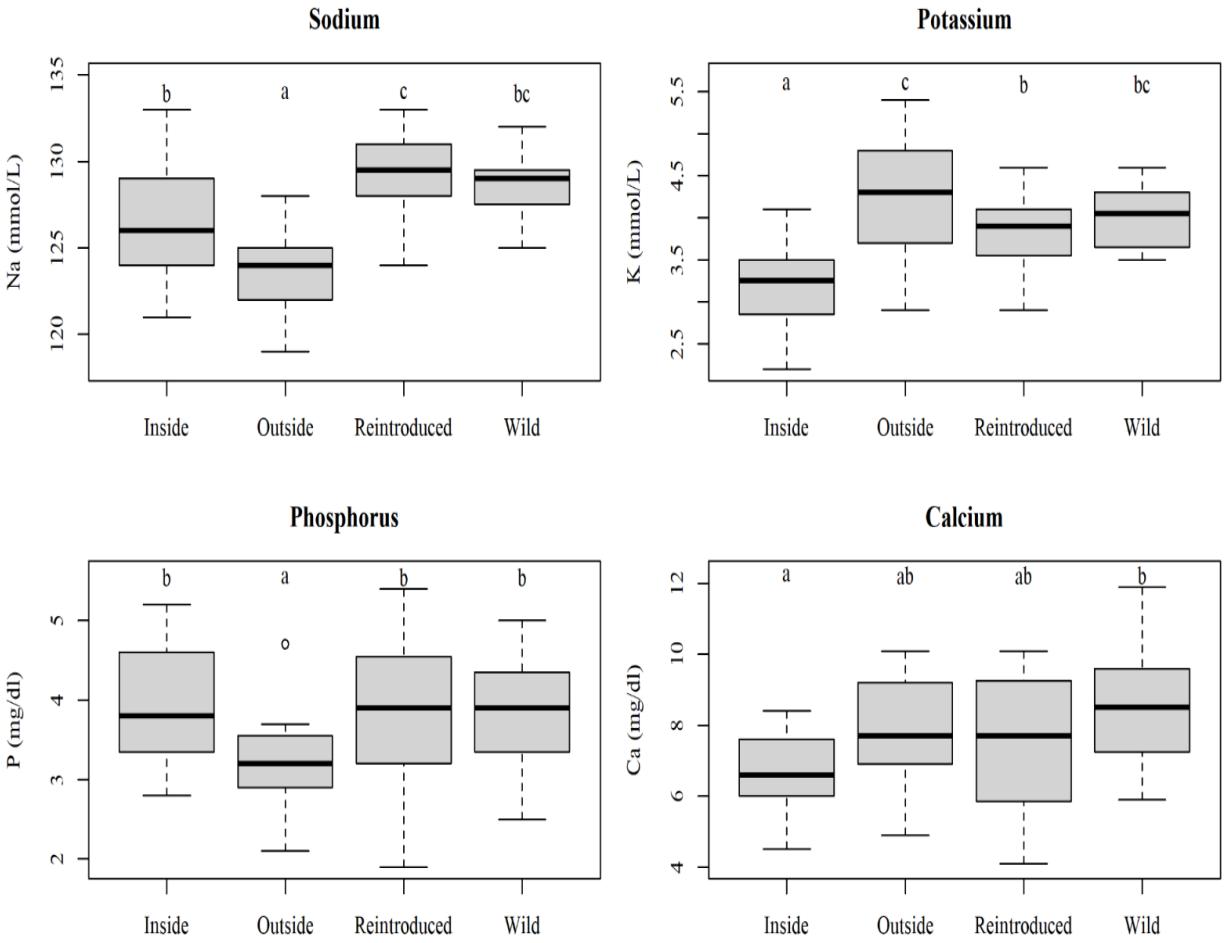

Figure 3. Box plots of ANOVA results for ionic concentrations: sodium, potassium, phosphorus, and calcium. Letters denote significant differences between sites. The first and third quartiles are represented by the bounds of the box (respectively) with the median shown as the dark line in the center of the box.

