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Variation in Parasite Prevalence of Kinosternid Turtles across Multiple Hydration Regimes

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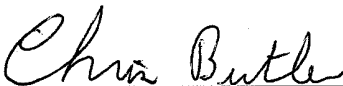Kristen Elizabeth Bliss

Oklahoma City, OK

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ABSTRACT

Complex ecological interactions or physiological adaptations increasing immunity may limit parasite establishment in some host populations. The Madrean Sky Islands are regarded as a series of complex ecological systems rife with speciation and biodiversity. These desert ecosystems are subject to extreme abiotic fluctuation. The Sonoran mud turtle (*Kinosternon sonoriense*) is one of the only semi-aquatic turtle species capable of surviving in these habitats. Fecal analysis (n=217), blood smears (n=18), and dissection (n=4) were utilized to characterize the interpopulational variation in parasite prevalence among three closely-related Kinosternid species. These species occupy environments along a gradient with varying degrees of hydration and aquatic permanence. *Kinosternon sonoriense* populations occupying extreme habitats within the Madrean Sky Islands had a significantly lower parasite prevalence than yellow mud turtle (*Kinosternon flavescens*) populations (p = < 0.001, 95% CI [14.688, 153.306]) and eastern mud turtle (*Kinosternon subrubrum*) populations (p = 0.04762, 95% CI [0.814, 15.598]). White blood cell estimates among species were also significantly different (df= 2, p = < 0.05) providing further evidence that *Kinosternon flavescens* and *Kinosternon subrubrum* harbor larger parasite communities than *Kinosternon sonoriense* populations. Necropsy of *Kinosternon sonoriense* yielded no evidence of parasitism or pathology. Differential habitat use as well as the extreme environment itself likely contributes to these differences. However, further study should be conducted on parasite-host interactions in extreme environments to better understand the ecological mechanisms preventing parasite establishment in the Madrean Sky Islands. Understanding the ecology of *Kinosternon sonoriense* and the utilization of ephemeral aquatic habitats will aid in future conservation efforts in the Southwestern United States.

I.   Introduction

Parasitism is one of the most common lifestyles in the animal kingdom and it is present, at some level, in nearly every ecosystem (Price et al. 1980; De Meeus and Renaud, 2002; Lafferty et al. 2006; Lafferty, 2010). However, within many ecosystems parasites are distributed unevenly and in depauperate patterns not fully understood (Poulin and Guegan, 2000). In free-living species, a latitudinal diversity gradient exists where community biodiversity decreases when moving towards the poles (Pianka, 1966). Varying degrees of predation, competition, temperature, and environmental stability have been used to explain species distributions, but there has yet to be any consensus among these hypotheses (Rohde, 1992). Factors such as energy availability or ecological time may better explain latitudinal diversity gradients among free-living species, however, large-scale models of parasite distributions have been largely ignored (Pianka, 1966; Rohde, 1992; Guernier et al., 2014). Until recently, uneven parasite distributions were regarded as random (Rohde, 1992). However, the organization of parasite community structure and distribution are now thought to be influenced by colonization-extinction processes (Poulin and Guegan, 2000). Colonization and extinction processes are affected by ecological interactions related to isolation, area, and habitat type (Wright et al., 1997; Poulin and Guegan, 2000; Hillebrand et al., 2001). Parasites may drop out of host populations due to complex ecological interactions or through physiologic adaptations reducing parasite success (Aho, 1995; Tobler et al., 2007; Eberhardt et al., 2013; Moeller et al., 2013). For example, differential habitat use among hosts may act as an effective parasite avoidance strategy (Hausfater and Meade, 1982; Thomas and Jones, 1993).

Adaptations dictating species survival in extreme or edge-of-range habitats are often physiologically costly (Sexton et al., 2009). Coping with constant environmental stress must come with ecological advantages allowing organisms to devote resources to growth and reproduction (Tobler et al., 2007; Sexton et al., 2009). Data regarding low parasite frequency within extreme environments are lacking (De Buron and Morand, 2004). However, markedly lower rates of parasitic infection have been observed in some fish (Tobler et al., 2007) and bird (Figuerola, 1999; Mendes et al., 2005) species occupying extreme habitats, as well as in some ocean environments (De Buron and Morand, 2004). Parasites may be adversely affected in extreme environments via direct die-off from the environment itself (Tobler et al., 2007) or indirectly if intermediate hosts are adversely affected (Esch et al., 1979; Mendes et al., 2005). Low parasite prevalence has also been demonstrated within species rapidly expanding or on the periphery of their geographic range (Phillips et al., 2010). The mechanism by which this occurs seems to be a combination of founder-effect and prolonged periods of low host densities (Phillips et al., 2010). A small subset of the host species is less likely to contain the entire parasite community of the parental population (Phillips et al., 2010). This is especially true of parasite species that exert a substantial energy cost upon the host (Phillips et al., 2010).

Geographically isolated or fragmented host populations are less frequently parasitized because they are more difficult to colonize (Anderson and May, 1978; May and Anderson, 1978; Arneberg et al., 1998). An uneven or highly fragmented distribution of hosts may lead to small parasite communities that are low in species richness and may never successfully become established in a population (Kuris and Lafferty, 1994; Lafferty et al., 1994; Byers et al., 2008). Host population size has a profound effect on parasite success (Lyles and Dobson, 1993). A threshold number of hosts within a population are required for a parasite species to become

established (Anderson and May, 1978). Once above this threshold, parasite prevalence increases at a rate largely determined by fecundity and transmission efficiency (Anderson and May, 1978; Dobson and Pacala, 1992). In large host populations, density-dependent transmission strategies further increase parasite abundance (Anderson and May, 1978; Dobson and Pacala, 1992). When comparing native versus invasive host species, trematode prevalence was significantly lower among invasive populations (Torchin et al., 2003). Small founding populations and complex parasite lifecycles often cause many parasite species to become lost during habitat colonization (Torchin et al., 2003). Host population densities play a central role in effective parasite transmission, and parasite species richness may also be positively linked with disease transmission rates (Arneberg, 2002). Thus, a substantial ecological cost emerges when increasing host population density.

Physiological host adaptations also inhibit parasite success (Paterson et al., 1998; Eberhardt et al., 2013; Moeller et al., 2013). Parasite avoidance is likely the single strongest selective pressure acting upon complex vertebrate immunity (Apanius et al., 1997; Sommer, 2005; Zimmerman et al., 2010; Eizaguirre et al., 2012; Stiebens et al., 2013). Heritable parasite resistance is thought to have strong genetic and environmental components among both wild and domestic populations (Uller et al., 2003; Ditchkoff et al., 2005; Raberg et al., 2009; Stiebens et al., 2013). Given that a heavy parasite burden generates stress upon individuals, it would be advantageous for rapid and effective immune response to alleviate this stress and allow individuals to devote greater resources for growth and reproduction (Ditchkoff et al., 2005).

Immune function is greatly influenced by resource availability and individual fitness (Stiebens et al., 2013). Decreased individual fitness leads to cyclical pathogenesis in some species (Eberhardt et al., 2013). Individuals with lowered immunity become more susceptible to

acute infection, and acute infection further weakens the individual's immune system, allowing for greater pathogenesis (Eberhardt et al., 2013). In the capybara (*Hydrochoerys hydrochaeris*), studies have linked lowered resource availability and increased stress to an increase in innate immune response and subsequent decrease in overall parasite prevalence (Eberhardt et al., 2013). Similarly, in Gila monsters (*Heloderma suspectum*) of the Sonoran desert, bouts of dehydration trigger a more robust and rapid innate immune response (Moeller et al., 2013). In both cases, for animals to survive periods of drought or starvation it is evolutionarily advantageous to divert limited resources to innate immunity.

All animals show some capacity for innate immunity and in higher vertebrates adaptive immunity is also present (Zimmerman et al., 2010). Innate immunity does not depend on prior exposure. It plays a key role in an organism's initial defense against parasitism via distinct plasma proteins and cell signaling pathways (Janeway et al., 2001; Roberts and Janovy, 2008; Zimmerman et al., 2010). Immunity has a significant energy cost and in many animals chronic stress characterized by sustained periods of increased hormone production ultimately leads to marked immunosuppression (Dhabhar and Mcewen, 1997; El-Lethey et al., 2003). However, acute physiologic stress characterized by short periods of hormone exposure may function to increase immune activity by increasing leukocyte concentration and antibody sensitivity (Dhabhar and Mcewen, 1997; Eberhardt et al., 2013; Moeller et al., 2013). This response can be measured by monitoring the corresponding fluctuations in white blood cell concentration, specifically, circulating eosinophils (Eberhardt et al., 2013). Increased eosinophil concentration is particularly effective against parasites, often leading to a marked decrease in parasite loads within animals subjected to acute physical stressors such as starvation (Eberhardt et al., 2013).

Mean intensities and prevalence of multiple parasite species drop during periods of drought, as some parasite species are closely linked with water permanence (Singleton, 1985). This association may be due to a combination of factors, including definitive host utilization of the permanent water within the habitat, the semiarid environment itself, and the direct versus indirect lifecycles of the parasites (Singleton, 1985). When indirect parasite life cycles are interrupted the ability to effectively reproduce and infect new hosts by means of horizontal transmission is largely diminished (Lafferty, 1997; Lafferty and Kuris, 1999). This decreases opportunities for new individuals to become infected or for infection within the host to remain stable (Singleton, 1985; Lafferty, 1997; Lafferty and Kuris, 1999). Seasonal parasite fluctuations may be partly tied to internal mechanisms during host dormancy periods, however, most of the evidence for temporal parasite fluctuation attributes these cycles to abiotic factors such as temperature, rainfall, and decreased host densities (Lass and Ebert, 2006). Host dormancy periods reduce contact among populations and thus reduce the ability for parasite populations to recruit and infect new hosts (Lass and Ebert, 2006).

Turtles characteristically possess a rich helminth community within both captive and wild populations (Aho, 1995). Likewise, they present excellent opportunities to study parasite-host interactions within the animal kingdom given the diversity in life history characteristics, habitat utilization, foraging behavior, and reproductive modes (Aho, 1995). Similar to mammalian models, there are marked differences in temporal parasite prevalence within many reptile systems (Hilsinger et al., 2011; Martinez-Bakker and Helm, 2015). Family Kinosternidae occupies a number of xeric environments throughout the Southwestern United States (Seidel et al., 1986). In broad studies, Kinosternidae typically has a less complex helminth community

structure than other semi-aquatic turtles (Aho, 1995). However, causation for this finding has yet to be explored (Aho, 1995).

Pilot Study

The original purpose of my study was to address whether parasitism was correlated with interpopulational variation among Sonoran mud turtle (*Kinosternon sonoriense*) populations in the Peloncillo Mountains, New Mexico. Investigating the interplay between abiotic factors and host biology would yield important life history information about *Kinosternon sonoriense*, and give a preliminary picture of the helminth fauna in this extreme habitat.

Fecal samples were opportunistically collected between May 2012 and August 2012 (n=34) and processed using formalin-ethyl-acetate sedimentation and zinc-sulfate flotation (Foreyt, 2001). All slides were analyzed using light microscopy, however, no evidence of gastrointestinal helminths was found. Samples were again collected May 2013 through August 2013 (n=92) throughout the Peloncillo range, as well as the Pajarito, Santa Catalina, Chiricahua, Galiuro, and Huachuca Mountains. Newly collected fecal specimens were processed using Parasep fecal concentrators, a closed system specifically designed to detect low levels of parasitic infection (Zeeshan et al., 2011). As a control, fecal samples from a Loggerhead sea turtle (*Caretta caretta*) were obtained from the Shoals Marine Lab and analyzed using both ethyl-acetate sedimentation and the Parasep system. Low levels of *Cymatocarpus solearis* eggs (Class: Trematoda) were found in samples concentrated using the Parasep system. Given the increased power of detection using the Parasep system, all further fecal samples were exclusively analyzed using this method.

