HEAD-STARTING AS A CONSERVATION TOOL FOR GOPHER TORTOISES (GOPHERUS POLYPHEMUS)

by

DANIEL PATRICK QUINN

(Under the Direction of Tracey D. Tuberville and Jeffrey A. Hepinstall-Cymerman)

ABSTRACT

Gopher tortoise (Gopherus polyphemus) populations are declining throughout their range and conservation action increasingly requires active management manipulations to mitigate losses. This thesis aimed to evaluate head-starting as a population augmentation tool for a depleted population of gopher tortoises at the Yuchi Wildlife Management Area in Burke County, GA. Specifically, we focused on diet and health in captivity and movement and survivorship after release. Different captive diet supplements fed during head-starting showed little differences in growth and plasma chemistry metrics. Plasma chemistry values were also similar to other head-start and wild gopher tortoise populations. After a soft-release, we radio-tracked two cohorts of head-starts (N = 41) 1-3 times per week for up to 486 days. For our first cohort of head-starts, we documented survivorship rates of 72.7% to first dormancy, 60.6% annually, and 48.4% to second dormancy. Our second cohort had an estimated survivorship of 70.0% to first dormancy at our northern release area. Due to issues with fire ants, we were unable to follow our soft-release methods for our second cohort at our southern release area and only 7.1% of head-starts survived to first dormancy. No head-start moved further than 122.0 m from their release site. We also compared radio-telemetered hatchling (n=10) and head-start (n=10)movement and survivorship after hard release at adult burrows for 62 days. Hatchling and head-starts did not demonstrate significantly different movement patterns. 88.9% of hard-released head-starts

survived to dormancy compared to 50.0% of hatchlings; however, this was not significantly different (P = 0.11). Our results suggest that head-start tortoises can be reared on simple diets of leafy greens with a commercial supplement and that head-starting could be a beneficial conservation tool for future augmentation efforts given that threats are mitigated prior to release.

INDEX WORDS: Plasma chemistry, Captive diet, Movement, Radio telemetry, Survivorship, Tortoise HEAD-STARTING AS A CONSERVATION TOOL FOR GOPHER TORTOISES (GOPHERUS POLYPHEMUS)

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DEDICATION

I would like to dedicate this thesis to my family, especially my parents, Jill, Steve, and Kari for all of their love and support. I especially thank you for the advice over the years, especially that I should endeavor to find a profession that I am truly passionate about. These words have been with me my entire academic career and I will take them with me into the future.

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CHAPTER 1

INTRODUCTION

Effective management of imperiled species requires land managers and scientists to evaluate threats to populations while gaining access to the appropriate tools to mitigate them. Anthropogenic threats have been well documented as the principal cause of many declining wildlife populations (Lande 1998) with habitat degradation cited as the single most significant cause of declines (Groombridge 1992, Fahrig 1997, Alford and Richards 1999). Due to the scale of anthropogenic disturbance, declines in wildlife populations are now occurring globally (Gibbons et al. 2000, Butchart et al. 2010) and conservation action is rapidly requiring active, manipulative management solutions to mitigate losses. Conversion of natural habitat for anthropogenic use is likely to increase in the future, thus increasing stress on already depleted populations recover, but only once habitat has been restored and anthropogenic threats mitigated (Congdon et al. 1993; Snyder et al. 1996; Philippart 1995). Population augmentation, in which new individuals are added to populations to increase their size, is one such technique. The ecological endpoint of population augmentation is to release healthy animals that will naturally assimilate with the recipient wild population and eventually increase breeding stock.

Individuals used for population augmentation may be wild-caught, displaced waif animals, or animals born in captivity. These animals are then translocated - intentionally released at a within-range location different from their capture location in order to 'establish, reestablish, or augment a population' (Griffith et al. 1989). The success of reintroduction and translocation programs such as the gray wolves (*Canis lupis*) in Yellowstone (Ripple and Beschta 2012), the Arabian oryx (*Oryx leucoryx*) in Oman (i.e., for the first 24 years prior to intensive poaching; Spalton et al. 1999), and Gorillas (*Gorilla gorilla*) in Central Africa (King et al. 2012) demonstrate that population augmentation can be a viable and necessary conservation tool. Historically however, many translocation programs have failed (Griffith et al. 1989) due in large part to failure to consider constraints (e.g., habitat requirements, disease, population genetics, etc.) that should be addressed before releasing animals into the wild (Snyder et al. 1996, Seigel and Dodd 2000). Even when constraints are appropriately considered, released animals must be monitored to determine the short and long-term efficacy of continuing programs for population management purposes.

While a wide variety of species have been used for augmenting wild populations (Soorae 2008), turtles (i.e., members of the order Testudines) have been one of the most prominent taxonomic groups for augmentation efforts including Kemp's Ridleys (Lepidochelys kempii; Caillouet Jr. et al. 2015), redbelly turtles (Pseudemys rubriventris, Haskell et al. 2014), diamond-backed terrapins (Malaclemys terrapin, Herlands et al. 2004), gopher tortoises (Gopherus polyphemus, Tuberville et al. 2005), desert tortoises (Gopherus agassizii, Field et al. 2007), Blanding's turtles (Buhlmann et al. 2015), Galapagos tortoises (Geochelone gigantea; Hambler 1994), western pond turtles (Actinemys marmorata, Vander Haegen et al. 2009) and European pond turtles (Emys orbicularis, Mitrus 2005). Turtles are relatively long-lived, late to mature, and produce few offspring that survive to adulthood, making populations particularly susceptible to changes in adult survivorship (Heppell et al. 1996). Anthropogenic threats have increased adult mortality and now 42% of all extant turtle species are listed as threatened according to the IUCN red list (Baillie et al. 2004). While habitat degradation is often the most significant threat (Gibbons et al. 2000), turtles are also at threatened by vehicle-induced mortality (Wood and Herlands 1997, Gibbs and Shriver 2002, Crawford et al. 2014), over-exploitation by humans (Sharma 1999; Nijman and Shepherd 2015), invasive species introduction (e.g., fire ants; Allen et al. 2001), subsidized predator populations (e.g., raccoons, Procyon lotor; Browne and Hecnar 2007; Prange et al. 2003) and communicable diseases

(e.g., fibropapillomatosis, iridovirus, and upper respiratory tract disease caused by mycoplasma infections; Foley 2005; Johnson et al. 2008; Brown et al. 2001).

The same life history traits that make turtle populations susceptible to high adult mortality also make them suitable candidates for population augmentation. Once threats are mitigated, increasing the number of adults should dramatically increase population viability. However, if threats remain unaddressed, efforts to increase adult population sizes will almost certainly fail. However, augmentation projects could be susceptible to unforeseen issues (e.g., survival, site fidelity, health, fecundity, etc.). Therefore, if organizations plan to use augmentation techniques, they must also evaluate its efficacy. As the most widely relocated and translocated herpetofaunal species (Seigel and Dodd 2000), the gopher tortoise (*Gopherus polyphemus*) is an ideal candidate for evaluating the role that augmentation tools could play in population conservation.

The gopher tortoise is one of only five extant tortoise species in North America. Their range extends along the southeastern sandhills and coastal plain of the United States where they reside in well drained, sandy soils. They are well known for their ability to construct large burrows which can reach upwards of 20 m long and 3 m below the surface (Ashton and Ashton 2008). A large number of species use these burrows as refuge (i.e. upwards of 360 species; Jackson and Milstrey 1989) making the gopher tortoise a critical ecological component of these ecosystems. However, gopher tortoise populations are declining throughout their range predominately due to habitat loss and habitat degradation from land development and fire suppression (Auffenberg and Franz 1982; Diemer 1986). The longleaf pine ecosystem, in which many gopher tortoise populations reside, has been reduced by 98% of its historic range (Noss et al. 1995). Subsequently, many extant gopher tortoises are federally listed as threatened west of the Tombigbee and Mobile rivers in Alabama. They are also listed as a future candidate in the remainder of their range to the east (USFWS 2013). The decline of this ecologically important species

warrants research into conservation efforts aimed at mitigating threats and increasing the viability of populations.

In July of 2013 The Gopher Tortoise Council submitted a report defining the minimum viable population size (MVP – "threshold number of individuals in a population below which an unacceptable risk of extirpation exists"; Shaffer 1981) for gopher tortoises at 250 individuals based on studies that examined population viability models with parameters including environmental conditions, age class specific mortality, initial population size, and survivorship in the presence of upper respiratory tract infections (Abercrombie 1981; Cox et al. 1987; Miller 2001; Root and Barnes 2006; Tuberville et al. 2009). In addition to the new threshold, the council determined that populations must have a density equal to or higher than 0.4 tortoises per hectare to ensure successful mating (Guyer et al. 2012), an equal sex ratio, active recruitment, variable age classes, and must exist on a landscape with no major constraints to movement. Many populations throughout the range fall short of one or more of these criteria, and many fail to meet even the minimum size threshold (Georgia DNR, unpublished data), and are therefore designated as non-viable.

Population augmentation is now a necessary tool to help depleted tortoise populations recover when current numbers and/or recruitment are insufficient to maintain viability and threats that caused the decline have been mitigated. Although adult tortoises outweigh the influence of younger counterparts when boosting populations (Congdon et al. 1993; Heppell et al. 1996), recent findings suggest that juvenile life stages should be the next priority if adult survivorship is high (Miller 2001; Pike et al. 2008; Tuberville et al. 2008). These findings indicate that where populations are depleted, increasing juvenile survivorship should take priority if adult cohorts are stable. By addressing these concerns through empirical studies, augmentation techniques may enable declining gopher tortoise populations to obtain viable and stable levels, ensuring persistence into the future.

Prior studies have established new populations or augmented depleted populations of gopher tortoises by translocating wild adults and sub-adults (Heise and Epperson 2005; Tuberville et al. 2005; Ashton and Burke 2007). However, translocated tortoises are often waifs or displaced due to anthropogenic disturbance (Burke 1989; Tuberville et al. 2011). These tortoises are obtained sporadically, and are an unreliable source for planned conservation efforts. Obtaining non-displaced adult gopher tortoises for translocation is rarely feasible without significantly affecting donor populations. Although translocating hatchlings might have less of an impact on donor populations and offer a more predictable source of animals, this age class has the highest mortality rates due to their increased vulnerability to predation (Ashton and Ashton 2008; Heppell et al. 1996; Tuberville et al. 2015). With these factors in mind, head-starting hatchlings to larger age classes may offer a solution to translocation concerns.