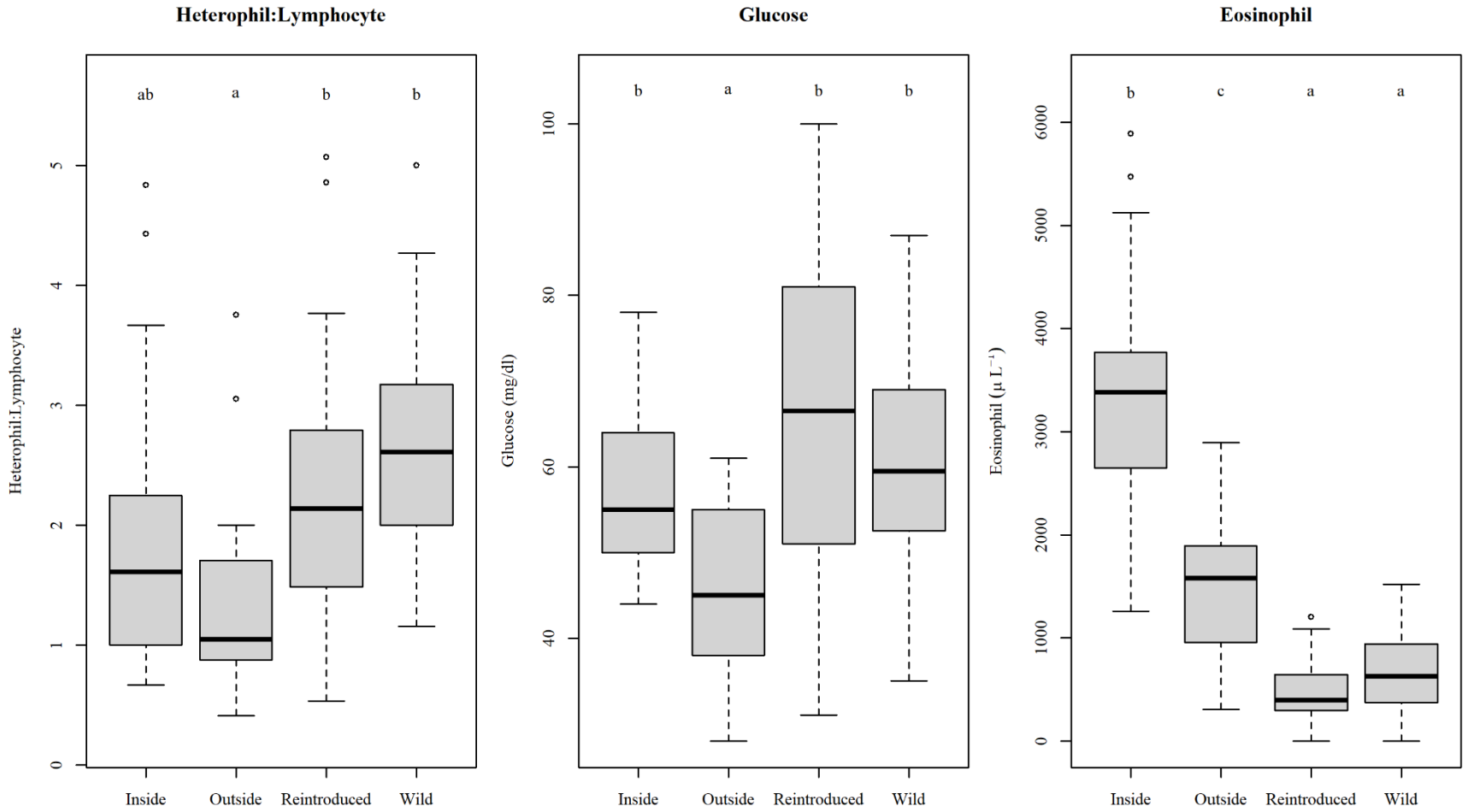

Figure 4. Box plots of ANOVA results for stress indicators: heterophil to lymphocyte ratio, glucose, and eosinophils. Letters denote significant differences between sites. The first and third quartiles are represented by the bounds of the box (respectively) with the median shown as the dark line in the center of the box.