Despite a robust sample set by 2013, parasite prevalence for *Kinosternon sonoriense* populations from multiple areas within New Mexico and Arizona was collectively less than 5%

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which is well below published findings for similar semi-aquatic turtle species (Thatcher, 1963; Herban and Yaeger, 1969; McAllister et al., 2008; Bursey and Brooks, 2011). In order to test the methodology used within the study and to determine whether this was a genus-wide finding, two other species of *Kinosternon* were sampled in 2014.

Based on the results from the pilot study, I modified my original thesis proposal to reflect my new objectives. The objectives of this study within the final sampling year were: 1) to characterize what physiological or environmental factors may limit helminths from utilizing the Sonoran mud turtle (*Kinosternon sonoriense*) as a host, and 2) examine whether these limitations were habitat or species-specific by collecting data from two additional mud turtle species, the eastern mud turtle (*Kinosternon subrubrum*) and the yellow mud turtle (*Kinosternon flavescens*). In order to address these questions, I collected and analyzed fecal material and blood samples to characterize the parasite community from wild populations of all three species in over twenty localities during my final sampling year. Blood samples from all three mud turtle species were also used to characterize innate immune response and investigate whether immunity differed among species.

II.   Materials and Methods

Study Organisms

The Sonoran mud turtle (*Kinosternon sonoriense*) is a near-threatened aquatic turtle inhabiting southwestern New Mexico, southern Arizona, northern Sonora, and western Chihuahua, Mexico (Iverson, 1991a; van Dijk, 2011). *Kinosternon sonoriense* persists in a stochastic and arid environment along an aquatic habitat permanence gradient (van Loben Sels et al., 1997; Stone, 2001; Ligon and Stone, 2003; Hall and Steidl, 2007; Hensley et al., 2010; Stone et al., 2015). Adult females reach 111 mm in midline carapace length (MCL) on average while

males reach 103 mm (Stone et al. 2001). Hatching occurs from July through September, and clutch size is three to eight for most populations within the United States (Degenhardt et. al. 2005). As opportunistic carnivores, *Kinosternon sonoriense* feeds on aquatic insects, copepods, small crustaceans, worms, fish, and amphibians (Ernst and Barbour, 1989). When these resources are scarce there is a shift towards more herbivorous feeding habits using riparian vegetation as a primary food source (Hulse, 1974). Individuals inhabiting ephemeral or intermittent aquatic habitats exhibit high levels of terrestrial activity, including seasonal migration, seasonal estivation, and terrestrial movements within and among drainages (Stone, 2001; Hall and Steidl, 2007; Stone et al., 2015). Populations within the Peloncillo Mountains demonstrate asynchronous aquatic activity, so that only a fraction of the population is active at any given time regardless of water levels (Stone et al., 2001; Stone et al. 2015).

The yellow mud turtle (*Kinosternon flavescens*) occurs throughout the Central and Southern United States; as far north as Iowa and Nebraska and south through Oklahoma, Texas, and Northern Mexico (Iverson, 1991b; Ernst and Lovich, 2009). *Kinosternon flavescens* prefers intermittent or ephemeral bodies of water within desert or prairie habitats (Christiansen et al. 1985; Ernst and Lovich, 2009; van Dijk, 2011). Males reach 115 mm in MCL on average and females reach 105 mm (Iverson, 1989). Individuals are largely terrestrial, estivating for long periods underground and subsisting on lipid reserves during the winter months sometimes up to 450 m away from bodies of water (Iverson, 1991b; Ernst and Lovich, 2009). In contrast to *Kinosternon sonoriense*, *Kinosternon flavescens* spends a larger portion of each year estivating underground (Ernst and Lovich, 2009). Individuals have been observed estivating continually for up to two years (Rose, 1980). However, like many Kinosternids, *Kinosternon flavescens* feeds primarily underwater (Ernst and Lovich, 2009). Individuals are largely opportunistic carnivores

with feeding habits similar to those *Kinosternon sonoriense* (Ernst and Barbour, 1989; Ernst and Lovich, 2009). In Nebraska, mating occurs in the spring and ends in mid-May (Iverson, 1991b). Females begin nesting at the end of May, and nesting peaks in the beginning of June (Iverson, 1991b; Ernst and Lovich, 2009). Females produce one clutch per year of up to six eggs on average, while some females forego reproduction seasonally depending on resources (Iverson, 1991b).

The eastern mud turtle (*Kinosternon subrubrum*) is distributed throughout the Eastern and Central United States, occurring as far north as Pennsylvania and New York, south into Florida, and as far west as Oklahoma, Texas, and Illinois (Iverson, 1991a; Ernst and Lovich, 2009). Individuals prefer slow-moving streams, shallow canals, ditches, sloughs, marshes, farm ponds, and lakes with soft to muddy bottoms and abundant aquatic vegetation (Ernst and Lovich, 2009; van Dijk, 2011). Midline carapace length of adult females is 88 mm on average while adult males reach 86 mm (Gibbons, 1983). *Kinosternon subrubrum* is the least terrestrial of my three study species (Mahmoud, 1969). However, *Kinosternon subrubrum* still exhibits terrestrial behavior, hibernating on land during the winter months and estivating in the summer during periods of drought (Gibbons, 1983). Individuals become largely inactive beginning in late October and begin to emerge in early to mid-April (Gibbons, 1983). *Kinosternon subrubrum* is omnivorous, feeding on colonial ciliates, planaria, earthworms, snails, small fish, tadpoles, carrion, green algae, and aquatic vegetation (Mahmoud, 1968). Mating occurs from mid-March until May, and nesting females are capable of laying multiple clutches per year, typically May through June or later (Gibbons, 1983; Gibbons et al., 1983).

Study Area

The Madrean Sky Islands are a series of 40 isolated mountain ranges located between the Mogollon Rim, transecting the state of Arizona, and the northern edge of the Sierra Madre Occidental, Figure 1 (Warshall, 1995). The Madrean Sky Islands are characterized by steep ecological gradation with greater species richness, more niche specialists, and more clinal variation than many other New World environments (Warshall, 1995). Elevation fluctuates from 600 to 3,000 m with scrub desert in the lowlands and coniferous forest at high elevations (Warshall, 1995; Skroch, 2008). A 100-125 mm precipitation increase occurs in this region for every 305 m in upward elevation (Lowe, 1964). Lowland urban areas across the region have an average precipitation of 28.45 cm (Western Regional Climate Center, 2016). Wet and dry seasons are distinct and characteristic for the semi-arid climate. The monsoon season from July through August typically follows periods of often severe annual drought between May and June (Western Regional Climate Center, 2016). Abiotic factors such as temperature and precipitation may greatly fluctuate across even small areas within short time periods (Coe et al., 2012). The majority of *Kinosternon sonoriense* individuals within my study were sampled from one of three drainages within the Peloncillo Mountains: the Clanton Draw, Cloverdale Creek, and Foster Draw, Figure 2 (Stone et al., 2011).
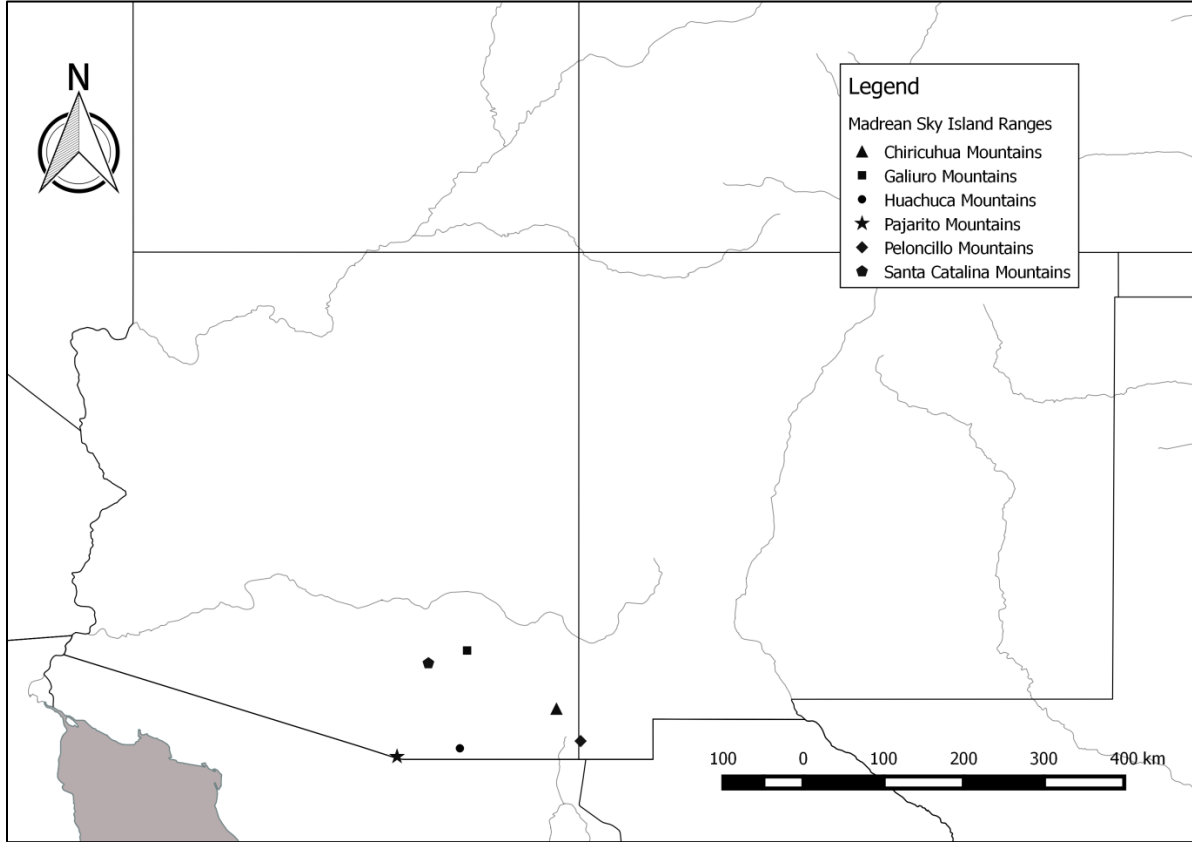
Figure 1: Mountain Ranges Sampled for *Kinosternon sonoriense*: this map depicts all the mountain ranges within the Madrean Sky Island complex sampled for *Kinosternon sonoriense* (n=142) in southern New Mexico and Arizona.