Head-starting - the practice of protecting especially vulnerable life stages of a species to increase the likelihood of survivorship for conservation purposes (Burke 2015) - is one potential management tool that could be used to boost depleted populations. Head-starting allows hatchlings to reach larger size classes, reducing vulnerability to predation due to their larger size and harder shells relative to hatchlings (Heppell et al. 1996; O'Brien et al. 2005). At the end of the head-start period, tortoises are released into the wild, typically at the size of 2-3 year old wild juveniles. By using headstarted tortoises, conservation organizations can predict and control the magnitude of manipulation. Head-starting can also ensure that donor populations are not drastically affected by collecting only certain proportions of the youngest cohort while retaining reproductive adults within the donor population. Furthermore, by locating and protecting nests for head-starting efforts, donor populations could increase recruitment of hatchlings by protecting nests that would otherwise succumb to predation (Quinn et al. 2016).

Head-starting is considered a potentially viable management tool for depleted turtle populations by multiple state and federal agencies including the U.S. Fish and Wildlife Service (Buhlmann et al. 2015). However, research is needed to determine the efficacy of head-starting and striking a balance between efficiency and effectiveness is critical for future head-starting efforts. Potential effects of captivity during the head-starting period are a prominent concern for head-started turtles. Captive conditions often differ substantially from the environment experienced by wild conspecifics, and could negatively impact head-start behavior, physiology, and health (Bowen et al. 1994, Mortimer 1995), reducing the efficacy of head-start survival and reproduction in the recipient population.

Although the impetus for head-starting is to reach larger sizes to increase survival in the wild, accelerated growth in turtles is also associated with health concerns (McArthur and Barrows 2004). For example, desert tortoises (*G. agassizii*) head-started to the size of wild eleven-year old in just one year suffered from significant shell deformities (Jackson et al. 1976). The shell deformities were so extreme that it is doubtful that these individuals were able to thrive after release into the wild. While other environmental variables (i.e., humidity, temperature, water availability, etc.) may play an important role in accelerated growth rates, diet is thought to be the main cause of accelerated growth in captive turtles (Ritz et al. 2012). Without adequate nutrients to keep up with accelerated growth rates, shell deformities in captive turtles is usually metabolic bone disease (MBD) caused by inadequate nutrition (Boyer 1996). MBD encompasses a variety of clinical syndromes that result in abnormal bone structure and blood calcium levels. Although non-communicable, it is still one of the most common diseases in captive turtles. MBD can be caused by a deficiency in dietary calcium and phosphorous as well as a deficiency in Vitamin D3 due to insufficient access to ultraviolet light (Avery 1993; Jacobson

1994; Boyer 1996). Other health issues may also arise from inadequate diet including obesity, hypovitiminosis A, gout, iodine deficiency, and steatitis (McArthur and Barrows 2004).

Due to the complexity of diets and the interactions of other environmental conditions, the precise effects of captive diet are difficult to discern. While little is known about wild juvenile diets in general, foraging preference has been documented at one site in west-central Florida (MacDonald and Mushinsky 1988; Mushinsky et al. 2003). Juvenile tortoises were documented foraging primarily on herbaceous plants (e.g., *Liatris* and *Dyschoriste*) and less on fibrous plants like grasses (*Poaceae*) during the summer months. However, grasses may constitute a more substantial portion of diets during colder months (Garner and Landers 1981; Mushinsky et al. 2003). Organizations interested in future head-starting programs will need to understand what diets will allow tortoises to grow quickly, while remaining healthy. To date, little research has been conducted on head-start diet and nutrition (but see Erickson 2015).

Tuberville et al. (2015) demonstrated that head-started tortoises could be reared safely in captivity and that survivorship was lowest the first year post-release. However, this study was not designed to test the effectiveness of head-starting as a management technique nor was it designed to evaluate potential risks associated with captive husbandry. This thesis aims to determine the efficacy of using juvenile head-started gopher tortoise to augment a depleted population on managed land by evaluating pre-release growth and health metrics and post-release performance metrics. In Ch. 2 I focus on pre-release metrics, specifically growth and plasma chemistry of tortoises fed different supplemental diets while being head-started at the Savannah River Ecology Laboratory in Aiken, South Carolina. I also compare growth and plasma parameters to head-started juveniles, wild juveniles, and wild adult tortoises from St. Catherines Island, GA. In Ch. 3 I focus on post-release performance of head-started gopher tortoises. Specifically, I document head-start movements, displacement from release, and

survivorship by using radio-telemetry at the Yuchi Wildlife Management Area (YWMA) in Burke County, Georgia, USA. I also compare movement and survival metrics between hatchlings and a sub-set of headstarts to evaluate head-start performance in relation to hatchlings.

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CHAPTER 2

DIET SUPPLEMENT EFFECTS AND HEALTH INDICES OF HEAD-STARTED GOPHER TORTOISES

INTRODUCTION

Head-starting- the practice of protecting especially vulnerable life stages of a species to increase the likelihood of survivorship for conservation purposes (Burke, 2015) - has become increasingly popular as a conservation tool for threatened and endangered species (Seigel and Dodd, 2000; Snyder et al., 1996). Turtle species (i.e., members of the order Testudines) are declining globally, with 42% of all extant species listed as threatened according to the IUCN red list (Baillie et al., 2004). Consequently, many conservation efforts are focused on recovering depleted turtle populations. Turtles are long-lived, late to mature, and demonstrate high adult survivorship, all of which prevent depleted populations from recovering quickly (Congdon et al., 1993). Turtles also demonstrate the lowest survivorship in the first year of life (i.e., the hatchling stage) when they are the most vulnerable to predators due to their small size and relatively soft shells (Ashton and Ashton, 2008; Heppell et al., 1996). Therefore, turtle populations are naturally the most dependent on adult survivorship. However, once threats that caused population declines have been mitigated, it is difficult for conservation efforts to further increase adult survivorship because it is naturally very high. Because juvenile survival is normally low, focusing management plans on this life-stage should be the next focus of management efforts (Tuberville et al. 2009). By head-starting hatchlings through the vulnerable hatchling stage, it may be possible to restore depleted populations using larger, older individuals that are less vulnerable to predation. The Turtle Survival Alliance (TSA) alone is involved in head-starting of 11 separate species for conservation efforts (Burke, 2015). Now that head-starting is viewed as a potentially viable management tool, understanding

how to maximize growth while maintaining health during the head-starting process is imperative for increasing post-release performance. While many head-starting projects attempt to evaluate postrelease performance in the wild (Buhlmann *et al.*, 2015; Nagy *et al.*, 2015; Tuberville *et al.*, 2015; Vander Haegen *et al.*, 2009), few have documented how the head-starting process affects individuals prior to release.

Captive settings often differ substantially from the environment experienced by wild conspecifics (e.g., diet, ambient temperature, sunlight, substrate, etc.) which may negatively affect physiological health of head-started turtles (Bowen *et al.*, 1994). Captive diet, in particular, plays an important role in turtle growth and health (Avery *et al.*, 1993; Ritz *et al.*, 2012). While larger body sizes are correlated with higher survival in the wild, accelerated growth in captivity has also been correlated with nutritional diseases caused by dietary deficiencies. The most common nutritional diseases include obesity, hypo- and hyper-vitaminosis A, gout, steatitis, goitre, shell pyramiding, and metabolic bone disease (McArthur and Barrows, 2004).

Shell deformities are particularly common in captive turtles. Shell pyramiding, in which the carapacial scutes become malformed after accelerated growth, has been linked with decreases in humidity and, to a lesser extent, increased dietary protein (Wiesner and Iben, 2003). Shell pyramiding may also be associated with metabolic bone disease, an umbrella term for a variety of pathological conditions that affect bone strength, structure, and mass. The suspected etiology of MBD in turtle species is usually linked with malnutrition (Boyer, 1996; Jacobson, 1994). Although non-communicable, MBD is one of the most common diseases in captive turtles and is often indicative of poor husbandry conditions (Rosskopf and Shindo, 2003). Head-started turtles raised in captivity and fed unnatural diets may be susceptible to MBD and other nutritional diseases. For example, desert tortoises (*Gopherus agassizii*) head-started to the size of eleven year old wild conspecifics in just one year suffered from considerable shell pyramiding associated with their accelerated growth (Jackson *et al.*, 1976). It is

doubtful that tortoises suffering from such extreme deformities could survive, much less thrive, in a wild population. It is critical then, that appropriate diets are fed to head-started turtles in order to mitigate the risk of nutritional diseases. However, due to the complexity of diets and the interactions with other environmental conditions, the precise effects of captive diet are often difficult to discern. Therefore, effects of captive settings need to be evaluated to help ensure head-starting quality and efficacy.

Historically, the gopher tortoise (*Gopherus polyphemus*) is one of the most frequently used species for population augmentations (Seigel and Dodd, 2000), making them ideal candidates for head-starting initiatives. The gopher tortoise is native to the Southeastern United States and is declining throughout its range (Auffenberg and Franz, 1982). They are federally-listed west of the Tombigbee and Mobile rivers in Alabama but habitat restoration efforts are underway, making head-starting an attractive management tool to help boost depleted populations. To date, little work has been done on head-starting in gopher tortoises and little is known about the effects of captive diet on head-starts (but see Erickson, 2015 and Holbrook *et al.*, 2015). Gopher tortoises are almost exclusively herbivorous, foraging on a wide variety of understory plant species. Wild juvenile gopher tortoises have been documented selectively foraging on legumes and herbaceous plants that are high in protein. High fiber plants like grasses make up a smaller portion of their diet compared to adults (Garner and Landers, 1981; MacDonald and Mushinsky, 1998; Mushinsky *et al.*, 2003). However, head-starting programs typically choose diets that are affordable, readily obtainable, and are therefore likely to be non-native to the species range (but see Holbrook *et al.*, 2015). Incorporating native forage species into diets during captivity could benefit head-start growth and health.

While growth can be assessed using standard morphological measurements (Jolicoeur and Mosimann, 1960), physiological health is more difficult to quantify. Plasma chemistry panels are a potentially useful tool to help assess physiological status by evaluating the components of plasma (i.e., plasma analytes) which can act as indicators of nutrition status and organ and tissue pathology.

Although plasma chemistry panels alone cannot provide definitive health diagnoses, they can be used to indicate physiological differences between individuals or groups of animals. Blood and plasma chemistry screening has already been used in turtles for assessing individual physiological status (Bolten and Bjorndal, 1992; Jacobson *et al.*, 1991; Perpiñan *et al.* 2008; Rosskopf Jr and Woerpel, 1982; Snoddy *et al.* 2009) and could be useful for comparing effects of different dietary regimens. Furthermore, plasma chemistry values can be used to compare head-started tortoises to reference values from healthy, wild individuals. While blood and plasma reference values for wild and healthy adult gopher tortoises have been documented (Hernandez *et al.*, 2010; Taylor and Jacobson, 1982), no blood or plasma reference values currently exist for free-ranging juveniles.