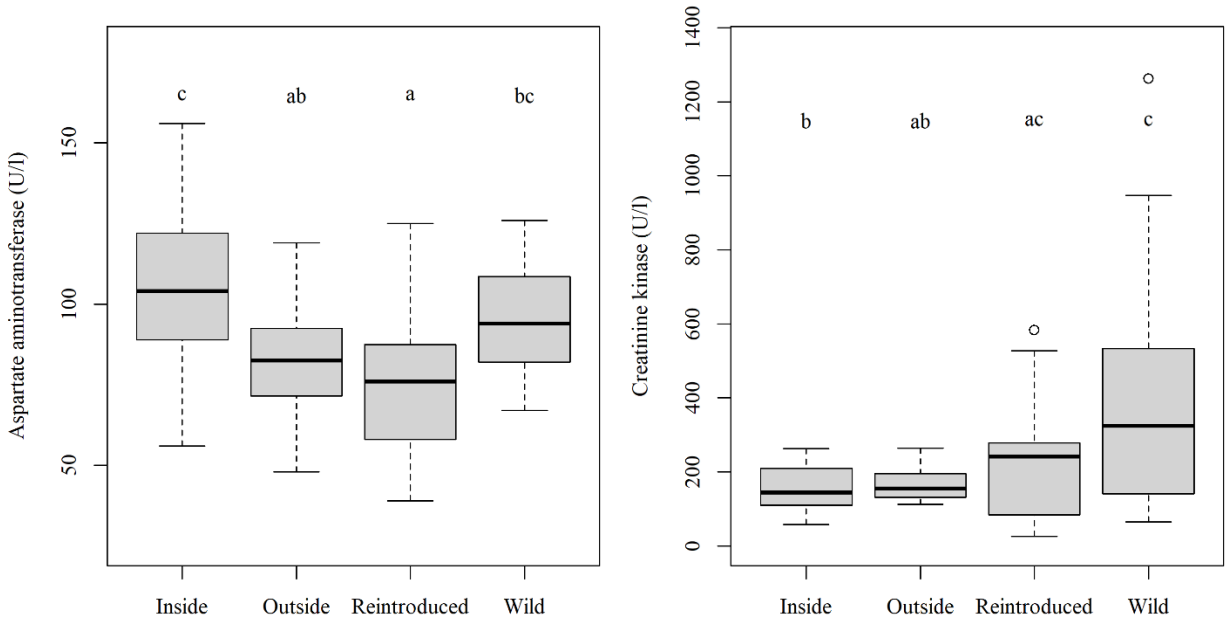

Figure 5. Box plots of ANOVA results for indicators of physical exertion: aspartate aminotransferase and creatinine kinase. Letters denote significant differences between sites. The first and third quartiles are represented by the bounds of the box (respectively) with the median shown as the dark line in the center of the box.