THE PELONCILLO MOUNTAINS
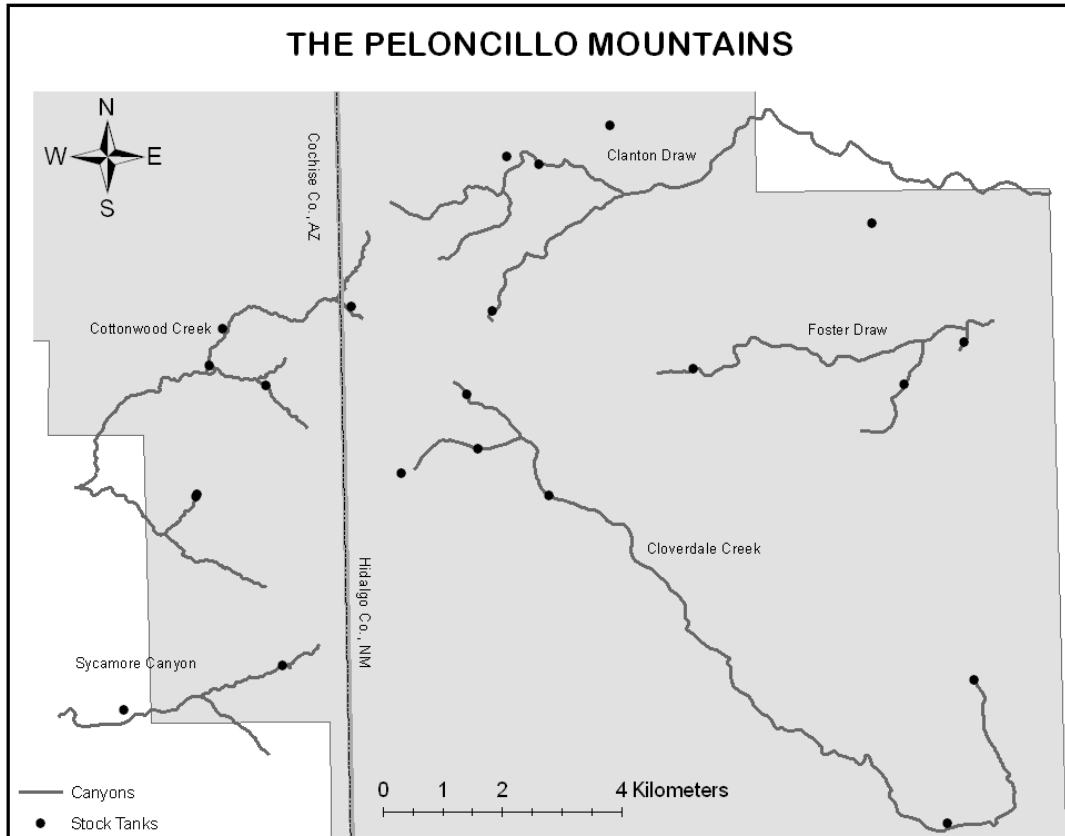
Figure 2: The Main Drainages of the Peloncillo Mountains Study Area: this map depicts the three main drainages utilized within the Peloncillo Mountains to sample *Kinosternon sonoriense* (n=103). (From the top) Clanton Draw, Foster Draw, and Cloverdale Creek are represented along with the stock tanks most often sampled. This map was originally created for use in Stanilla (2009).

Gimlet Lake is located within the Crescent Lake National Wildlife Refuge in the Nebraska sand hills (Figure 3). Gimlet Lake is a shallow, 330,000 m^2 natural lake that is surrounded annually by a 900 m drift fence (Iverson, 1991b), Figure 4. The fence intercepts the migration of *Kinosternon flavescens* inhabiting the permanent lake during the late spring and early summer. During the late summer to early fall, individuals migrate back to the surrounding sand dunes located 30 m from Gimlet Lake (Iverson, 1991b). The refuge area surrounding Gimlet Lake is at roughly 1,000 m elevation and largely made up of grassland with rolling sand dunes and

intermittent to permanent wetlands. Annual precipitation for this area typically reaches 45 cm with the majority of rainfall occurring May through August (Western Regional Climate Center, 2016).
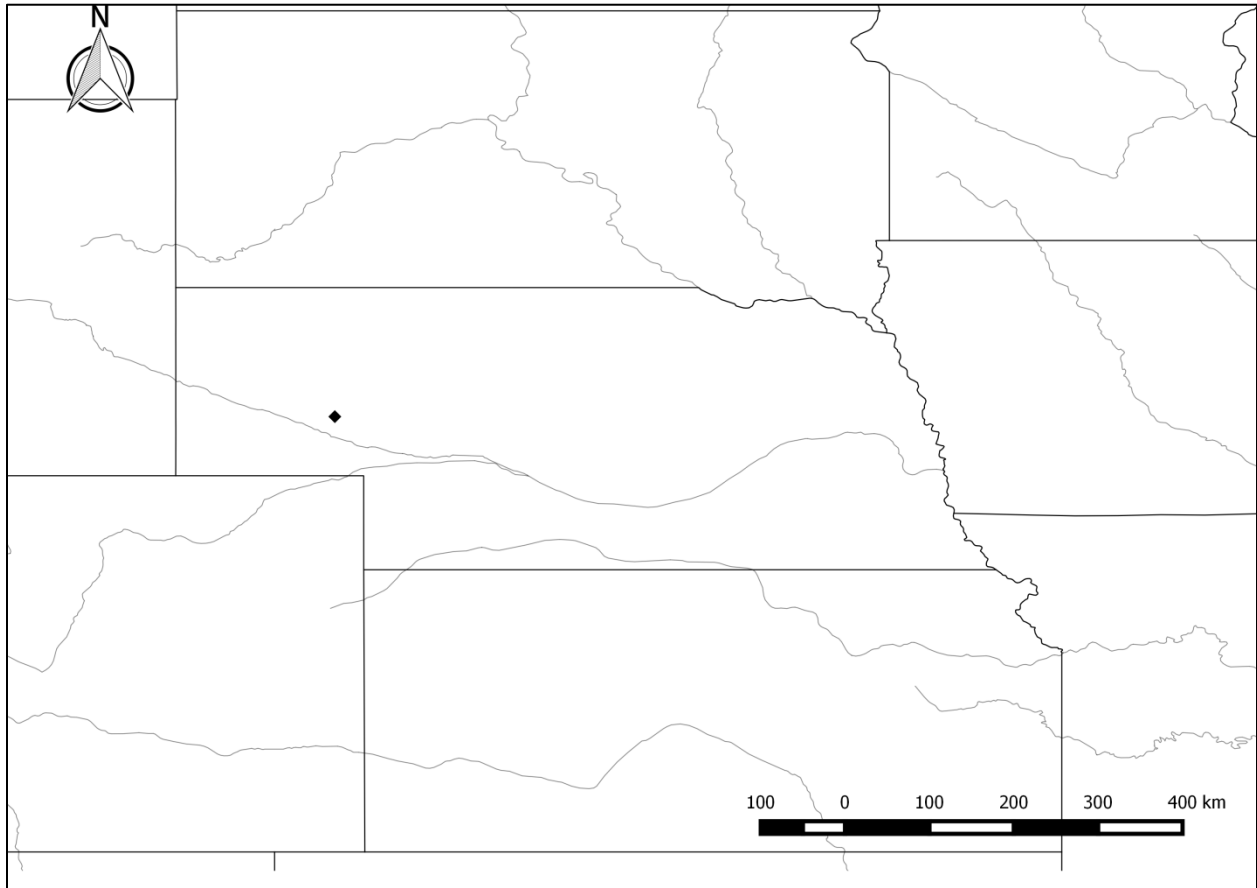
Figure 3: *Kinosternon flavescens* **Study Site:** this map depicts the location of Gimlet Lake, located in Garden County, Nebraska. Gimlet Lake was the location used to sample *Kinosternon flavescens* individuals (n=44).

Figure 4: Satellite Photo of Gimlet Lake: this aerial satellite photo depicts the Gimlet Lake area sampled for *Kinosternon flavescens* (Google Earth, 2016a).

The Muddy Boggy River is a 175 km permanent tributary of the Red River, crossing through

five counties within southeastern Oklahoma's cross-timbers region (Figure 5). The surrounding

area also contains part of the Ouachita Mountains to the north and cypress swamps to the

southeast (Oklahoma Climatological Survey, 2016). Muddy Boggy River is relatively clear and

slow-moving (USGS, 2016). Much of the area surrounding the Muddy Boggy has been either

converted for agricultural use or permanently flooded and maintained as reservoirs (Pigg, 1977).

Oak and hickory bottomland hardwood forest dominate the lowland floodplain area along the

river in Atoka County where my sampling was focused (USGS, 2016). Elevation for the area is

on average 60 m (USGS, 2016). Atoka County receives, on average, 119 cm of precipitation

annually, the majority of which occurs May through July (Oklahoma Climatological Survey,

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2016). Approximately 100 km from the river's head, a relatively shallow, slow-moving stream

termed "Boggy Cutoff" extends three kilometers to the southeast (USGS, 2016), Figure 6.

Several localities along Boggy Cutoff were sampled for *Kinosternon subrubrum* individuals. A

naturally-occurring oxbow and permanent farm pond both roughly 50 m from Boggy Cutoff

were also sampled for *Kinosternon subrubrum* individuals (Figure 6).
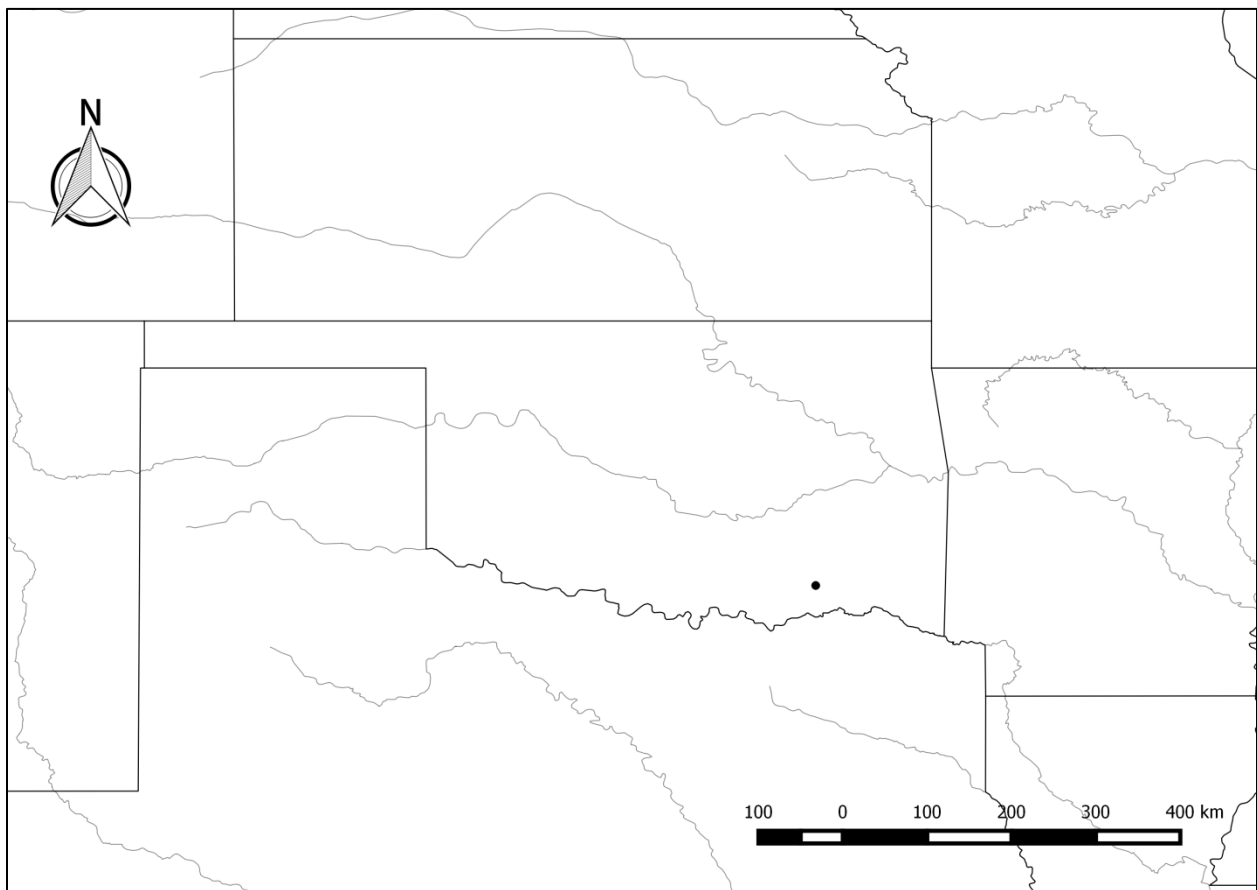
Figure 5: *Kinosternon subrubrum* Study Area: this map depicts the location of Muddy Boggy
Cutoff used to sample *Kinosternon subrubrum* individuals (n=31).