From Fall 2014 through Spring 2015, we head-started 98 gopher tortoise hatchlings at the Savannah River Ecology Laboratory in Aiken, South Carolina. For seven months we implemented a study to evaluate the effects of three different supplemental diet regimens on growth metrics and plasma chemistry values of these tortoises. During the same time period, we head-started 44 gopher tortoises at St. Catherines Island, GA and obtained plasma chemistry data for wild adult and juvenile gopher tortoises. The aim of our research was to determine how diet affects health parameters of head-started gopher tortoises and how these metrics compare to wild conspecifics. Specifically, our objectives were to: (1) document and compare growth metrics and plasma chemistry parameters among head-started gopher tortoises offered different combinations of leafy greens, sod grass, and natural vegetation, and (2) compare growth metrics and plasma parameters of head-started tortoises to wild juvenile and adult tortoises at St. Catherine's Island, GA.

METHODS

Hatchling Husbandry:

From 27 June–28 July 2014, we searched for and collected gopher tortoise nests at: St. Catherines Island (SCI) in Liberty County (see Tuberville *et al.*, 2008 for detailed site description), Reed Bingham State Park (RBSP) in Cook County, and the Yuchi Wildlife Management Area (YWMA) in Burke County, Georgia (see Bauder *et al.*, 2014). For a detailed description of egg collection and incubation methods, see Quinn *et al.* (2016). Tortoises collected from RBSP and YWMA were hatched and reared at the University of Georgia's Savannah River Ecology Laboratory (SREL) in Aiken, South Carolina. Tortoises collected from SCI were head-started separately on SCI and were not included in the diet manipulation component of this study. However, all hatchlings from SCI and SREL were head-started for subsequent release at YWMA (see chapter 3).

We reared tortoises at SREL in a climate controlled animal holding facility with ambient temperature maintained at 24°C. The animal holding facility is a concrete building partitioned into multiple concrete stalls and a translucent fiberglass roof that allowed natural sunlight to filter through for natural day-night cycles. We distributed tortoises among 15, 190 L (50 gal) oval Rubbermaid® bins (Newell Rubbermaid, Atlanta, Georgia, USA) inside of three-walled concrete stalls measuring 5.0 m long by 1.5 m wide (Figure 2.1). Each bin housed 6-7 tortoises, and we kept tortoises within each bin separate from other bins. We filled tubs with 3 cm of substrate, making sure to evenly cover the bottom of the tub, using a mixture of approximately 75% Harvest[™] Organic Garden Soil (Harvest Power, Waltham, Massachusetts, USA) and 25% sterile Quikrete® Play Sand (The QUIKRETE® Companies, Atlanta, Georgia, USA). We made artificial hide structures by cutting 10.2 cm (4 in) diameter plastic corrugated tubing in half along its length to make two half-pipes, and then cut hide structures into approximately 16.5 cm length segments. We placed two hide structures inside each rearing bin (Figure 2.1). Approximately 25 cm above the substrate of each bin, we suspended a Zoo Med[®] Mini Combo Deep Dome Lamp Fixture (Zoo Med Laboratories Inc., San Luis Obispo, California) with two bulbs for day and night. We controlled lights with automated timers that alternated each bulb for 12 hours a day to ensure a constant heat gradient. We used a 100w Zoo Med[®] PowerSun mercury vapor bulb during the day time (0700 – 1900 h) to ensure adequate access to a temperature gradient as well as UVB and UVA radiation for Vitamin D synthesis and normal behavior (Adkins *et al.*, 2003). We used a 50w Exoterra[®] Infrared Basking Spot (Rolf C. Hagen Group, Montreal, Canada) to provide heat during the night time (1900-0700 h). We placed hardware cloth over the tubs to prevent the lamps from accidentally falling into the bins. Lights kept basking areas between 21- 35°C. We soaked all tortoises (grouped by husbandry bin) in small plastic tubs three times a week to ensure adequate hydration.

All tortoises were fed a base diet of commercially-available leafy vegetables consisting of turnip greens, mustard greens, kale, romaine lettuce, and green leaf lettuce. However, the exact mixture of food varied based on availability. The leafy vegetables were cut into small pieces and mixed with rehydrated Zoo Med® Grassland Tortoise Diet food pellets for added nutrients. We chose this as a base diet for all tortoises because it reflects common tortoise dietary protocols (Holbrook *et al.*, 2015). We first soaked 35 g of the Zoo Med® food pellets in warm water to rehydrate and soften them and then mixed them in with approximately 600 g of leafy vegetables. We made plates of food using 40 g of the leafy vegetable-pellet mixture and fed the mixture to all bins for every feeding. Initially, we fed tortoises three days a week, but switched to five days a week on 20 January 2015 to adjust for increased food intake as the tortoises grew. Although availability of each leafy vegetable varied throughout the headstarting period, during any one feeding we gave all tortoises the same vegetable mixture.

Experimental Diet Treatments

From 02 November 2014 through 28 May 2015 (207 d) we conducted a diet supplement experiment to determine the effects that different diets might have on growth and plasma parameters. We randomly selected hatchlings for placement into one of 15 bins in the three stalls (i.e., five bins per stall) in the animal rearing facility at SREL. Because both incubation environment (e.g., temperature, humidity, water availability, etc.) and parental effects (e.g., genetics, maternal body size and condition, energetic provisioning to reproduction, etc.) have been shown to alter initial size and growth rates (Janzen, 1993; Janzen *et al.*, 1990; Nafus *et al.*, 2015; Packard *et al.*, 1987), we used clutch as a blocking factor (i.e., separated siblings so that none were in the same bins) to prevent a "clutch effect" that could bias growth and plasma parameters due to similarities among siblings. We also weighted selection to ensure that all treatments received the same number of individuals (n = 32 per treatment). We then randomly selected supplemental diet treatments for each bin but weighted selection to ensure that treatments were even (i.e., five tubs per treatment) and that treatments were interspersed between stalls (i.e., to prevent isolative segregation; Hurlbert, 1984).

We used the following supplemental diet treatments to evaluate the effects of diet on growth and plasma chemistry: 1. *Control*, 2. *Sod* and, 3. *Natural* (Figure 2.2). The *Control* treatment consisted only of the base diet of leafy vegetables (Table 2.1) with Zoo Med[®] Grassland Tortoise Diet food pellets. We fed tortoises in the *Sod* treatment with the control diet supplemented with a mat of sod grass obtained from a local vendor. The sod mat measured approximately 30 by 60 cm. Initially, we used Bermuda sod (*Cynodon dactylo*) but later switched to Centipede sod (*Eremochloa ophiuroides*) to see if tortoises would prefer this. Although we changed grass species of sod during the study, sod type was consistent across replicate bins receiving the sod treatment. We fed tortoises receiving the *Natural* treatment the control diet supplemented with a 30 x 60 cm plot of native plants. We filled the native plots to approximately 5 cm with potting soil and grew seven species of native plants (Table 2.1). We

selected plant species based on species and genera documented as palatable to gopher tortoises (Ashton and Ashton, 2008; MacDonald and Mushinsky, 1998; Mushinsky *et al.*, 2003) and native to the future head-start release site (i.e., YWMA). We purchased all native plant seeds from Roundstone Seed, LLC (Upton, Kentucky, USA) as a multi-species composite mixture. We cold-stratified seeds mixed in with vermiculite for approximately one month in a refrigerator at approximately 2.8°C, and germinated them in the potting pallets for one to two months in a greenhouse before placing them in the tortoise bins. All living vegetation was watered three days a week at the same time when tortoises were soaked.

Health Metrics

Growth –To determine the effects of diet on tortoise growth, we used a DeltaRange[®] Mettler PE 3600 gram scale (Mettler Toledo, Columbus, Ohio, USA) to measure mass to the nearest 0.1 g. We used dial calipers (Mitutoyo, Aurora, Illinois, USA) to measure midline carapace length (CL), maximum carapace width (CW), and maximum shell height (SH) to the nearest 0.1 mm. We took all measurements at hatching, just prior to initiation of the diet treatment study, and monthly thereafter until our last measurement before release on 28 May 2015. We calculated shell volume (SV) as cm³ using the following equation by (Loehr *et al.*, 2004):

$$SV = \pi \times CL \times CW \times SH/6000$$

We used SV because, unlike single measurements of size, it accounts for shell shape by using measurements for width, length, and height. We determined body condition index scores (BCI; g/cm³) using the same equation as Nagy *et al.* (2002).

$$BCI = (Mass/(CL \times CW \times SH))$$

We also measured shell hardness indices (SHI) for tortoises just prior to release at the YWMA using methods similar to Nagy *et al.* (2011), using the equation:

$$SHI = 100 (CSH/USH)$$

where CSH is compressed shell height and USH is uncompressed shell height (i.e., SHI = 100 represents a completely hard shell). We used a Starrett[®] 3732XFL-4 inch electronic micrometer (L.S. Starrett Company, Athol, Massachusetts, USA) to measure USH and CSH in the middle of the second vertebral scute. We measured USH as using the micrometer when the spindle touched the second vertebral scute. To measure CSH, we advanced the micrometer spindle until the micrometer ratchet slipped and continued to slip after three rotations. We used a 5.08 cm (i.e., 2.0 in) insert attached with a custom made plastic collar to the non-advancing end (Nagy *et al.*, 2011) to allow measurement of tortoises between 2.5 – 5.0 cm. We only collected SHI scores just prior to release because young hatchlings might have been susceptible to injury from compression by the micrometer. All other growth metrics and body condition score were taken monthly, after 2 November 2014, until the final measurements were taken at the termination of the study (i.e., 28 May 2015; 207 day study period).

Plasma Chemistry – From 26 May – 2 Jun 2015, we collected blood from 45 randomly selected tortoises from each diet treatment (i.e., 14 from *Control*, 16 from *Sod*, and 15 from *Natural*) just prior to release. We heparinized 25-gauge, 5/8" needles then flushed needles of excess heparin to limit dilution. We then collected 0.25-0.75 mL of whole blood from the subcarapacial venous sinus (Hernandez-Divers *et al.*, 2002), making sure not to collect more than 0.5 mL for every 100 g of tortoise mass (Terry Norton, DVM, Diplomate ACZM; personal communication). We collected blood after tortoises had fasted for \ge 12 hours. We first cleaned the integument around the venipuncture site with a cotton tip swab with rubbing alcohol, ensuring that the swab was not heavily soaked to prevent dripping onto the tortoises head. For each individual, we transferred 0.5-0.75 mL whole blood to a microhematocrit tube, and centrifuged it at 10,000 RPM for five minutes to determine packed cell volume (PCV) visually using a ZIPocrit Reader Card (JorVet[™], Loveland, Colorado, USA). It is difficult to avoid dilution of small blood samples by heparin and the subcarapacial venous sinus is prone to lymph contamination (Barrows *et al.*, 2004). Low PCV values in clinically healthy animals may indicate dilution of the sample. Therefore, if PCV fell below 20%, we excluded the sample from analyses. Otherwise, we centrifuged the remaining blood in 1.5 mL or 2.0 mL microcentrifuge tubes for approximately 3 min at 6000 RPM. From the separated sample, we pipetted off the plasma, which we stored at -60 °C for up to three months. To analyze further plasma chemistry values, we thawed plasma samples and placed 100 µL into a VetScan® Avian Reptilian Profile Plus rotor which we analyzed with a VetScan® VS2 (Abaxis, Inc., Union City, California, USA). Rotors evaluated plasma chemistry values for Aspartate Aminotransferase (AST), Creatine Kinsase (CK), Uric Acid (UA), Glucose (Glu), Calcium (Ca), Phosphorous (P), Potassium (K+), Sodium (NA+), and Total Protein (i.e., the amount of albumin and globulin in the plasma; TP).