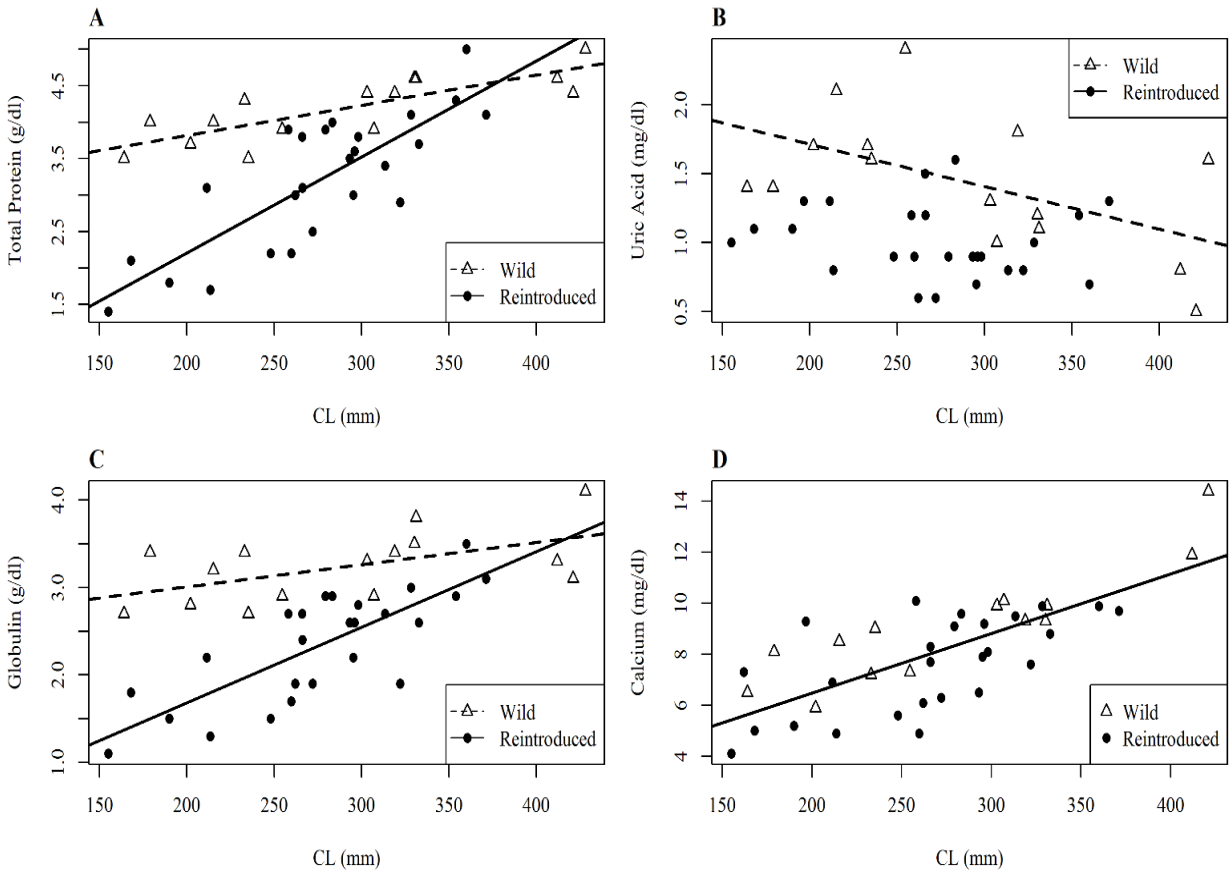

Figure 6. Linear regression relationship of several protein analyte concentrations (A–C) and calcium concentration (D) with carapace length (CL) in reintroduced and wild populations of Alligator Snapping Turtles. A. Total protein concentration to carapace length—there was a significant relationship in both reintroduced and wild populations ($R^2 = 0.6416$, $P < 0.001$ and $R^2 = 0.6295$, $P < 0.001$ respectively). B. Uric acid concentration to carapace length—there was no significant relationship among the reintroduced population, but there was for the wild population ($R^2 = 0.2407$; $P = 0.036$). C. Relationship of globulin concentration to carapace length—there was a significant relationship between globulin concentration and carapace length among the reintroduced ($R^2 = 0.5908$; $P < 0.001$), but not wild turtles ($P = 0.2468$). D. Relationship of calcium concentration to carapace length of reintroduced and wild Alligator Snapping Turtles combined ($R^2 = 0.5361$; $P < 0.001$).

SUMMARY

Surveying potential reintroduction sites to assess the turtle communities is an essential step in the process for reintroducing Alligator Snapping Turtles. I examined the turtle communities at nine sites along the Verdigris, Elk, Fall, and Caney rivers and their tributaries in Kansas. I did not detect the presence of Alligator Snapping Turtles at any of the sites sampled, but several sites had robust turtle communities.