Figure 6: Satellite Photo of Boggy Cutoff: this aerial satellite photo depicts the Boggy Cutoff area. The beginning point of the stream is marked with a pin as 'Boggy Cutoff'. Two other points were marked delineating the localities most heavily sampled for *Kinosternon subrubrum* (Google Earth, 2016b).

The sampling localities chosen for this study vary along a hydration gradient with respect to rainfall amounts and aquatic habitat permanence (Table 1). *Kinosternon sonoriense* populations and sub-populations were sampled within both permanent (n= 15) and intermittent or ephemeral (n=127) bodies of water. *Kinosternon sonoriense* individuals sampled within the Madrean Sky Islands were subject to extreme temperature and rainfall fluctuations (Stone et al. 2001; Hensley et al. 2010; Stone et al. 2015). Terrestrial movements were common but unpredictable given the variable abiotic fluctuation within the environment and asynchronous activity of individuals (Stone et al., 2015). *Kinosternon flavescens* and *Kinosternon subrubrum* populations in my

study, occur in large permanent bodies of water, Table 1 (Iverson, 1991b; Day Ligon, personal communication, 2014). Movements from estivation sites into the water are largely predictable given the comparatively little annual variation in temperature and rainfall amounts (Western Regional Climate Center, 2016). Individuals exhibit synchronous aquatic habitat use as sub-populations migrate and utilize both Gimlet Lake and Boggy Cutoff within the same activity period each year (Iverson, 1991b; personal observation, 2014). *Kinosternon flavescens* also exhibits a higher degree of terrestrial activity than *Kinosternon subrubrum* (Mahmoud, 1969; Iverson, 1991b), Table 1.

Based upon ecological differences in the habitat and habitat use among the three mud turtle species in my study, predictions can be made with respect to general trends in parasite prevalence among species. If estivation duration or degree of terrestrial activity negatively impacts parasite colonization then parasite prevalence will be low in *Kinosternon sonoriense* and *Kinosternon flavescens* and high in *Kinosternon subrubrum*, Table 2 (Mahmoud, 1969). If asynchronous aquatic habitat use lowers encounter probability and transmission rates then *Kinosternon sonoriense* will have the lowest parasite prevalence among the three mud turtle species (Table 2). Similarly, if unpredictable aquatic habitat availability negatively impacts parasite survival then *Kinosternon sonoriense* will have the lowest parasite prevalence among the three mud turtle species in this study (Table 2).

Table 1: Habitat and Species Characteristics of Three Mud Turtle Populations: this table summarizes the differences among sampling localities and species within this study. The annual variation in terrestrial movements for each population, the degree of terrestrial activity exhibited, propensity for aggregation or asynchronous vs synchronous aquatic habitat use, the overall permanence of the aquatic habitats utilized by each population, if habitats are fragmented, maximum usable aquatic habitat for each population expressed in surface area (m^2), and average yearly rainfall (cm).

Species	Movements	Terrestrial Activity	Aggregation	Permanence	Is Habitat Fragmented?	Maximum Size of Habitat (m^2)	Average Yearly Rainfall (cm)
Kinosternon sonoriense	Unpredictable	High	Low	Ephemeral	Yes	4,000	< 30
Kinosternon flavescens	Predictable	High	High	Permanent	No	220,000	45
Kinosternon subrubrum	Predictable	Low	High	Permanent	No	Continuous	119

Table 2: Ecological Predictions for Patterns of Parasite Prevalence of Three Mud Turtle Populations: this table outlines the general trends expected in parasite prevalence among mud turtle populations within this study. The three major ecological interactions among populations correspond with predicted helminth prevalence for each population.

Species	If Estivation Duration or Terrestrial Activity Prevents Parasites	If Asynchronous Aquatic Habitat Use Prevents Parasites	If Unpredictable Aquatic Habitat Availability Prevents Parasites
Kinosternon sonoriense	Low Prevalence	Low Prevalence	Low Prevalence
Kinosternon flavescens	Low Prevalence	High Prevalence	High Prevalence
Kinosternon subrubrum	High Prevalence	High Prevalence	High Prevalence

All three mud turtle species within this study are closely related (Iverson, 1991a). Physiological differences were assumed to be negligible except in terms of immunity. Little is known about the immunity of Kinosternids. However, general predictions based upon turtle (Aguirre et al., 1995; Casal et al., 2009), reptile (Davis et al., 2008; Moeller et al., 2013), and mammalian models (Eberhardt et al., 2013) may be made. If *Kinosternon sonoriense* is more parasite resistant due to increased innate immunity then WBC counts will be higher in individuals without a detectable parasite load. On the other hand, if *Kinosternon sonoriense* lacks heightened immunity WBC counts will be normal or lower in individuals without a detectable parasite load.

Sample Collection

Kinosternon sonoriense and *Kinosternon flavescens* fecal samples were collected opportunistically as part of two long-term mark-recapture studies (Iverson, 1991; Stone 2001; Ligon and Stone 2003; Iverson et al., 2009; Stone et. al. 2011). *Kinosternon subrubrum* fecal samples were collected opportunistically as part of multiple graduate theses (McKnight et al., 2015). Individuals were captured via one of three methods: hoop nets baited with sardines, funnel traps, or by hand (Stanila, 2009; Stone et. al., 2011; McKnight et al., 2015). All captures were given a unique set of marks filed into the marginal scutes of the carapace and shell dimensions were recorded to the nearest 0.1 mm using dial calipers (Stanila, 2009; Stone et. al. 2011). Fecal material was collected on-site using standardized Thermo Scientific ® 15 mL collection vials containing either Zinc-PVA or 10% buffered Formalin solution.

Samples collected in Zinc-PVA or 10% buffered Formalin were concentrated per manufacturer's instructions using the Mini Parasep ® SF system. Mini Parasep SF is a closed,

disposable concentration system used for concentrating helminth and protozoan cysts. Using the Parasep system, helminth eggs were detectable at lower levels than traditional sedimentation techniques (Zeeshan et al., 2011). Once samples were concentrated, supernatant was placed onto a clean glass slide containing iodine and examined under light microscopy for helminth eggs (Foreyt 2001). Eggs were counted and subsequently measured to the nearest 0.1 μm for comparison with published morphology and size data for identification (Foreyt, 2001; Rasband 2012). Independent confirmation for each helminth class was employed as often as possible.

Blood samples were collected at the time of capture from the sub-carapacial sinus located between the nuchal of the carapace and the neck from *Kinosternon sonoriense* (n=10), *Kinosternon flavescens* (n=10), and *Kinosternon subrubrum* (n=10) (Avery and Vitt 1984; Bennett et al., 1986; Gabriel and Gregory 2006). A 0.22 gauge needle was used to obtain 0.05-0.1 ml of blood and a thin smear was made on site according to the techniques outlined by Center for Disease Control protocols (CDC, 2015). Slides were fixed in the field using absolute methanol (Warhurst and Williams, 1996). Giemsa staining was used on all thin smears according to manufacturer's instructions. Slides were examined under light microscopy for the presence of haemoparasites. White blood cell (WBC) counts were obtained using the estimation techniques outlined in Jacobson (2007).

Opportunistic dissection was employed when two *Kinosternon sonoriense* individuals were found freshly dead within the study area. In addition, two *Kinosternon sonoriense* museum specimens were also dissected. At the time of necropsy, all four turtles had been injected with 10% buffered formalin to preserve organs and soft tissues and stored in 70% ethyl alcohol. All necropsies were performed under the direct supervision of a veterinary pathologist experienced in parasitological identification. All diagnostic findings were confirmed with the New Hampshire

State Veterinary Diagnostics Laboratory (NHSVD). Histopathological preparation and subsequent staining was performed on all organs and soft tissues by the NHSVD. Intestinal scrapings, stomach contents, urinary bladder secretions, and cloacal swabs were also examined via direct microscopy.

Data Analyses

The parasitic infection within *Kinosternon sonoriense*, *Kinosternon subrubrum*, and *Kinosternon flavescens* was quantified based on measures of parasite prevalence and intensity. Prevalence is the sum of infected individuals with a particular parasite species divided by the total number of hosts examined (Bush et al., 1997). Intensity was used as a measure of the severity of infection per individual host (Bush et al., 1997). Intensity is the number of individual parasites of a single species per host (Bush et al., 1997). I used the programs R and Quantitative Parasitology 3.0 (QP 3.0) for statistical analyses (Rozsa et al., 2000; R core team 2015).

Prevalence and intensity statistics were calculated using QP 3.0 (Rozsa et al., 2000). Prevalence, intensity, and WBC estimates were tested in R for normality using a Shapiro-Wilk test and none were normally distributed. A Fisher's exact test with Bonferroni correction for multiple tests was used to examine interspecific variation in prevalence using two by two contingency tables in the program R (MacDonald and Gardner, 2000; Rozsa et al., 2000; R Core Team, 2015). Confidence intervals were generated using Wald's adjusted method for sample sizes less than 1000 individuals and based on a skewed binomial distribution using QP 3.0 (Rozsa et al., 2000; Reiczigel, 2003). A Kruskal-Wallis rank sum test was used to test intensity and WBC estimates for interspecific differences among species (R Core Team, 2015).

Results

Fecal Analysis

Overall parasite prevalence was lowest for *Kinosternon sonoriense* at 4.96% (n= 142). Interspecific variation in parasite prevalence was significantly lower than *Kinosternon flavescens* (p = < 0.001, 95% CI [14.688, 153.306]) or *Kinosternon subrubrum* (p = 0.04762, 95% CI [0.814, 15.598]). Six of 142 samples contained at least one helminth egg. Eggs were light brown in color and measured 35-42.5 μm long by 15-22.5 μm wide with a prominent operculum (Figure 7). Five of the positive samples were taken from the Peloncillo Mountains and one positive sample was taken from the Pajaritos (Table 3). One parasite species was recovered and identified to Class (Figure 7). No published parasite species list exists for *Kinosternon sonoriense* and no adult parasite specimens were recovered in necropsy or fecal analysis, making identification lower than Class Trematoda unreliable.

Median intensity of infection among *Kinosternon sonoriense* individuals was the lowest of all three species (1.00 (n= 6) 95% CI [1.00 - 6.00]). Median intensity of infection was highest in *Kinosternon flavescens* (5.00 (n=31) 95% CI [5.00-13.00]), Table 3. Interspecific differences were also compared using the intensity of all positive individuals. Intensity of infection was significantly different among species (χ^2 =10.098, df = 2, p < 0.05), Table 3.

Figure 7: Trematode Egg from *Kinosternon sonoriense*: this egg measures 35 x 17.5 µm. Magnification was 40X and the operculum is indicated by the arrow.

Table 3: Descriptive Statistics for Trematode Prevalence and Intensity in Three Species of Mud Turtle: this table depicts the descriptive statistics calculated for parasite prevalence and intensity with corresponding confidence intervals. 'NA' indicated the number of infected individuals was too small to generate a confidence interval. Populations sampled are listed by locality and species with sample sizes.