Because there are no plasma chemistry reference values for wild one-year old juvenile gopher tortoises, we compared values from tortoises head-started at SREL (*SREL-HS*; 5.8 – 8.3 cm CL) to values obtained from: 1) similar-aged gopher tortoises head-started at SCI (*SCI-HS*) but not part of the diet study (*SCI-HS*; 7.4 – 9.7 cm CL); 2) wild juvenile gopher tortoises from SCI (*Wild Juv*; 11.6 – 23.7 cm CL; Terry Norton, unpublished data); and 3) wild adult gopher tortoises from SCI (*Wild Adult*; 23.9 – 34.7 cm CL; Terry Norton, unpublished data). Head-started tortoise plasma chemistry values from SCI were obtained using the same protocol as head-started tortoises at SREL. However, total protein values from all wild tortoises from SCI were measured by refractometer and chemistry profiles were performed on plasma samples using standard dry-slide determinations with a Kodak 700XRTM chemical analyzer by the Department of Pathology, University of Miami (Miami, Florida, USA; Terry Norton, personal communication). Head-start tortoises from SCI were reared on two large sand tables with infrared lights, cardboard hide structures, and native sands from the island for substrate. Head-starts from SCI were fed
ad libitum six days a week with spring mix and other leafy vegetables similar to those in our *Control* diet treatment (Table 2.1) along with rehydrated Zoo Med[®] Grassland Tortoise Diet. Head-start tortoises from SCI were also soaked three times per week and brought outside on warm days above 22°C.

Statistical Analysis

We used a multivariate analysis of variance (MANOVA) to compare initial mass, CL, SV, and BCI of each diet treatment group to detect any differences in initial mass and size prior to initiation of the diet study. We calculated the daily rate of change of all four variables by subtracting final values collected on 28 May 2015 from initial values collected on 02 November 2014 and divided by the number of days between these two dates (207 d). We used a MANOVA to compare average daily rates of change among treatments. We compared final SHI among treatment groups using a one-way ANOVA. We compared mean CL rate of change among months for tortoises head-started at SREL using a one-way ANOVA. We compared days spent in captivity, initial mass, final mass and rate of change of mass between SREL and SCI head-starts using Student's T-tests. We tested all metrics for normality using a Shapiro-Wilk's test. If data were not normal, we natural log-transformed them if it allowed us to meet the assumption of normality.

We compared plasma chemistry metrics among diet treatment groups using one-way analysis of variance (ANOVA) for each metric. We also compared plasma chemistry metrics among head-start tortoises and wild juveniles and wild adults. For all statistical comparisons between head-starts and wild tortoises, we used one-way ANOVAs when assumptions of normality could be met. If normality could not be met even after transforming the data, we used non-parametric Kruskal-Wallace tests to compare plasma chemistry values. To prevent an increase in our type I error rate by running multiple univariate tests instead of one multivariate test, we used a Bonferroni correction of our critical alpha value, dividing 0.05 by the number of tests (n=10) for a critical alpha value of 0.005 (Bonferroni, 1935; Shaffer, 1995). However, because plasma chemistry collection between head-started and wild tortoises was not part of

a controlled study, we also evaluated whether or not there was overlap in data ranges for plasma chemistry values that were significantly different. While not a robust test, comparing data range overlap enabled us to assess what values may have been the most biologically significant.

The total protein minimum detection limit on the VetScan[®] rotors was relatively high (i.e., 2.0 g/dL). As a conservative approach, any TP values that could not be determined because they fell below 2.0 were adjusted to half the minimum detection limit (Antweiler and Taylor, 2008). All growth and plasma chemistry data were recorded as means ± 1 SE. We analyzed all statistical tests using Program R (Version 3.1.0; R Development Core Team 2014). All methods followed protocols approved by the University of Georgia Institutional Animal Care and Use Committee (# A2014 08-006-Y1-A0), GA Department of Natural Resources (29-WJH-14-93), and GA state parks permit #172014.

Results

We collected 157 eggs from all three field sites (see Quinn et al. 2016 for further details): 49 from SCI (6 clutches), 63 from RBSP (9 clutches) and 45 from YWMA (7 clutches). Of these, 144 hatched (91.7% overall hatch success). Forty-four tortoises hatched and were head-started on SCI (89.8% hatch success). Fifty-eight tortoises hatched from RBSP (92.1% hatch success) and 42 hatched from YWMA (93.3% hatch success) were head-started at SREL and were the primary focus of this study. One clutch only contained two hatchlings and was not included in the diet study in order to ensure that each clutch was represented in each of the three treatments. Therefore, we used only 96 tortoises in total for experimental trials (n = 32 tortoises per treatment). Tortoises at SREL and SCI were head-started for an average of 275.7 \pm 0.9 days and 275.7 \pm 0.7 days in captivity before we released them on 5 June 2015 at YWMA for a population augmentation study (See Ch. 3). Days spent in captivity was not significantly different between the head-starting sites (t = 0.08, df =1, P = 0.93). Survivorship in captivity was 100% for both SREL and SCI.

Tortoises reared at SREL had a mean hatching mass of 32.4 ± 0.5 g, CL of 49.9 ± 0.3 mm, shell volume of 30.1 ± 0.5 cm³, and BCI of 0.57 ± 0.004 g/cm³. We recorded "initial" size metrics (not to be confused with hatching size metrics taken just after hatching) of all tortoises from SREL at the onset of the diet study (i.e., 2-Nov-14). Tortoises used in the diet supplement study had a mean initial mass of 36.9 ± 0.6 g, CL of 52.1 ± 0.3 mm, shell volume of 32.7 ± 0.6 cm³, and BCl of 0.59 ± 0.004 g/cm³. Initial size was significantly different among clutches (Wilk's λ = 0.16, F_{14,79} = 3.14, P < 0.001) but not treatment groups (Wilk's λ = 0.97, F_{2, 79} = 0.34, P = 0.95), suggesting that while tortoise size was dependent on their relation to one another, there was no significant size difference among tortoises selected for each treatment group. Tortoises at SREL had a final mean mass of 88.1 ± 1.7 g, CL of 72.2 ± 0.5 mm, shell volume of 79.8 \pm 1.5 cm³, and BCI of 0.58 \pm 0.003 g/cm³ (Figure 2.3). Mean SHI for all tortoises at termination of the study was 61.0 ± 1.3 . Final shell hardness was significantly different among diet supplement treatments ($F_{2,79}$ = 8.75, P < 0.001), with Sod (55.0 ± 2.6) significantly lower than Control $(65.2 \pm 1.7; P < 0.001)$ and *Natural* $(62.9 \pm 2.1; P = 0.007)$ treatments (Figure 2.4). Shell hardness also differed significantly among clutches ($F_{14,79} = 4.15 P < 0.001$). There was a significant difference in daily growth rate (Table 2.2) among treatments (Wilk's λ = 0.70, F_{2,79} = 3.44, P < 0.001; Table 2.2). We analyzed the univariate daily growth metrics separately using ANOVAs. However, no single growth metric was significant (all P > 0.05) among treatments suggesting that some combination of metrics may have been significant. For both initial metrics and rate of change MANOVAs, clutch (i.e., blocking factor) was significant (P < 0.001 for both), suggesting an effect of clutch on initial size and change in size over time.

Supplemental Diet and Plasma Chemistry

With an unadjusted P-value, there was a significant difference among diet treatment groups and glucose ($F_{2,29} = 3.79$, P = 0.04), total protein ($F_{2,26} = 5.12$, P = 0.01), and phosphorus ($F_{2,26} = 6.06$ P =

0.007; Table 2.3). However, neither diet treatment nor clutch had a significant effect on plasma chemistry values with our adjusted alpha value of 0.005, although phosphorous was nearly significant at P = 0.007. Phosphorus was highest in the *Natural* treatment (3.55 ± 0.1 mg/dL), compared to *Sod* (3.31 ± 0.09 mg/dL) and *Control* (3.14 ± 0.1 mg/dL). A post-hoc Tukey HSD analysis revealed that the *Natural* diet was significantly different from the *Control* (P = 0.004) but not *Sod* (P = 0.1). Our blocking factor (i.e., clutch) was not significant for any plasma chemistry parameter (P > 0.005; Bonferroni correction), suggesting that parental effects did not have a significant effect on any of the plasma chemistry parameters we tested. Total protein was under the detection limit (i.e., < 2.0 g/dL) for six of 14 *Control* (42.9%), 10 of 16 *Sod* (62.5%), and three of 15 *Natural* (20%).

SREL Head-start Growth

Because we found no significantly different growth metrics (except final shell hardness) among diet supplements, we pooled all other growth metrics from the SREL diet study beginning with the first measurements taken just after hatching and final measurements collected on 28 May 2015. We also added the two tortoises from SREL that were not included in the diet experiment study. These two tortoises received the same diet treatment as our *Control* animals. To adjust for the time spent in captivity due to different hatching dates, we report metrics as mean change per day (d) \pm 1 SE. After spending an average of 267.8 \pm 0.9 days in captivity at SREL, tortoises reached a mean CL of 72.3 \pm 0.5 mm (0.08 \pm 0.002 mm/d), mean mass of 88.6 \pm 1.7 g (0.21 \pm 0.005 g/d), mean SV of 80.1 \pm 1.5 cm³ (0.18 \pm 0.004 cm³/d), and BCI of 0.58 \pm 0.0 g/cm³. Although mean BCI increased from hatching to the end of the head-starting period, the mean change was only 0.01 g/cm³ and daily rates were too low to be reported. Mean CL growth rate varied significantly among months (F_{6, 678} = 152.3, P < 0.001). A post-hoc Tukey HSD test showed that growth during February, April, and May were the same, but that all other months had significantly different growth rates (*P* < 0.05; Figure 2.5). From lowest to highest, the mean daily growth rates was 0.004 \pm 0.004 mm/d in November (mean of 0.4 mm in 29 days), 0.03 \pm 0.003

mm/d in December (mean of 1.3 mm in 34 days), 0.07 ± 0.007 in January (mean of 2.1 mm in 28 days), 0.13 \pm 0.004 mm/day in May (mean of 3.3 mm in 24 days), 0.13 ± 0.008 mm/day in February (mean of 3.9 mm in 28 days), 0.15 ± 0.003 mm/day in April (mean of 5.0 mm in 32 days) and 0.19 ± 0.01 mm/d in March (mean of 6.03 mm in 31 days).