Observing these turtle communities in Kansas in addition to extensive post-monitoring efforts of the Caney River turtle community—which Alligator Snapping Turtles have been reintroduced on the Oklahoma side—allowed me to make comparisons between potential reintroduction sites and an arguably successful reintroduction site. Of the sites I sampled, the Verdigris River near Coffeyville appears to be the most suitable reintroduction site in Kansas. Capture rates were somewhat low; however, diversity was high and similar to the community which Alligator Snapping Turtles have been reintroduced. Several sites were eliminated as suitable reintroduction sites due to high human impact and impoundments that would cause translocated turtles to move upstream and out of their historical range.

Wildlife reintroduction initiatives have been implemented around the world for at-risk and extirpated species, but the outcomes of most of these populations was vastly understudied until the early 2000s (Seddon et al. 2007). Thankfully, this has not been the case for the reintroduced population of Alligator Snapping Turtles at the Caney River in Oklahoma. Previous studies have shown that this population initially survived their release and even tended to grow quicker after release than their captive conspecifics (Anthony et al. 2015). The continual recapture of this species each year after release—even during flood years when it is challenging

to catch any species—further indicates they are surviving in this system. The next questions to answer was how well they were surviving and how does that compare to wild conspecifics. Additionally, I wanted to know how the captive head-start Alligator Snapping Turtles at Tishomingo National Fish Hatchery compared to free-ranging reintroduced and wild populations. I accomplished these goals using health assessments.

While no population or individual was apparently unhealthy, there were differences found among populations which were primarily derived from ontogenetic effects and apparent differences in diet. The wild population—especially the smaller individuals—were eating a higher protein diet compared to turtles of the same size from the reintroduced population on the Caney River, indicating earlier forage success of high protein food items in the wild population. Between the two captive populations the major differences were in ionic concentrations that were driven primarily by diet—pelleted food for the indoor housed turtles and natural vegetation forage for the outdoor housed turtles. Understanding these dietary differences will improve rearing and management techniques for this species in captivity and before the release of head-started individuals.

Knowing there is a lack of apparent health issues in the reintroduced population—at least in comparison to the wild population and previously published reference ranges (Chaffin et al. 2008)—indicates Alligator Snapping Turtles are a suitable species for reintroduction when all the reintroduction criteria are met (e.g. robust turtle community, habitat requirements, range requirements, etc.). This will further increase future reintroduction initiatives, including the potential consideration for releases of Alligator Snapping Turtles in Kansas.

ADDITIONAL REFERENCES

Christopher, M.M., K.H. Berry, I.R. Wallis, K.A. Nagy, B.T. Henen, and C.C. Peterson. 1999. Reference intervals and physiologic alteration in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert. Journal of Wildlife Diseases, 35:212–238.

Flanagan, J. 2000. Disease and health considerations. *In* Turtle Conservation. M.W. Klemens (ed.). Smithsonian Institution Press, Washington, D.C., pp. 85–95.

Gibbon, J.W., D.E. Scott, T.J. Ryan, K.A. Buhlmann, T.D. Tuberville, B.S. Metts, J.L. Green, T. Mills, Y. Leiden, S. Poppy, and C.T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. BioScience, 50:653–666.

IUCN/SSC. 2013. Guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission, Gland, Switzerland.

Kansas Department of Wildlife and Parks. 1986. Summary of nongame task force actions relative to developing three species lists: species in need of conservation, threatened, and endangered. Kansas Department of Wildlife and Parks Nongame Task Force Report, Pratt, Kansas, pp. 27.

Milton, S.L., and P.L. Lutz. 2003. Physiological and genetic responses to environmental stress. *In* The biology of sea turtles, volume II. P.L. Lutz, J.A. Musick, J. Wyneken (eds.). CRC Press, Boca Raton, Florida, pp. 163–197.