Species	Locality	n	Infected	Prevalence (%)	95% CI (%)	Intensity Min-Max	Median Intensity	95% CI
K. sonoriense	Peloncillo Mountains, NM	103	5	4.85	1.94 - 11.01	1.0 - 6.0	1.00	1.00 - 6.00
	Pajarito Mountains, AZ	28	1	3.57	0.19 - 17.48	1.0	1.00	NA
	Galiuro Mountains, AZ	5	0	0.00	-	-	-	-
	Santa Catalina Mountains, AZ	4	0	0.00	-	-	-	-
	Chiricuhua Mountains, AZ	1	0	0.00	-	-	-	-
	Huachuca Mountains, AZ	1	0	0.00	-	-	-	-
K. flavescens	Garden County, NE	44	31	70.46	55.72 - 82.09	1.0 - 305.0	5.00	5.00 - 13.00
K. subrubrum	Atoka County, OK	31	5	16.13	6.58 - 33.63	1.0 - 15.0	1.00	1.00 - 15.00

Parasite prevalence was highest for *Kinosternon flavescens* (70.46% n=44). One parasite

species was identified to Class (Figure 8). However, no adults were found within fecal samples

so classification higher than Class Trematoda was not attempted. Some species lists do exist for

Kinosternon flavescens, but measurements of eggs alone are not sufficient to make a reliable

identification.

Figure 8: Trematode Egg from *Kinosternon flavescens*: this egg was recovered from individual 1200+309 and measured 40 x 17.5 μm. The operculum is indicated by the arrow.

Parasite prevalence was intermediate in *Kinosternon subrubrum* (16.13% n=31) and

significantly lower than *Kinosternon flavescens* (p = < 0.001, 95% CI [3.513, 48.884]). At least

two parasite species were found and identified to Class (Figures 9 and 10). As with *Kinosternon*

flavescens, parasite species lists do exist for this turtle, however, no adults were recovered from fecal samples. Identification of eggs lower than Class Trematoda was not attempted.

Figure 9: Trematode Egg from *Kinosternon subrubrum*: this egg measured 100 x 65 µm. The arrow indicates the location of the operculum which is barely visible under the debris.
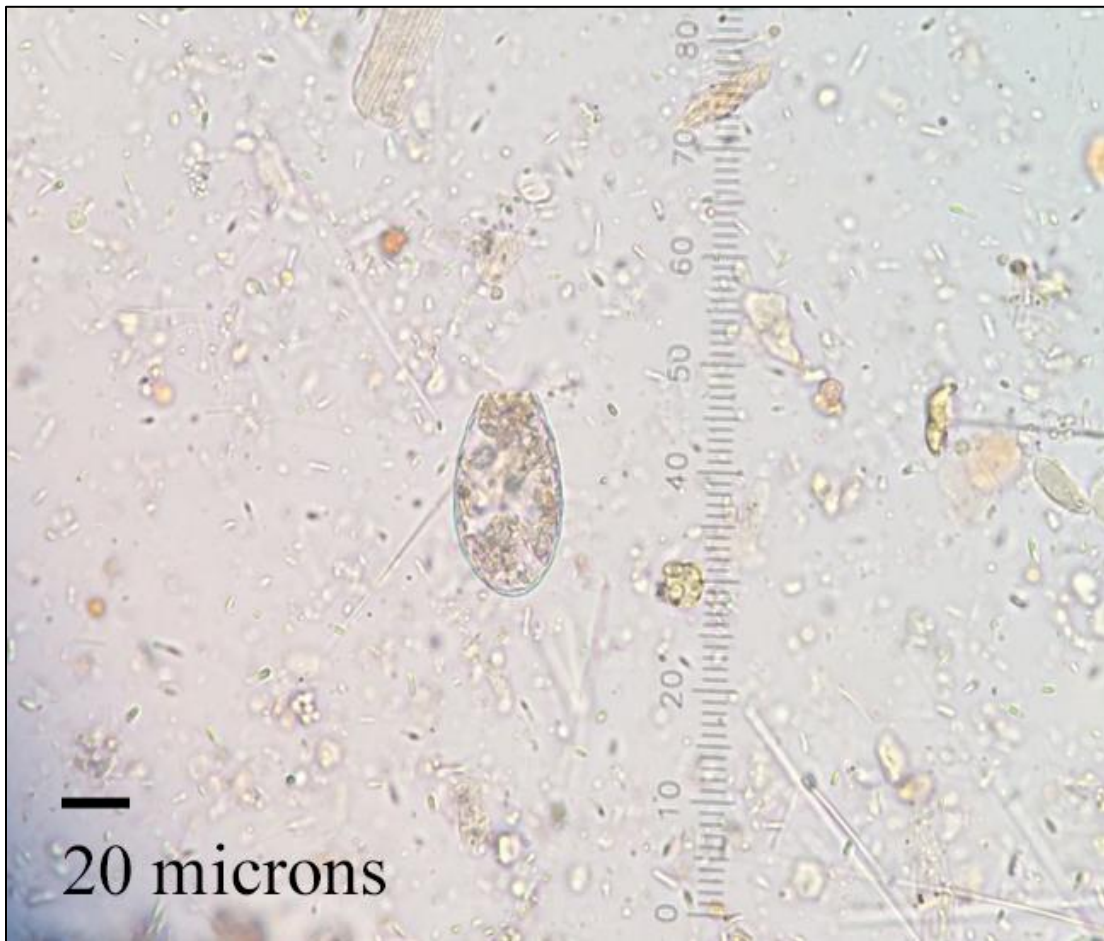
Figure 10: Second Trematode Egg from _Kinosternon subrubrum_: this egg was found in the same individual and measured 47.5 x 25 μm. The operculum seems to be either bent backwards out of view or damaged.

Blood Analysis

   Kinosternon sonoriense had the lowest WBC estimates of all three species with a median cell count of 12, 320 cells/ µl (n=7), Table 4. The interspecific variation among species was significant (χ^2= 8.3179, df= 2, p = < 0.05, Figure 11). *Kinosternon subrubrum* had the highest WBC estimates with a median of 23, 600 cells/ µl. However, small sample sizes could make this a poor estimate (n= 2). Sample size for *Kinosternon subrubrum* was lower than in the other two species given the poor smear quality from field conditions. Ten samples were collected but eight were thrown out of the analysis given the unreliability of the slides. *Kinosternon flavescens* WBC estimates fell between the other two species with a median WBC estimate of 18, 400 cells/ µl (n=9). *Kinosternon flavescens* was the only species positive for haemogregarine parasites (Genus: *Haemogregarina*), Figure 12.

Table 4: White Blood Cell (WBC) Estimates by Species: this table summarizes WBC count estimates by species and gives the range for the estimates. A positive (+) or negative (-) result for haemogregarine parasites is also indicated. *Kinosternon subrubrum* blood smears did not contain erythrocytes that could be effectively scanned for parasites given the lysing of cells and poor quality of the slide, indicated as "NA".

Species	n	Median (cells/ μl)	Min - Max (cells/μl)	Haemogregarine
Kinosternon sonoriense	7	12, 320	8, 000 - 17, 120	-
Kinosternon flavescens	9	18, 400	10, 560 - 38, 720	+
Kinosternon subrubrum	2	23, 600	18, 400 - 24, 960	NA

WBC Estimate by Species
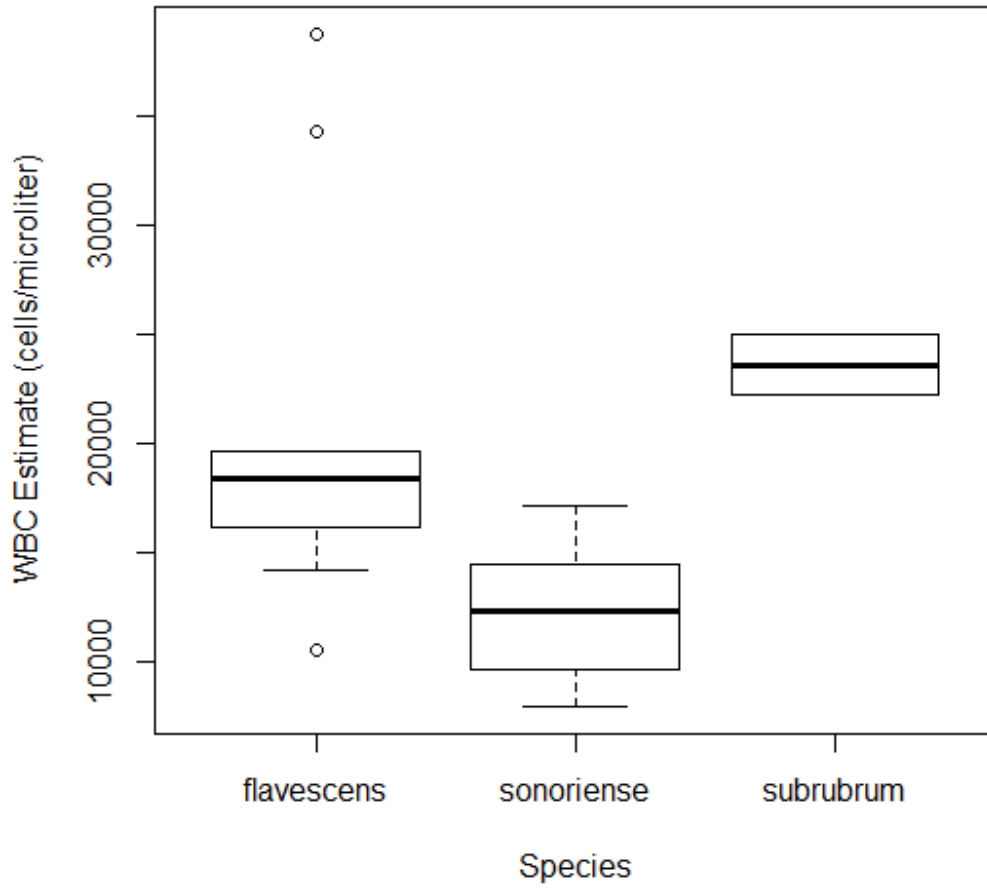
Figure 11: Boxplot of WBC Estimates for Three Species of Mud Turtle: this figure generated in R depicts the differences found in WBC estimates for all three species in the study. The interspecific variation among species was significant (χ^2= 8.3179, df= 2, p = < 0.05).
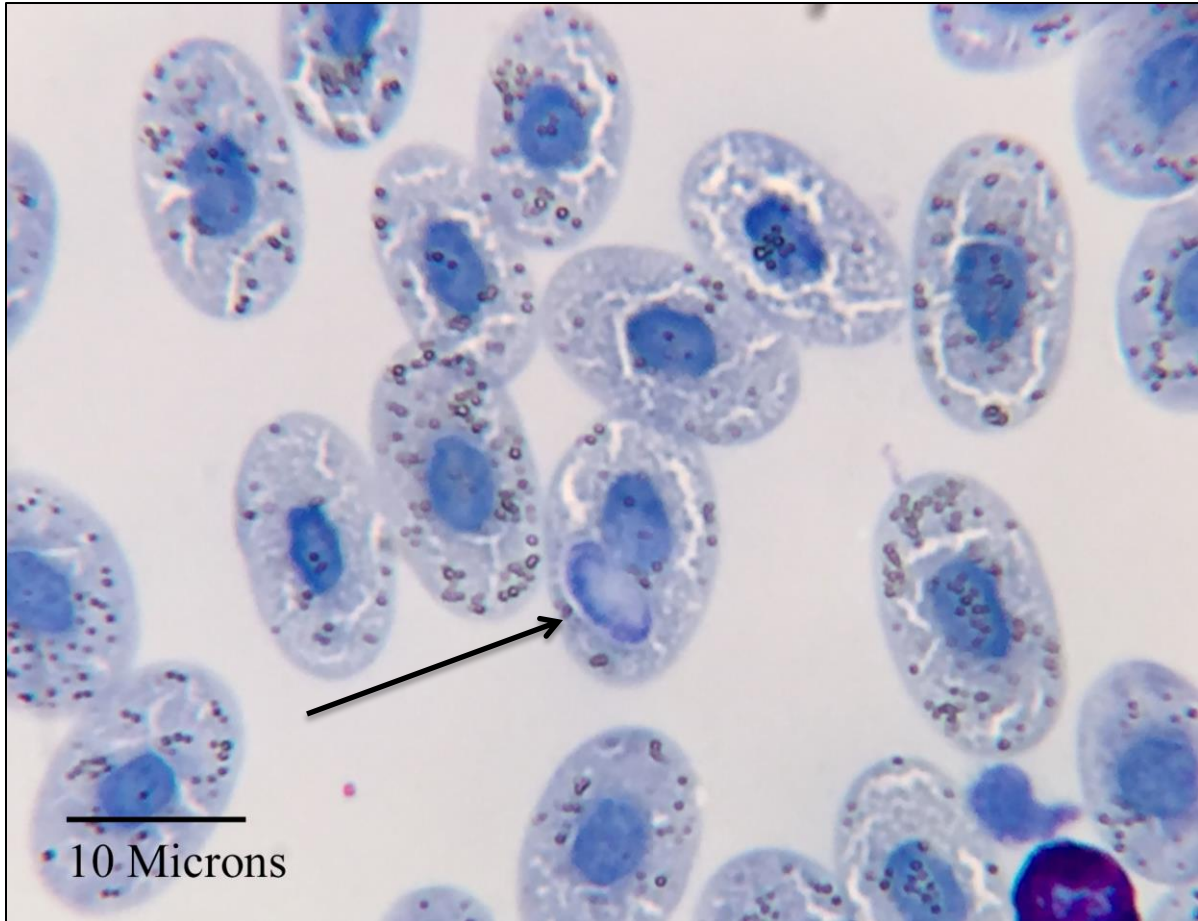
Figure 12: Haemogregarine Parasite of *Kinosternon flavescens*: this photo shows a haemogregarine parasite in a *Kinosternon flavescens* erythrocyte. The arrow indicates the parasite.