SREL and SCI Growth Comparison

Although we did not collect other shell morphometric data for SCI head-start tortoises, we did collect mass data. Initial mass was 33.2 ± 0.5 g for SCI head-starts and 32.4 ± 0.5 g for SREL head-starts, which was not significantly different (t = 0.98, df = 140, *P* = 0.33). However, SCI head-starts had a significantly higher final mass (134.4 ± 4.5 g) than SREL head-starts (88.6 ± 1.7g; t = 11.7, df =140, *P* < 0.001). Because tortoises were in captivity for different lengths of time based on their hatching date, we also adjusted the change in mass by the number of days each individual spent in captivity. Rate of mass increase was significantly greater for SCI head-starts (0.38 ± 0.02 g/d) than for SREL head-starts (0.21 ± 0.005 g/d; t = 12.6, df = 140, *P* < 0.001) indicating that SCI tortoise gained mass more quickly in captivity than SREL tortoises (Figure 2.6).

Plasma Chemistry Comparisons among Tortoise Groups

Because clutch was not a significant factor in our analysis, we combined plasma chemistry data from all SREL head-starts (*SREL-HS*) to compare to SCI head-starts (*SCI-HS*), SCI wild juveniles (*Wild Juv*), and SCI wild adults (*Wild Adult*). Total protein was under the detection limit for 19 of 45 *SREL-HS* (42%), and three of 15 *SCI-HS* (20%). Only Ca and K+ were normally distributed among the populations. We natural-log transformed CK, UA, and GLU to meet the assumption of normality for parametric tests. However, AST, CA, P, NA+, PCV, and TP were not normally distributed even after transforming data, and were therefore analyzed non-parametrically (i.e., Kruskal-Wallace and Dunn's nonparametric comparison post-hoc test).

We found no significant differences among groups from SREL and SCI for Ca, Glu, Na+, and CK (P > 0.005) but we did find significant differences in AST, UA, P, K+, TP, and PCV (P < 0.005; Table 2.4). We conducted post-hoc tests for each plasma parameter to document differences and similarities among populations (Figure 2.7) and report non-transformed values next to p-values. Mean AST values were significantly higher for both Wild Adult ($314.0 \pm 64.0 \text{ U/L}$) and Wild Juv ($276.8 \pm 33.4 \text{ U/L}$) compared to SREL-HS (111.0 ± 4.7 U/L; P < 0.001) and SCI-HS (117.8 ± 7.1 U/L; P < 0.001). Mean natural logtransformed UA values were significantly higher for Wild Adult ($4.1 \pm 0.3 \text{ mg/dL}$) compared to SREL-HS $(1.4 \pm 0.1 \text{ mg/dL}; P < 0.001)$ and SCI-HS $(2.1 \pm 0.2 \text{ mg/dL}; P = 0.001)$. Mean natural log-transformed UA values were also significantly higher for Wild Juv ($2.9 \pm 0.4 \text{ mg/dL}$) compared to SREL-HS (P < 0.001). Mean P values were significantly higher for SCI-HS ($4.3 \pm 0.2 \text{ mg/dL}$) compared to SREL-HS (3.3 ± 0.1 mg/dL; P < 0.001). Mean K+ values were significantly higher for SCI-HS (5.9 ± 0.1 mmol/L) compared to Wild Juv (5.0 \pm 0.2 mmol/L; P < 0.001). Mean TP values were significantly higher for both Wild Adult (4.3 \pm 0.2 g/dL) and Wild Juv (3.2 \pm 0.2 g/dL) compared to SREL-HS (1.7 \pm 0.09 g/dL; P < 0.001) and SCI-HS $(2.0 \pm 0.1 \text{ g/dL}; P < 0.001)$. Mean PCV values were significantly higher for both *Wild Adult* (28.6 ± 1.0 %) and Wild Juv (26.7 \pm 1.2 %) compared to SREL-HS (22.3 \pm 0.5 %; P < 0.001) and SCI-HS (21.4 \pm 0.8 %; P < 0.001). However, because our plasma chemistry values among groups were not part of a controlled study, we also evaluated untransformed data for range overlap (Figure 2.8). The only ranges that did not overlap were for TP of Wild Adult (range: 3-6) with SREL-HS (range: 2-2.8) and SCI-HS (range: 2.1-2.8).

Discussion

Effects of Diet Supplement on Head-start Growth and Plasma Chemistry

Supplemental diet treatments had little effect on head-start growth. Of the five metrics we tested for growth, only final SHI was significantly different among treatments. The *Sod* treatment had a mean SHI 15.6% lower than *Control* and 12.6% lower than *Natural* treatments. However, we are hesitant to suggest

that this disparity is due to actual ingestion of the sod grass because no tortoises were ever witnessed foraging on it. Rather, we suspect the difference was due to the tortoises' inclination to hide underneath the sod mat. By hiding under mats, it's possible that tortoises simply didn't feed as much. However, tortoises in the *Sod* treatment were larger in mass at the end of the study than the *Natural* treatment and had a larger CL on average than both the *Control* and *Natural* treatments. Furthermore, the rate of growth in mass and CL were similar for the *Sod* treatment relative to both *Control* and *Natural* treatments. Another explanation for differences in SHI could be that the sod itself was compressing the shell when tortoises were hiding underneath it. Based on the equation for SHI, we would expect that smaller shell heights with the same ability to resist compression would have a smaller SHI score. Therefore, if the weight of the sod affected shell height, then it is possible that it also affected SHI scores even if the shell was just as resistant to compression as other treatments. Interestingly, we did note that the average shell height was smaller for *Sod* (35.3 mm) compared to *Control* (36.9 mm) and *Natural* (36.2 mm) treatments at the end of the study.

Supplemental diet treatments had no effect on head-start plasma chemistry values which may be due to the fact that the base diet of leafy greens with rehydrated diet pellets made up the bulk of food for all treatment groups. Therefore, regardless of treatment, tortoises may have had relatively similar nutrient intake. Furthermore, not all dietary components were selectively ingested. As previously mentioned, no tortoises were witnessed foraging on grass in the *Sod* treatments. While tortoises in the *Natural* treatment group did forage on some of the vegetation in the natural foraging plots, they were never seen eating any of the grass species. It is surprising that there was no evidence of our head-started tortoises selectively foraging on any grasses. Although ingested at lower rates than adults, free-ranging juvenile tortoises have been documented foraging on grasses (MacDonald and Mushinsky, 1998; Mushinsky *et al.*, 2003). However, the juveniles in these prior studies were older and larger (i.e., 8-15 years old) than the head-starts in this study and may not reflect the exact dietary preferences of

hatchlings and young juveniles. Further exacerbating issues with forage, it was not always possible to grow the *Natural* treatment plots quickly enough to replace old ones. Natural vegetation plots took approximately two months to grow with one month required to cold-stratify seeds and approximately one month of germination for plants to grow large enough to offer substantial forage. Between foraging and trampling, the tortoises would often destroy the plots of natural vegetation within two to three weeks while we were only able to replace plots every three to four weeks. Ultimately, we were unable to replace all plots as quickly as necessary to ensure tortoises had a constant supply of natural forage. The challenges associated with offering the diet supplements we selected and the fact that commercially available greens comprised the bulk of all the diets likely contributed to the similarity in growth and plasma chemistry metrics among treatment groups. Given that our treatment groups showed few differences in growth and plasma chemistry, a basic diet made from suitable commercial produce may be the best option simply because it is logistically simple. Furthermore, none of our tortoises suffered from any clinical signs of stress or illness (i.e., tortoises appeared healthy and behaved normally) prior to release, suggesting that the basic diet is not only logistically simple, but nutritionally viable.

We observed no clutch effects on plasma chemistry values but we did observe clutch effects on both initial size and subsequent growth. Although simple genetic heritability can affect growth in turtles (Steyermark and Spotila, 2001), other factors may have influenced growth of our hatchlings before eggs were found and collected. Incubation environment (e.g., nest temperature, substrate moisture, etc.) can influence hatching size (Gutzke *et al.*, 1987; Brooks *et al.*, 1990). Maternal characteristics such as maternal body size and body condition can also affect egg and hatchling size which has been positively correlated with post-hatching growth rates (Congdon *et al.*, 1983; Brooks *et al.*, 1990; Nafus et al., 2015). Therefore, growth of our head-started tortoises were likely biased by the maternal or environmental effects that each clutch was subject to in the nest before being collected, which is why we chose to use

"clutch" as a blocking factor. However, no studies have been conducted currently that could explain why plasma chemistry values were not significantly different among clutches.

Head-start and wild tortoise comparison

Mass at hatching was not significantly different for *SREL-HS* and *SCI-HS*. However, *SCI-HS* grew faster, gaining an average of 45.8 g (65.9%) more weight than *SREL-HS*. The most likely explanation for increased growth in *SCI-HS* relative to *SREL-HS* is the difference in feeding and husbandry protocols. Whereas we gave *SREL-HS* tortoises their base diet of 40 g of food three to five times a week, *SCI-HS* tortoises were fed a base diet of spring mix and other leafy greens *ad libitum* two to three times a day, six days a week. Furthermore, *SCI-HS* were placed outdoors on warm days, allowing them to bask in natural ultraviolet light which may have helped accelerate growth. Although *SREL-HS* and *SCI-HS* grew at different rates and were raised under different captive conditions, the only plasma chemistry value that was significantly different between them was phosphorous. *SCI-HS* had slightly higher phosphorus levels (mean = 4.3 mg/dL) compared to *SREL-HS* (mean = 3.3 mg/dL). However the difference was not extreme (1.0 mg/dL) and may not be biologically or clinically significant, especially given that normal ranges for reptiles are between 1 and 5 mg/dI (Campbell, 1996). Furthermore, phosphorous is primarily important in relation to calcium (i.e., Ca:P ratio) and it is generally agreed that a Ca:P ratio of at least 2:1 is necessary for normal growth (Kass *et al.*, 1982). Despite differences in phosphorus, both *SCI-HS* and *SREL-HS* had Ca:P ratios above this threshold.