Moll, E.O., and D. Moll. 2000. Conservation of river turtles. *In* Turtle Conservation. M.W. Klemens (ed.). Smithsonian Institution Press, Washington, D.C., pp. 126–155.

Perpiñán, D., S.M. Hernandez-Divers, K.S. Latimer, T. Akre, C. Hagen, K.A. Buhlmann, and S.J. Hernandez-Divers. 2008. Hematology of the Pascagoula map turtle (*Graptemys gibbonsi*) and the southeast Asian box turtle (*Cuora amboinensis*). Journal of Zoo and Wildlife Medicine, 39:460–463.

Seddon, P.J., D.P. Armstrong, and R.F. Maloney. 2007. Developing the science of reintroduction biology. Conservation Biology, 21:303–312.

Seddon, P.J. 1999. Persistence without intervention: assessing success in wildlife reintroductions. Trends in Ecology & Evolution, 14:503.

APPENDICES

Appendix A. CITI training certificates for Basic Wildlife Researcher Animal Care and Use (A-1), Working with Fish (A-2), and Working with Reptiles (A-3). This research was initially performed under protocol 17-028.0-A (approved 23 May 2017) which was renewed 17 April 2019 as protocol 19-015.0-A.

1

CITI PROGRAM

Completion Date 23-Apr-2018
Expiration Date 22-Apr-2021
Record ID 26831052

This is to certify that:

Samantha Hannabass

Has completed the following CITI Program course:

Animal Care and Use (Curriculum Group)
Researcher - Wildlife (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

Missouri State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w0e5a795a-f973-4e0a-92cf-d8b1e1119550-26831052

Appendix A continued.

2

CITI PROGRAM

Completion Date 23-Apr-2018
Expiration Date N/A
Record ID 26839821

This is to certify that:

Samantha Hannabass

Has completed the following CITI Program course:

Working With Fish in Research Settings (Curriculum Group)
Working with Fish (Course Learner Group)
1 - Lab Animal Research (Stage)

Under requirements set by:

Missouri State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w99e372f4-aafd-43a5-b794-1db979867a21-26839821

Appendix A continued.

3

CITI PROGRAM

Completion Date 23-Apr-2018
Expiration Date N/A
Record ID 26831054

This is to certify that:

Samantha Hannabass

Has completed the following CITI Program course:

Working with Reptiles in a Research Setting (Curriculum Group)
Working with Reptiles (Course Learner Group)
1 - Lab Animal Research (Stage)

Under requirements set by:

Missouri State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w88a1530a-d920-419b-8c57-7580d1af75da-26831054

Appendix B. Total number of each species captured on the Caney River in Oklahoma—between Hulah Lake and the Kansas border—that were also captured in Kansas in 2017–2019. These data were used to calculate catch per unit effort, Shannon diversity index and species evenness, and Bray-Curtis similarity indices to compare a reintroduced population with the sites sampled in Kansas. Alligator Snapping Turtles (*Macrochelys temminckii*) were also captured at the Caney River each year but were not captured in Kansas and were omitted.

Species	2017	2018	2019	Total
Apalone spinifera	236	178	26	440
Graptemys ouachitensis	17	92	60	169
Sternotherus odoratus	11	15	0	26
Trachemys scripta	628	592	93	1313
Chelydra serpentina	3	20	2	25
Chrysemys picta	0	0	0	0
Pseudemys concinna	11	0	4	15

Appendix C. C-1. An Alligator Snapping Turtle from the wild population included in the health assessment with a healed tail amputation, likely from a previous boat propeller injury. C-2. A large hypomelanistic Alligator Snapping Turtle in a tank with individuals from the same year class. C-3. Visible skin shedding on an Alligator Snapping Turtle from the outdoor captive group. C-4. An individual from the indoor captive group exhibiting spinal and shell syphosis. C-5. Scarring on the tail of an Alligator Snapping Turtle from the wild population. C-6. An abscess on a hind foot of an indoor captive individual.