Necropsy

Kinosternon sonoriense specimens were necropsied (n=4) on two occasions. However, no evidence of parasitism was found from gross dissection or histopathological examination. All organs and soft tissues were removed from the body and dissected for signs of necrosis, encysted parasites, adult helminth attachment, or pathology. All individuals dissected were in relatively good body condition with no obvious signs of infection or distress noted in my examinations.

The GI tract and lungs were searched extensively as these are common areas of attachment and infection for helminths and pentastomids. Some individuals showed signs of more decomposition than others. Visual inspection was difficult for individual UCO1809 as much of the soft tissue in and around the lungs had rotted (Appendix: Figure A1), but histological examination revealed no signs of pathology or parasitic infection (Appendix: Figure A2). The lungs from the remaining individuals were better preserved and were visually inspected for signs of pathology (Appendix: Figure A3).

The GI tract was also dissected and searched for adult parasites and signs of any necrosis or pathology (Appendix: Figures A4 and A5). Histological examination of the urinary bladder in individual UCO1810 (Appendix: Figure A6) revealed some inflammation caused by cystitis, however, leukocyte aggregation was limited to the submucosal layer and localized. No submucosal inflammation was observed in any other individuals. Three locations within the intestines were sampled as well but revealed no sign of inflammation or pathology (Appendix: Figure A7). Scrapings from the intestines, urinary bladder, and cloaca were also examined under direct light microscopy, but I found no sign of parasitism or pathology.

III.   Discussion

Kinosternid turtles characteristically harbor a number of parasite species (Thatcher, 1963; Herban and Yaeger, 1969; McAllister et al., 2008; Bursey and Brooks, 2011). However, the parasite species richness of Kinosternidae is relatively low when compared to other semi-aquatic turtles (Aho, 1995). If the results are put within this context for both *Kinosternon flavescens* and *Kinosternon subrubrum* they are relatively unremarkable. However, the parasite prevalence of *Kinosternon sonoriense* is surprisingly low (Thatcher, 1963; Herban and Yaeger, 1969; McAllister et al., 2008; Bursey and Brooks, 2011). Necropsy and blood analyses support these

conclusions. Based on the predictions made within this study, either ecological or physiological mechanisms could explain low parasite prevalence in *Kinosternon sonoriense*. Complex ecological interactions within the Madrean Sky Islands or heightened immunity may inhibit parasite colonization and establishment.

White blood cell (WBC) counts may be important indicators for increased immune response in animals (Jacobson, 2007). Elevated levels of circulating white blood cells often indicate pathology in reptiles (Knotkova et al., 2005). Elevated WBC counts are positively correlated with known pathology in turtles (Aguirre et al., 1995; Knotkova et al., 2005; Casal et al., 2009). White blood cell estimation supports the results of fecal analyses given *Kinosternon sonoriense* individuals had the lowest WBC estimates of the three species within this study. Healthy individuals with low parasite burdens should have correspondingly normal WBC estimates. While hematology data for Kinosternid turtles are scarce in the literature, WBC estimates in freshwater turtles are typically between 7,000 and 15,000 cells/ µl (Innis et al., 2007; Jacobson, 2007; Deem et al., 2009). *Kinosternon sonoriense* had estimated WBC counts between 8,000 and 17,120 cells/ µl which were lower than both *Kinosternon flavescens* and *Kinosternon subrubrum* populations. Within this study, higher parasite prevalence was positively correlated with high levels of circulating white blood cells. Therefore, none of the species within this study appear to have an increased innate immune response to helminths resulting in parasite resistance.

Within the Madrean Sky Islands, *Kinosternon sonoriense* populations inhabit geographically isolated mountain ranges that are ecological islands high in biodiversity (Bodner et al., 2003; Hensley et al., 2010; Stone et al., 2015). Glaciation during the Pleistocene caused isolation and subsequent divergence among many species (Hewitt, 2004; Shepard and Burbrink, 2008). Periods of connectedness facilitated by the melting of glaciers caused dispersal and range

expansion (Knowles, 2000; Shepard and Burbrink, 2008). High species diversity and low levels of endemism within these communities illustrate strong selective pressure and resulting rapid differentiation within these populations (McCormack et al., 2009; Perez-Alquicira et al., 2010). Due to historic isolation, rates of extinction among populations inhabiting these ranges must be low to maintain populations given the assumed low rates of immigration (Lomolino and Weiser, 2001). Parasite species becoming locally extinct in host populations would have few opportunities to re-colonize these isolated montane communities (Dobson and Pacala, 1992; Apanius et al., 2000). Extinction and colonization events are important for determining the absence of a parasite species within an island community (Dobson and Pacala, 1992). Unique attributes of individual island habitats, complex ecological interactions, and the spatial or temporal heterogeneity of the habitats themselves may also shape parasite community structure (Apanius et al., 2000).

The habitats within the Madrean Sky Island complex are prone to annual fluctuation in water availability and temperature (Coe et al., 2012). Most annual precipitation occurs during the summer monsoons that follow the dry season. The distribution of rainfall is highly variable both spatially and temporally (Coe et al., 2012). In Blackwater Canyon, the best-studied site in the Peloncillo Mountains, the surface area of aquatic habitat fluctuates from zero to 375 m^2 during the dry season to over 4,000 m^2 during a typical monsoon season (Stone et al., 2001; Stone et al., 2015). Flash-flooding occurs regularly and portions of canyon systems flow for short periods during most years (Stone et al., 2015). In contrast, Gimlet Lake and Boggy Cutoff are not subject to annual drying or substantial fluctuation in precipitation levels. Over a 17-year-period Gimlet Lake maintained an average surface area of 220,000 m^2 with the recorded minimum being 70,000 m^2 (Gosselin et al., 2000). Similarly, gauge height for the Muddy Boggy River never fell

below 2 m from 2007 to 2016 (USGS, 2016). While height of this river annually fluctuates according to season, average depth over a nine year period was 6 m (USGS, 2016).

Variations in temperature and precipitation affect the viability, distribution, and transmission efficiency of many parasite species (Stromberg, 1997; Mas-Coma et al., 2009). Parasites that utilize indirect life cycles and depend upon free-living stages for infection are particularly susceptible to environmental fluctuation (Mendes et al., 2005; Tobler et al., 2007; Mas-Coma et al., 2009; Martinez and Marino, 2011). Parasites may be sensitive to desiccation and temperature fluctuation as water is often necessary for infection and temperature may directly affect parasite emergence and metabolism (Singleton, 1985; Mas-Coma et al., 2009; Macnab and Barber, 2011). Intermediate hosts may also be adversely affected by drought or flooding events (Mostafa, 2009; Hoverman et al., 2011). Parasites may be directly removed from the environment through large-scale die-off of free-living stages (Esch et al., 1979; Tobler et al., 2007) or indirectly removed via the distribution and survival of intermediate hosts (Esch, 1971; Aho, 1995). Specific ecological interactions among parasites, intermediate hosts, and definitive hosts are complex (Martinez and Marino, 2011). Models for disease transmission amidst abiotic fluctuation are largely species and habitat specific (Mas-Coma et al., 2009; Pech et al., 2010; Martinez and Marino, 2011). Further investigation within the Madrean Sky Islands is warranted to characterize whether these interactions may be affecting parasite community structure and transmission.

Kinosternon sonoriense makes routine movements out of intermittent tanks into ephemeral canyon pools during the wet season (Stone et al., 2001; Stone et al., 2015). Data from Blackwater Canyon indicate individuals belong to one of three movement classes (Stone et al., 2001; Stone et al., 2015). Half the individuals in the population utilize an intermittent tank and

ephemeral pools, with a high frequency of movements from the intermittent tank to ephemeral pools during the wet season, and back to the tank during the dry season (Stone et al., 2015). Other turtles appear to either utilize ephemeral pools or the intermittent tank exclusively (Stone et al., 2015). Seasonal movements away from larger intermittent aquatic environments may affect parasite transmission (Stone et al., 2001).

Ephemeral aquatic environments often contain a less diverse parasite community than permanent aquatic environments (Dunsmore and Didzinski, 1968; Edwards and Bush, 1989; Ebert et al., 2001; Altermatt and Ebert, 2010; Readel, 2011). Younger aquatic communities have less exposure to parasites than do older, more permanent communities (Dobson and Pacala, 1992; Ebert et al., 2001). The probability of encountering a large portion of the parasite community during the hydroperiod of an ephemeral pool is low (Ebert et al., 2001). Invertebrate diversity within ephemeral aquatic habitats is also lower when compared with intermittent or permanent bodies of water (Feminella, 1996; Welborn et al., 1996; Williams, 1996). Aquatic habitat characteristics such as hydroperiod dictate parasite community structure because ephemeral pools may lack the intermediate hosts necessary to satisfy the indirect life cycle constraints of many trematodes (Esch, 1971; Marcogliese and Cone, 1991; Aho, 1995).

Many populations of *Kinosternon flavescens* and *Kinosternon subrubrum* make routine movements between terrestrial estivation sites and seasonal aquatic habitats (Gibbons, 1983; Iverson, 1991b; Steen et al., 2007). However, the populations used within this study make movements between permanent aquatic habitats and terrestrial sites used for mid-summer estivation and winter hibernation (Mahmoud, 1969; Iverson, 1991b).Within the Gimlet Lake population, individuals sampled exclusively utilize a permanent aquatic habitat (Iverson, 1991b; John Iverson, personal communication, 2014). No long-term data exists for the Boggy Cutoff

population, but individuals in similar habitats have been observed frequently making overland movements to ephemeral water (Stone et al., 1993; Riedle et al., 2015). Within this study, ephemeral pool utilization across these three species is likely varied with *Kinosternon sonoriense* populations displaying the highest degree of ephemeral habitat use and *Kinosternon flavescens* displaying the lowest degree of ephemeral habitat use. However, the timing of ephemeral aquatic habitat use also varies among these populations (Iverson, 1991b; Stone et al., 2001; Stone et al., 2015).