Plasma chemistry was very similar between wild and head-started tortoises. All plasma chemistry ranges overlapped between head-starts and wild juveniles. Only total protein (TP) failed to overlap between wild adult and head-started tortoises. Normal TP values for reptiles typically range between 3-8 g/dL (Campbell, 1996). However, gopher tortoise TP values are usually lower than 5 g/dL (Hernandez *et al.*, 2010; Taylor and Jacobson, 1982) and while a TP value < 2.0 g/dL would be concerning

for an adult gopher tortoise, values less than 2.0 g/dL are not uncommon for young juvenile tortoises (Terry Norton, DVM, Diplomate ACZM; personal communication). Although wild tortoises fell well within the normal range, 19 head-started tortoises (34%) from SCI and SREL fell below 2.0 g/dL threshold. Low total protein (i.e., hypoproteinemia) may be associated with malnutrition, malabsorption, maldigestion, protein losing enteropathies, blood loss, and chronic hepatic or renal disease (Campbell, 1996). Prior to release, all head-starts were clinically healthy, suggesting that chronic disease was not responsible for low TP values. While nutrition could play a role in low TP values, even some tortoises (20%) in the *Natural* treatment group had values below 2.0 g/dL. Furthermore, other plasma chemistry metrics did not indicate nutrition deficiencies for our head-starts. Like PCV, the most likely explanation for low TP values in clinically healthy animals may be dilution of our relatively small blood samples by either the heparin used to prevent blood clotting in the syringe or lymph contamination which the subcarapacial venous sinus is prone to (Barrows *et al.*, 2004). One other possible explanation for low TP values could be that the head-starts, held in captivity, were not inoculated with the hind-gut microbial community necessary for breaking down cellulose efficiently (Campbell, 1996; Lance and Morafka, 2001), decreasing the amount of nutrients that could be absorbed.

Although we statistically analyzed all plasma chemistry values among populations, the biological significance of any differences is circumstantial. Wild tortoise plasma chemistry values were analyzed from plasma collected from different sexes, during different years (i.e., 2010 through 2012), and during different times of the active season (i.e., May through August); all of which could have influenced plasma chemistry results (Dickinson *et al.*, 2002; Taylor and Jacobson, 1982). Additionally, *Wild Juv* (avg. mass = 1.07 kg; T. Norton, unpublished data) were much larger than our head-started juveniles (avg. mass = 0.203 kg). The potential confounding issues surrounding plasma chemistry values from wild, older tortoises may mean that they are inappropriate references for one-year old juveniles. However, these are the closest values we can achieve for comparison at the present time because no other published

reports have evaluated wild juvenile gopher tortoise plasma chemistry prior to this study. While it was difficult to statistically compare between wild and head-started tortoises, it may be beneficial to examine the values that were significantly different.

Elevated aspartate aminotransferase (AST) can help indicate hepatic and muscle tissue damage, especially if values are over 250 U/L (Campbell, 1996). Whereas all head-started tortoises were under 250 U/L (mean = 113.4 U/L), Wild Juv (mean = 276.6 U/L) and Wild Adult (mean = 314.0 U/L) were not. The elevated AST in wild tortoises may be due to physical stress or injuries from living in the wild. While elevated AST values could indicate some illness in these wild tortoises (Wilkinson, 2004), handling could also increase these values and shouldn't be cause for concern without a subsequent increase in creatine kinase (Terry Norton, DVM, Diplomate ACZM; personal communication) which was not detected. Uric acid (UA) was also higher for wild tortoises compared to head-starts. However, normal UA values fall between 0-10 mg/dl (Campbell, 1996) which all head-started and wild tortoises fell within. The differences may simply be due to dietary differences in urea and protein ingestion from different diets. Potassium (K+) values vary depending on the species but generally fall between 2-8 mmol/L for reptiles (Campbell, 1996) and have been documented between 2.9-7.0 mmol/L in wild gopher tortoises (Hernandez et al., 2010; Taylor and Jacobson, 1982). Again, neither wild nor head-started tortoises in our study fell outside this range. Differences in potassium may simply be due to minute differences in nutrition because of diets. Packed cell volume (PCV) was lower for SCI-HS compared to Wild Juv and Wild Adult as well as SREL-HS compared to Wild Adult. The normal range for most reptiles is between 20-40% (Campbell, 1996). Although all head-start and wild tortoise PCV averages fell within the normal ranges reported for gopher tortoises, eight Wild Adult and two Wild Juv tortoises were above 30% PCV. One Wild Juv was at 44% PCV and was likely dehydrated (Terry Norton, DVM, Diplomate ACZM; personal communication). Packed cell volume is a good indicator of hydration, and slightly high values may not be cause for alarm. Similar to TP, lower PCV in head-starts may be due to naturally low values in juveniles

along with diluted samples from heparin and lymph. Furthermore, it seems likely that head-started tortoises, soaked in water three days a week, may have also had lower PCV values because they were more hydrated than their wild counterparts that rely on rainfall and food for the majority of their water.

Conclusions

Multiple conservation agencies have used head-starting as a tool to augment depleted populations of turtles, the success of which depends on ensuring head-start health during captivity. While some environmental variables can be readily replicated to mimic natural conditions in captivity (i.e., water availability, temperature, humidity, etc.), natural diets are often impractical to replicate, especially for herbivorous species. Because a natural diet can be challenging to replicate in captivity, and because diet can affect the health and growth of head-started animals, studying the effects of captive diet is especially important. None of the experimental diets in our study strongly affected growth metrics or plasma chemistry parameters. Although other dietary treatments are open to evaluation, logistically simple diets like the Control diet of leafy vegetables sold commercially, can offer similar nutritional value relative to more logistically complicated diets. Finally, while some plasma parameters were different between wild and head-started tortoises, plasma chemistry values of head-started tortoises largely overlapped with wild gopher tortoises or fell within normal ranges, suggesting that short-term head-starting under the protocol used in our study did not result in substantial nutritional deficiencies. However, increases in some natural forage (i.e., legumes and forbs) and inoculation with natural hind-gut bacteria may be beneficial for growth in future head-starting efforts. However, future head-starting programs should be cognizant that longer captivity time periods may also entail different nutritional and dietary requirements.

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Table 2.1. Experimental diet treatments (*Control, Sod, and Natural*) for gopher tortoises (*Gopherus polyphemus*) and their constituents including family, genus, species and common name. All tortoises received the *Control* diet as a base. Tortoises selected for *Sod* and *Natural* diets were given the following vegetation as a supplement to the *Control* base.

Diet	Family	Scientific Name	Common Name	
Control	Brassicaceae	Brassica rapa	Turnip Greens	
	Brassicaceae	Brassica juncea	Mustard Greens	
	Brassicaceae	Brassica oleracea	Kale	
	Asteraceae	Lactuca sativa	Romaine Lettuce	
	Asteraceae	Lactuca sativa	Green Leaf Lettuce	
Sod	Poaceae	Cynodon dactylon	Bermuda grass	
(+Control)	Poaceae	Eremochloa ophiuroides	Centipede grass	
	Fabaceae	Mimosa nuttaillii	Sensitive Briar	
	Poaceae	Sorghastrum nutans	Indian Grass	
	Asteraceae	Rudbeckia hirta	Blackeyed Susan	
	Poaceae	Schizachyrium scoparium	Little Bluestem	
Natural (+Control)	Fabaceae	Chamaecrista fasciculate	Partridge Pea	
(+Control)	Fabaceae	Tephrosia virginiana	Goats Rue	
	Poaceae	Muhlenbergia	Muhly Grass	
	Asteraceae	Pityopsis graminifolia	Narrowleaf Silkgrass	
	Asteraceae	Liatris sp.	Blazing Star	

Table 2.2. Growth metrics among diet treatments for gopher tortoises (*Gopherus polyphemus*) head-started at the Savannah River Ecology Laboratory, Aiken, SC. Comparisons were made between the initial measurements (day 0 when study first began), final measurements (day 207 when study ended), and rate of change (change from initial to final, divided by 207 days). Mass, midline carapace length (CL), shell volume (SV), body condition index (BCI), and shell hardness (SHI) are reported (mean ± 1 SE). Shell hardness was only recorded at the end of the study.

	Diet Supplement	Mass (g)	CL (mm)	SV (cm ³)	BCI (g/cm ³)	SHI
Initial (02 Nov 2014)	Control	37.3 ± 1.1	52.4 ± 0.5	33.1 ± 1.0	0.59 ± 0.01	NA
	Sod	36.8 ± 1.1	51.9 ± 0.6	32.2 ± 1.0	0.60 ± 0.01	NA
	Natural	36.8 ± 1.0	52.1 ± 0.5	32.8 ± 0.9	0.59 ± 0.01	NA
Final (28 May 2015)	Control	90.4 ± 2.9	72.3 ± 0.9	81.7 ± 2.7	0.58 ± 0.00	65.2 ± 1.7
	Sod	87.3 ± 3.0	73.0 ± 0.9	79.0 ± 2.5	0.58 ± 0.00	55.0 ± 2.6
	Natural	86.5 ± 2.8	71.3 ± 0.9	78.6 ± 2.5	0.58 ± 0.00	62.9 ± 2.1
Rate (Unit per day)	Control	0.26 ± 0.01	0.10 ± 0.00	0.23 ± 0.01^{a}	-0.01 ± 0.01	NA
	Sod	0.24 ± 0.01	0.10 ± 0.00	0.23 ± 0.01 ^a	-0.02 ± 0.01	NA
	Natural	0.24 ± 0.01	0.09 ± 0.00	0.22 ± 0.01 ^a	-0.01 ± 0.01	NA

Table 2.3. Mean plasma chemistry values among diet treatments (*Control, Sod, and Natural*) of head-started gopher tortoises (*Gopherus polyphemus*) reared at the Savannah River Ecology Laboratory, Aiken, SC. Mean values represent untransformed data, but statistical analyses were conducted on natural log-transformed data where indicated. Comparisons were made among treatments using clutch as a blocking factor. We used univariate one-way ANOVAs to compare aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), glucose (GLU), calcium (Ca), phosphorous (P), sodium (Na+), potassium (K+), packed cell volume (PCV), and total protein (TP). To account for inflated Type I error rates, we adjusted our alpha value by dividing 0.05 by the number of tests (n = 10) to obtain an adjusted alpha value of 0.005.

Plasma Chemistry	Mean Values			Block (Clutch)		Treatment	
·	Control	Sod	Natural	Test Statistic (df)	P-Value	Test Statistic (df)	P-Value
AST (U/L)*	101.1	119.4	111.5	1.91 (13,26)	0.07	1.64 _(2,26)	0.21
CK (U/L)*	521.2	1054.6	667.1	1.10 (13,26)	0.41	0.33 (2,26)	0.72
UA (mg/dL)*	1.5	1.3	1.5	1.17 (13,26)	0.36	0.47 (2,29)	0.63
GLU (mg/dL)	87.2	95.8	103.4	0.30 (13,26)	0.99	3.79 _(2,29)	0.04
Ca (mg/dL)	11.0	11.2	11.3	1.34 _(13,25)	0.25	0.24 (2,25)	0.80
P (mg/dL)	3.1	3.3	3.6	2.41 _(13,26)	0.03	6.06 (2,26)	0.007
Na+ (mmol/L)**	134.5	136.1	134.1	1.08 (13,26)	0.42	0.56 (2,26)	0.58
K+ (mmol/L)	5.4	5.7	5.7	1.61 (13,26)	0.15	1.40 (2,26)	0.26
PCV %	23.4	22.8	22.9	1.46 (13,22)	0.21	0.15 (2,22)	0.86
TP (g/dL)**	2.6	1.4	2.1	1.03 (13,26)	0.45	5.12 _(2,26)	0.01

* Data were natural log-transformed to meet assumption of normality

**Transformations did not produce normality

Table 2.4. Mean plasma chemistry values for head-started and wild gopher tortoises (*Gopherus polyphemus*): (1) Savannah River Ecology Laboratory head-starts (*SREL-HS*), (2) St. Catherines Island head-starts (*SCI-HS*), (3) wild juveniles (*Wild Juv*) from St. Catherines Island, and (4) wild adults (*Wild Adult*) from St. Catherines Island. Mean values are presented as untransformed data but statistical analyses were conducted on natural log- transformed data where indicated. We used ANOVAs and non-parametric Kruskal-Wallis tests when data could not be normalized by transforming. We compared aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), glucose (GLU), calcium (Ca), phosphorous (P), sodium (Na+), potassium (K+), packed cell volume (PCV), and total protein (TP) among populations. To account for inflated Type I error rates, we adjusted our alpha value by dividing 0.05 by the number of tests (n = 10) to obtain a new alpha value of 0.005. Significant comparisons are indicated in bold.

Plasma Chemistry	Mean Values				Analysis		
	SREL-HS	SCI-HS	Wild Juv.	Wild Adult	Test	Test Statistic (df)	P-Value
AST (U/L)	113.4	118.5	276.8	314.0	Kruskal-Wallis	55.5 ₍₃₎	< 0.001
CK (U/L)*	719.6	574.9	1305.1	968.8	One-Way ANOVA	0.38 (3,93)	0.77
UA (mg/dL)*	1.6	2.1	2.9	4.1	One-Way ANOVA	26.4 _(3,92)	< 0.001
GLU (mg/dL)*	95.9	95.0	105.6	108.9	One-Way ANOVA	1.80 (3,93)	0.15
Ca (mg/dL)	11.3	11.8	12.2	13.5	Kruskal-Wallis	9.70 ₍₃₎	0.02
P (mg/dL)	3.6	4.3	3.5	5.0	Kruskal-Wallis	18.4 ₍₃₎	< 0.001
Na+ (mmol/L)	135.4	136.6	137.0	137.7	Kruskal-Wallis	3.64 ₍₃₎	0.30
K+ (mmol/L)	5.7	5.9	5.0	5.2	One-Way ANOVA	7.1 _(3,93)	< 0.001
PCV %	23.0	21.5	26.7	28.7	Kruskal-Wallis	33.2 ₍₃₎	< 0.001
TP (g/dL)	1.8	2.1	3.2	4.4	Kruskal-Wallis	66.2 ₍₃₎	< 0.001

* Data were natural log transformed to meet assumption of normality



Figure 2.1. Gopher tortoise (*Gopherus polyphemus*) head-start rearing bins in one of three stalls in the animal holding facility at the University of Georgia's Savannah River Ecology Laboratory, Aiken, SC.



Figure 2.2. Gopher tortoise (*Gopherus polyphemus*) supplemental diet treatments at the University of Georgia's Savannah River Ecology Lab, Aiken, SC: (A) *Control* diet consisting of commercially-available leafy vegetables and Zoo Med Grassland Tortoise Diet. The diet plate was considered a base diet and was fed to all treatments, with the *Control* only receiving this diet plate; (B) Tub with *Sod* supplement; (C) Tub with *Natural* diet supplement plots.



Figure 2.3. Mean monthly gopher tortoise (*Gopherus polyphemus*) morphometric comparisons among diet treatments for A) mass (g); B) midline carapace length (mm); C) shell volume (cm³); and D) body condition (g/cm³). Data collection began on 02 November 2014 and was taken again on or near the first of each month until the final date, 207 days later on 28 May 2015. Each month represents data taken on or near the first of the month.



Figure 2.4. Mean shell hardness index (where a shell hardness of 100 = completely hard) among gopher tortoises (*Gopherus polyphemus*) fed different diet supplements. Shell hardness measurements were taken at the end of head-starting just prior to release on 28 May 2015.



Figure 2.5. Mean daily rate of change in straight carapace length (CL) per month for gopher tortoises (*Gopherus polyphemus*) head-started at the Savannah River Ecology Laboratory, Aiken, SC (Nov 2014-May 2015). Bars indicate 95% CI and letters designate significantly different values at alpha = 0.05.



Figure 2.6. Mean mass change per day from hatching in Fall 2014 to final measurement on 28 May 2015 between head-started gopher tortoises (*Gopherus polyphemus*) reared at the Savannah River Ecology Laboratory (*SREL-HS*) and St. Catherines Island (*SCI-HS*).



Figure 2.7. Comparisons of significantly different plasma chemistry values among head-started and wild gopher tortoises (*Gopherus polyphemus*): Head-starts at the Savannah River Ecology Laboratory head-starts (SREL-HS) and St. Catherines Island (SCI-HS), and wild juveniles (Wild Juv) and adults (Wild Adult) from St. Catherines Island. Bars indicate 99.5% CI and letters designate significantly different values at alpha = 0.005.



Figure 2.8. Boxplots of significant plasma chemistry values collected from head-started gopher tortoises (*Gopherus polyphemus*) at the Savannah River Ecology Laboratory (SREL-HS) and St. Catherines Island (SCI-HS) and Wild juveniles (Wild Juv) and wild adult tortoises (Wild Adult) from St. Catherines Island.

CHAPTER 3

POST-RELEASE MOVEMENT AND SURVIVORSHIP OF HEAD-STARTED GOPHER TORTOISES (GOPHERUS POLYPHEMUS)

INTRODUCTION

Habitat degradation is the leading cause of wildlife population declines world-wide (Groombridge 1992, Alford and Richards 1999), and recovery of critical habitat and mitigation of anthropogenic threats is the key to preventing extirpation and extinction of native species (Fahrig 1997). However, once land is obtained and restored, populations may be too depleted to recover naturally. Depleted populations are more vulnerable to stochastic episodes of mortality if they are not able to recover quickly or have not produced the number of individuals necessary to buffer the population. Land managers may need to consider manipulative conservation strategies that augment depleted populations by increasing the number of individuals in order to reach a viable size (Philippart 1995). Presently, 42% of all extant turtle species are listed as threatened according to the IUCN red list criteria (Baillie et al. 2004). In general, turtles have long life spans, are late-to-mature, and have innately low offspring survival (Congdon et al. 1993) that may make augmentation strategies particularly useful for ensuring the persistence of populations. The gopher tortoise (*Gopherus polyphemus*) is an ideal candidate for studying effects of augmenting populations because it is one of the most frequently used species for translocation and reintroduction programs (Seigel and Dodd 2000).

The gopher tortoise is the only tortoise species native to the southeastern United States, residing primarily in well-drained sandy soils of the coastal plain and sandhills. Tortoises are ecosystem engineers, constructing extensive burrows in sandy soils that act as refuge for the tortoises themselves

but also hundreds of other species (Jackson and Milstrey 1989). Gopher tortoises occupy long-leaf pine savannahs and coastal dunes, characterized by open canopies, which allows sunlight to reach the understory. However, habitat degradation caused largely by land development and fire suppression (i.e., leading to canopy closure), has led to displacement or extirpation of local populations (Auffenberg and Franz 1982). Already the longleaf pine (*Pinus palustris*) ecosystem, where many tortoise populations naturally occur, has disappeared by upwards of 98% of its historic range (Noss et al. 1995). Consequently, tortoise populations are declining throughout their range as well (Auffenberg and Franz 1982). They are now federally-listed threatened west of the Tombigbee and Mobile rivers in Alabama and are being considered for federal listing in the remainder of their range in the east (USFWS 2013).

In order to aid management and conservation efforts for tortoises, The Gopher Tortoise Council published a report that defined the minimum viable population (MVP) per reserve at 250 adult individuals (Gopher Tortoise Council 2013, 2014). The report was based on consensus from species experts as well as previously developed population viability models that collectively examined the influence of parameters such as environmental conditions, age- or class-specific mortality, initial population size, and disease-associated mortality (Abercrombie 1981; Cox et al. 1987; Miller 2001; Root and Barnes 2006; Tuberville et al. 2009). Many populations throughout the tortoise's range do not reach this 250 adult benchmark (Smith et al. 2009; GA DNR, unpublished data). Even with anthropogenic threats completely mitigated, these depleted populations are considered non-sustainable without augmentation.

Several studies have evaluated the use of translocated adult gopher tortoises displaced by development for augmenting populations (Heise and Epperson 2005; Tuberville et al. 2005; Ashton and Burke 2007), but the sporadic availability of adult tortoises for translocation makes them an unreliable source for planned management efforts. Hatchling tortoises, while more reliable for planned efforts, have a low annual survivorship—estimated at 12.8% according to a meta-analysis conducted by Perez-

Heydrich et al. (2012). Recent findings suggest that, provided adult survivorship is high, juvenile life stages should be the next focus of management efforts (Miller 2001; Pike et al. 2008; Tuberville et al. 2009). Head-starting - the practice of protecting especially vulnerable life stages of a species to increase the likelihood of survivorship for conservation purposes (Burke 2015) - may offer a suitable alternative to translocations of adults or hatchlings. Because head-starts are released at a later age, they should be larger in size and have a harder shell, both of which presumably reduce their vulnerability to predation compared to hatchlings (Heppell et al. 1996, O'Brien et al. 2005). Furthermore, head-starting tortoises in controlled environmental conditions allows them to continue activity, growing during the times when they would otherwise be dormant, and resulting in larger sizes compared to same-aged wild conspecifics. However, head-started tortoises released to augment populations need to be monitored and evaluated to determine their potential as a recovery tool (Snyder et al. 1996, Seigel and Dodd 2000). Although long-term monitoring is necessary for determining population recovery, short-term monitoring can determine if future head-starting efforts may be worth conducting. Specifically, post-release survivorship and movement are the two most immediate factors that can be evaluated initially after release. If released animals do not survive or if they move too far from the release site or off the managed land entirely, they will then be unable contribute to recovery of the population.

Although some studies have been conducted on head-starting in other turtle species (e.g., *Emys orbicularis*, Mitrus 2005; *Actinemys marmorata*, Haegen et al. 2009; *Gopherus agassizii*, Nagy et al. 2015; *Emydoidea blandingii*, Buhlmann et al. 2015), only Tuberville et al. (2015) has monitored head-started gopher tortoises post-release. Their study demonstrated that tortoises could be head-started successfully in captivity but that survivorship varied among cohorts (3.1% -100% survivorship). However, this study was not designed to test the effectiveness of head-starting as a management tool, necessitating future research on head-start efficacy for gopher tortoises.

In order to adequately assess head-starting as a potential management tool, we radio-tracked tortoises released into a depleted population at a managed site in Georgia, USA. Specifically, we conducted a study of head-start tortoise movement and survival post-release. Our goals were to: 1) document post-release movement and survival of two cohorts of head-started tortoises and 2) compare movement and survivorship between head-started tortoises and hatchlings.