Kinosternon sonoriense populations in the Peloncillo Mountains exhibit asynchronous aquatic habitat use (Stone et al., 2001; Stone et al., 2015). Data from Blackwater Canyon suggest only a fraction of this sub-population is active at any one time regardless of water levels or season (Stone et al., 2001; Stone et al., 2015). This asynchronous behavior may be present in other populations within the Madrean Sky Islands as data suggest similar patterns of aquatic habitat use (Hensley et al., 2010). Asynchronous activity may prevent a large portion of *Kinosternon sonoriense* populations from being heavily parasitized given individuals are only active in low densities throughout the year (Stone et al., 2001; Stone et al., 2015). Within both ephemeral and intermittent aquatic habitats, individuals appear to be active for short periods before returning to terrestrial estivation (Stone et al., 2015). Short activity periods by individuals at low population densities would make the probability of encountering a parasite low. Density-dependent transmission strategies among parasite species could fail in this environment given only a fraction of the population is active at once (Anderson and May 1978; Dobson and Pacala, 1992). *Kinosternon sonoriense* individuals that are active at a given time are often distributed throughout long stretches of canyon system further limiting both infection rates and horizontal transmission rates (Stone et al., 2015).

Kinosternon flavescens and *Kinosternon subrubrum* populations within this study synchronize aquatic habitat use and aggregate within permanent bodies of water (Iverson, 1991b). Annually, individuals migrate from terrestrial estivation sites to permanent water and populations remain active during the same time periods each year (Mahmoud, 1969; Iverson, 1991b). While little long-term data exist for the Boggy Cutoff population, no data suggest asynchronous aquatic habitat use in similar *Kinosternon subrubrum* populations (Mahmoud, 1969; Gibbons, 1983; Stone et al., 1993; Steen et al., 2007; Riedle et al., 2015). Both populations subsist at comparatively higher densities assuming synchronous aquatic activity.

Ecological barriers to parasite colonization have been observed within geographically isolated or fragmented host populations (Anderson and May, 1978; May and Anderson, 1978; Arneberg et al., 1998; Kuris and Lafferty, 1994; Lafferty et al., 1994; Byers et al., 2008), invasive host populations (Torchin et al., 2003), and within extreme habitats (Mendes et al., 2005; Tobler et al., 2007). Small host populations that subsist in low densities limit parasite transmission and lead to localized parasite extinction or low prevalence within the environment (Arneberg, 2002; Torchin et al., 2003). Invasive populations tend to have fewer parasites given small founding populations will carry only a fraction of the parasite community with them to new habitats (Torchin et al., 2003). This leads to low genetic diversity among parasite species, and new environments often lack intermediate hosts necessary to complete life cycles (Lyles and Dobson, 1993; Torchin et al., 2003). Abiotic fluctuations in rainfall or temperature also may inhibit parasite success (Roberts and Grenfell, 1992; Stromberg, 1997; Altizer et al., 2006). Droughts and flooding may alter host behavior or affect parasites directly by driving infectious stages into soil or washing them downstream (Stromberg, 1997).

These complex ecological interactions are often difficult to tease out of analyses to determine the precise mechanisms structuring parasite absence or abundance (Aho, 1995). Local parasite diversity may be largely dictated by local biotic and abiotic conditions so interactions among individual species become increasingly important (Aho, 1995). While it is possible to determine precise mechanisms for parasite occurrence in some habitats, long-term study of community processes, food web dynamics, and habitat use are vital when considering the many potential confounding factors inherently present (Pedersen and Fenton, 2007). Habitat area, type, and degree of isolation all influence the colonization-extinction processes governing parasite prevalence within hosts (Poulin and Guegan, 2000; Lafferty et al., 2010). But the variation in parasite communities among the same or closely-related species inhabiting different environments remains one of the most poorly understood aspects of parasite ecology (Timi and Poulin, 2003).

IV. Conclusions

The results of this study suggest that some combination of ecological mechanisms are preventing successful parasite establishment within *Kinosternon sonoriense* populations inhabiting the Madrean Sky Islands. While the precise mechanisms are unclear, further study is warranted to better understand the community ecology of extreme environments, parasite colonization of novel habitats, and the factors dictating parasite community structure in these areas. Parasite avoidance through differential habitat use has never been documented in turtles, but this strategy has been observed in fish (Poulin and Fitzgerald, 1989), birds (Mendes et al., 2005), bats (Reckardt and Kerth, 2007), primates (Hausfater and Meade, 1982), and invertebrates (Thomas and Jones, 1993; Ebert et al., 2001). Such insights may yield vital life history information and important co-evolutionary relationships between mud turtles and their parasites

in isolated habitats. Further, understanding the colonization-extinction dynamics of these island systems may help us understand the processes structuring these communities.

While little evidence for increased parasite resistance exists within the present study, further investigation is warranted. Co-evolutionary relationships between parasites and hosts are well-documented in the animal kingdom and illustrate historic selection pressures on both free-living animals and their parasites (Brandt et al., 2007; Dare et al., 2008; Eberhardt et al., 2013). Reptiles are particularly sensitive to temperature and rainfall fluctuations given their dependence upon ectothermy and osmostic balance for many physiological processes such as reproductive success, sex determination, metabolism, and immune function (Moeller et al., 2013; Urban et al., 2013). Therefore, some advantage must be present within the Madrean Sky Island environments to balance the energy expenditure necessary to regulate these physiologic processes. Understanding the effects that environmental stochasticity may have on mud turtles is essential to their long term survival in these areas. A low parasite burden would arguably increase fitness and help offset the stressors inherently present in extreme environments. Studying habitats that have undergone rapid speciation in response to historical changes in climate are vital in understanding what changes are likely to take place in the future. Studies should focus on the mechanisms dictating community structure in the Madrean Sky Islands so that effective conservation can be maintained for years to come.

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APPENDIX

Figure A1: Necropsy of *Kinosternon sonoriense*: this photo depicts the gross dissection of *Kinosternon sonoriense* individual UCO1809 performed at New Hampshire Diagnostic Veterinary Lab (NHDVL) and overseen by Dr. Inga Sidor, DVM. This specimen was less preserved than UCO1810; however, this is a section of lungs and GI tract. The red arrow indicates the location of the lungs. While difficult to determine given the state of decomposition, no signs of pathology were found.
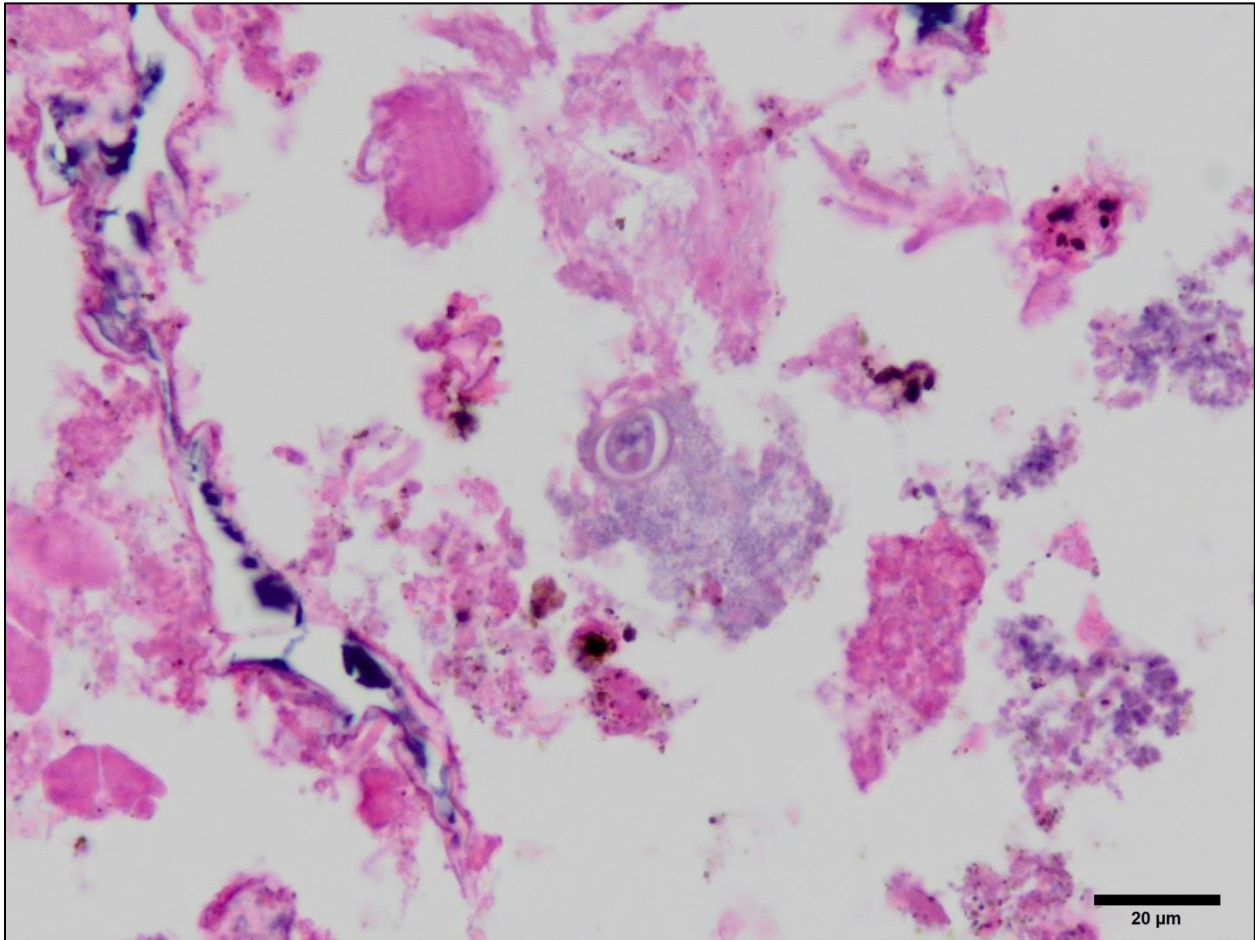
Figure A2: Lung Section from *Kinosternon sonoriense* Stained with H&E: this slide was made from the same piece of lung shown above at the NHVDL and examined for pathology by myself and Dr. Sidor. Deterioration of the tissues was noted, as well as post mortem fungal growth, however no evidence of parasitism or pathology was found.

Figure A3: Dissection of *Kinosternon sonoriense* Lungs: this photo shows the gross dissection of Kinosternon sonoriense individual UCO1810. This individual was in relatively good condition, as can be seen by the removed lung (top) and trachea with attached lung seen here. No evidence of pathology or parasitism was found in gross dissection or tissue histology.
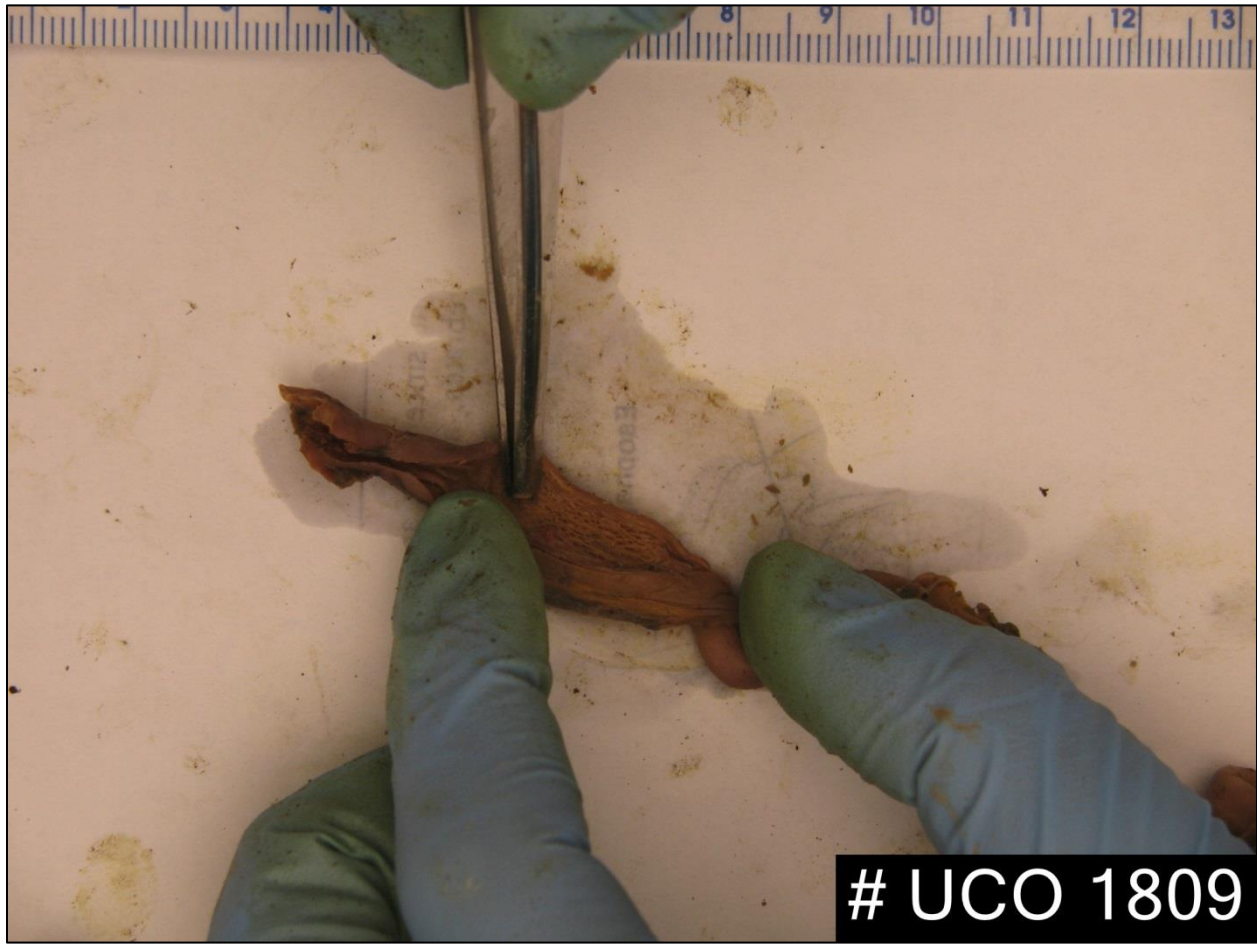
Figure A4: Dissection of GI Tract from *Kinosternon sonoriense*: this photo depicts the gross dissection of the lower GI tract from individual UCO1810. Above, parts of the large intestine are being dismantled in an effort to find adult helminths. Scrapings of the material pictured above were examined under microscopy, as well as histopathological slides made from sections of the entire GI tract, but no evidence of parasitism was found.

Figure A5: Second Dissection of GI Tract from *Kinosternon sonoriense*: this photo depicts the dissection of the gastrointestinal tract of individual UCO1810. No signs of pathology were found from gross dissection and no helminths were observed.
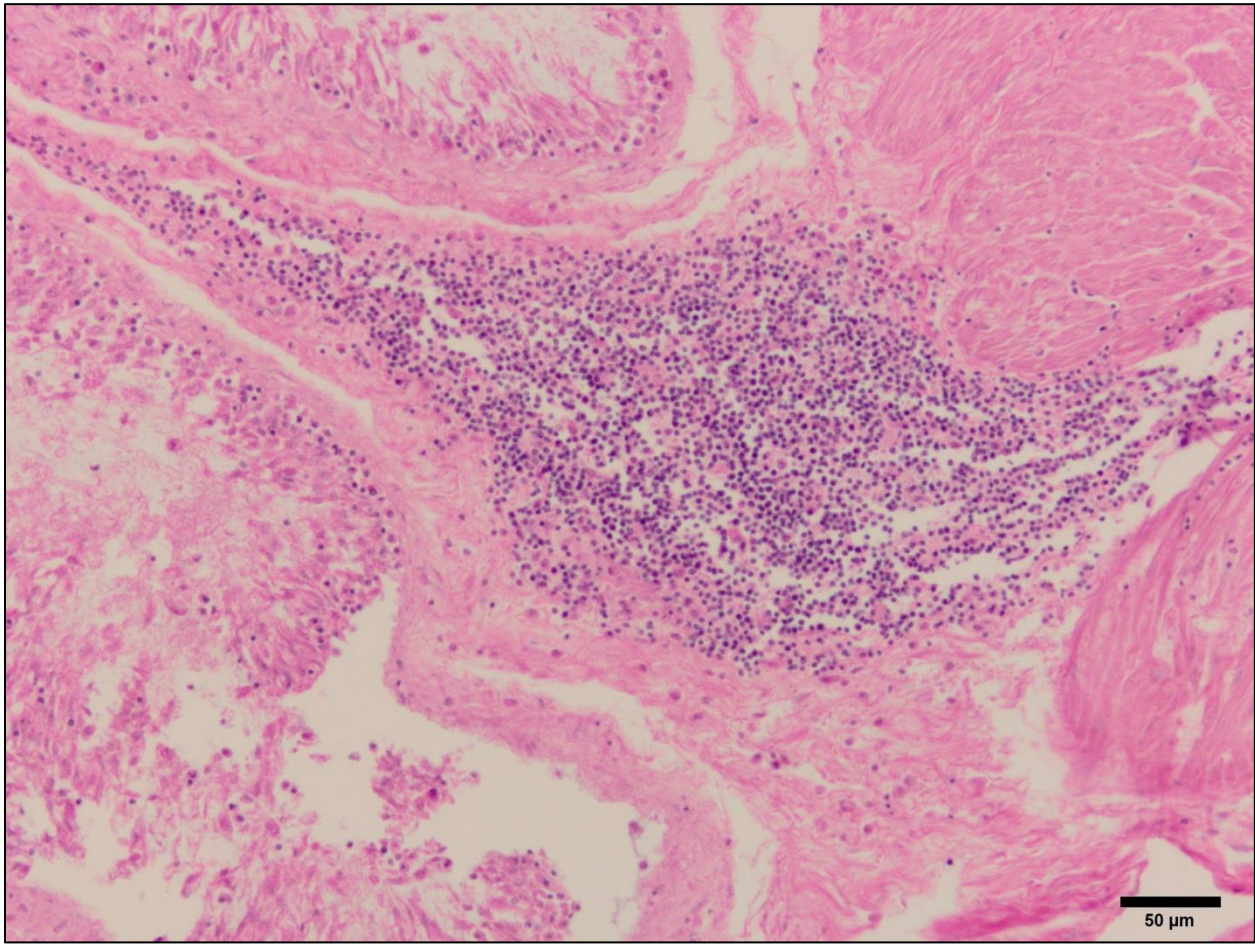
Figure A6: Slide of Urinary Bladder from *Kinosternon sonoriense*: this photo was taken of sections of urinary bladder (UCO1810) stained with H&E. Above, the inflammation was noted but seemed to be localized as no ulceration was found and leukocytes were confined to the submucosa. No evidence of pathology or parasitism was found.
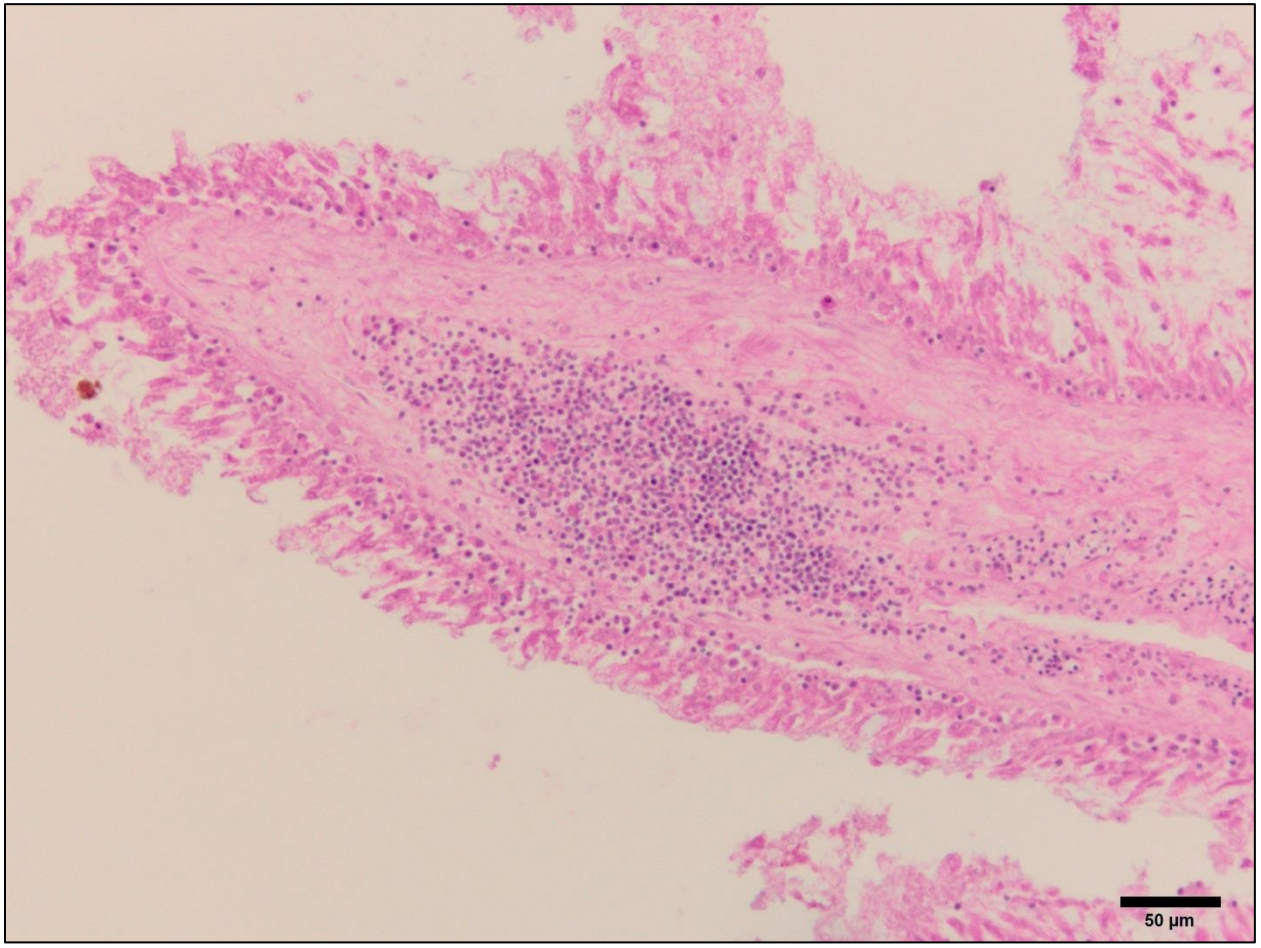
Figure A7: Slide of Distal Intestine Stained with H&E: pictured here is a circular fold of the small intestine with numerous villi present. No cysts or evidence of pathology were found.