Study Area

The Yuchi Wildlife Management Area (YWMA) was the recipient site for our head-started gopher tortoises. Yuchi WMA is a 3127 hectare tract of land just south of the Georgia piedmont Fall Line on the Upper Coastal Plain and near the northeastern edge of the gopher tortoise's range. YWMA is predominately composed of upland pine and pine/scrub oak mixtures, with several creek bottoms and wetlands adjacent to the Savannah River. The upland soils are well-drained sandy soils (i.e., Lakeland, Troup, Bonifay, Orangeburg, and Lucy), grading into poorly-drained flood plains (i.e., Osier, Chastain, and Shell Bluff). YWMA is currently owned and managed by the Georgia Department of Natural Resources (GA DNR), but was formerly private lands largely used for timber harvest prior to purchase by the state in 1988. Longleaf pine restoration has been and is still one of the main priorities at YWMA. Of the 3127 ha, approximately half are currently considered suitable habitat for gopher tortoises (Smith et al. 2009). Despite the large amount of suitable habitat, Smith et al. (2009) found few native tortoises while conducting line transect surveys. To date, only 27 native tortoises (89% adult, 4% subadult, 7% juvenile) have been counted during line transect surveys resulting in an estimate of only 44 native tortoises (GA DNR, unpublished data). Although not known with certainty, the reason for the low population density of this site was likely poor silviculture practices and tortoise harvest by the local public prior to purchase by the state (J. Jensen, GA DNR, pers. comm.). Once the state began protecting the land and restoring habitat, it was presumed that historic threats to the tortoise population were largely mitigated. The large amount of seemingly suitable habitat and absence of a viable population

makes the site an ideal candidate for examining the conservation potential of population augmentation. As part of a separate study conducted by the Orianne Society in collaboration with GA DNR, 18 translocated adult tortoises were released at YWMA in 2012 (Bauder et al. 2014) with an additional 19 released in 2013 (J. Jensen, GA DNR, pers. comm.). These releases brought the final population count to an estimated 81 adult and sub-adult tortoises with no reported accounts of hatchlings or young juveniles. The YWMA was subsequently chosen by GA DNR as a suitable recipient site to help assess the effectiveness of using head-started juveniles to augment populations on managed lands.

METHODS

Hatchlings and Pre-release Husbandry

We collected all hatchlings as eggs from the following populations in Georgia: St. Catherines Island (SCI) in Liberty County (see Tuberville et al. 2008 for detailed site description), Reed Bingham State Park (RBSP) in Cook County, and YWMA in Burke County (see Bauder et al. 2014). SCI is an ideal donor site because it supports a robust and non-native population that has been the focus of long-term monitoring to evaluate translocation as a management tool (Tuberville et al. 2008). RBSP supports a robust population having one of the highest densities of tortoises in Georgia (i.e., >3 tortoises/ha; Ballou 2013). For a detailed description of egg collection methods and hatching success, see Quinn et al. (2016). We head-started tortoises for 9-12 months. We felt this time period was sufficient for tortoises to grow to large enough sizes to affect survivorship (Buhlmann et al. 2015) while also ensuring that tortoises had sufficient time during warm months for constructing burrows and acquiring resources prior to winter dormancy. Tortoises hatched on SCI were head-started by SCI staff and fed *ad libitum* six days a week and soaked three times a week. We head-started tortoises hatched from eggs collected at RBSP and YWMA at the University of Georgia's Savannah River Ecology Laboratory (SREL; Aiken Co., SC) and fed them 3-5 times a week and soaked them three times a week (See Ch. 2 for husbandry details at SREL). Prior to release, we marked all tortoises in all treatments by notching a unique combination of marginal scutes (Ernst et al. 1974). We used dial calipers (Mitutoyo, Aurora, Illinois, USA) to measure midline carapace length (CL) and a DeltaRange[®] Mettler PE 3600 gram scale (Mettler Toledo, Columbus, Ohio, USA) to measure mass to the nearest 0.1 g of all tortoises prior to release. We calculated all morphometrics as mean ± 1 SE.

Release Areas

We selected two release areas centrally located within the YWMA to reduce the likelihood of tortoises wandering off the property. The release areas were located on either side of a sandy gravel road within YWMA (Figure 3.1) and were separated by 275 m between their center points. The northwest release area was adjacent to where translocated adult tortoises were released by Georgia DNR and the Orianne Society. The two release areas are in separate but adjacent management compartments that are similar in structure, with a sparse open canopy, absent mid-story and diverse understory, making them well suited for gopher tortoises (Aresco and Guyer 1999; Landers and Speake 1980; Nussear and Tuberville 2014). Georgia DNR managers partially cleared and burned the southeast release area in the winter/spring of 2013, but some older growth trees remain. In the late 2000s, land managers clearcut the northwestern release area and planted longleaf pine, which is now the dominant tree species.

Release Treatments

We conducted three releases of gopher tortoises to evaluate post-release movement and survivorship (Table 3.1). The release groups were as follows:

Release 1: (N=12), Represents tortoises from the 2013 cohort that we head-started for approximately 9 months at SCI and released at YWMA in summer 2014. We attached radio-transmitters (Advanced Telemetry Systems Model R1680, 3.6 g) to all 12 tortoises on the fourth vertebral scute using

WaterWeld epoxy (J-B Weld[®], Sulphur Springs, TX, USA). We released tortoises into two soft-release pens prior to release. Soft-release pens allowed tortoises to acclimate to their new environment while also allowing them to excavate their own burrows to promote site fidelity. We placed one soft-release pen in each release area, 280 m apart. Pens were made from chain link (3 m length x 3 m width x 1.8 m height; MidWest Black E-Coat Exercise Pens, MidwestPetProducts, Inc., Irvine, CA, USA; Figure 3.3A). We modified them to prevent escape of tortoises and trespass of predators by attaching aluminum flash sheeting around the perimeter of cages, sinking the pens into the ground approximately 15 cm, and placing 1.9 cm (0.75 in) mesh cloth over the tops to exclude avian predators. We constructed 5-10 starter burrows in both pens by pounding a 7.5 cm diameter pipe at an angle (~35 degrees) using a post driver. We randomly assigned 5-6 head-started tortoises per pen. On 30 May 2014 we released all 12 tortoises into the soft-release pens. We removed the pens 47 days later on 16 Jul 2014 and began tracking.

Release 2: (N=133) Represents tortoises from the 2014 cohort that we head-started for approximately 9 months at SREL and SCI and released at YWMA in summer 2015. We used the same transmitter package as *Release 1* for 30 tortoises. We created 28 small pens (1.2 m length, 1.2 m width, 0.6 m height) that we placed in 1-ha circular buffers in both release area separated by 274 m at their centers (Figure 3.1). We used a grid pattern in each buffer so that no pen was within 25m of another pen, however one pen had to be moved on the northwest side due presence of red imported fire ants (*Solenopisis invicta*). We constructed pens with galvanized hardware cloth (0.64 mm mesh; Jackson Wire International, Inc., Houston, Tx, USA; Figure 3.3B). We excavated a narrow, 15-cm deep trench in dirt to place the pen walls, which we reinforced with wooden stakes at all four corners to help add stability to the hardware cloth. We created 5-6 starter burrows using the same methods as *Release 1*. We randomly placed 5-6 tortoises in pens, but weighted selection to ensure that no pens contained tortoises from the same clutch. Once tortoises were placed in the pens, we folded over the sides of the pens and secured

them with zip ties to cover the top of the pens. Prior to soft release of the tortoises, we applied AMDRO[®] (Ambrands, Atlanta, GA, USA) fire ant bait around pens three weeks prior to and again one day before tortoises were placed in pens. On 5 June 2015, we released all 133 tortoises into soft-release pens (Table 3.1). We initially intended to remove pens after 30-50 days, but we removed pens from the southeast release area after only four days and staggered the release of the pens on the northwest pens (see results).

Release 3: (N= 20). For comparison of movement and survival between head-starts and hatchlings, we released head-started (*HS*; N=10) and neonates directly released (*DR*; N = 10) after hatching. The *HS* were retained from the 2014 cohort and head-started for approximately one year, whereas the *DR* were collected from the 2015 cohort and held in captivity for no more than 19 days. We used the same transmitter packages for all 10 *HS* tortoises as for head-starts in *Release 1* and *Release 2*, however, we attached the 10 *DR* tortoises with radio-transmitters (Advanced Telemetry Systems Model R1655, 1.2 g) on the fourth vertebral scute using Loctite[®] 5 min Epoxy (Henkel Corp., Düsseldorf, Germany; Figure 3.2). We conducted a hard-release on 14 September 2015 by placing one *HS/DR* pair at the entrance of pre-existing adult burrows. All releases occurred on the Northwest side of the gravel road. We placed tortoises just inside the burrow entrance and facing into the burrow. The tortoises were then free to move down into the burrow or move elsewhere on the landscape.

Post-Release Monitoring

For all three releases, we radio-tracked transmittered tortoises from their release dates (Table 3.1) through 15 November 2015 using a radio-receiver (Model R1000) and three-element Yagi antennae (Communications Specialists, Inc., Orange, California, USA). We tracked tortoises 2-3 times a week during the increased activity periods immediately after release (June-August) and then tracked tortoises at least once per week through the remainder of the study. We documented post-release movement
and site fidelity by recording each telemetry location to the nearest ± 3 m using a Garmin GPSMAP* 64 (Garmin International, Inc., Olathe, Kansas, USA). We marked all burrows used by transmittered tortoises by placing a uniquely numbered aluminum tag (Forestry Suppliers, Inc, Jackson, Mississippi, USA) adjacent to the burrow apron using a landscaping staple. When tortoises were in burrows, we documented the burrow ID, GPS location, and whether or not the tortoise had moved since its previous tracking event. If tortoises were above ground, we noted activity (e.g., resting, walking, foraging, excavating a new burrow) and survival status. If we suspected that tortoises were dead inside their burrows due to lack of movement, excess presence of fire ants, and/or lack of maintenance to burrow (i.e., collapsing burrow entrance, prolonged absence of tracks, excess foliage on apron) we scoped the burrow to determine the tortoise's status using a juvenile burrow scope (Environmental Management Systems, Inc. Canton, GA, USA). If tortoises were found deceased above or below ground, we attempted to determine the most likely cause of death by looking for damage to the shell and transmitter package. If a deceased tortoise showed damage to the structural integrity of the body or transmitter we assumed that a mammalian predator was the cause of death; otherwise we assumed fire ants to be the direct cause of death.

Winter dormancy, when tortoises are unlikely to move from their burrows, is largely dictated by temperature (McRae et al. 1981) which can fluctuate within and between years. We defined the 15th of November as the cutoff for evaluating movement and survival to winter dormancy based on evidence from other studies that tortoises in Georgia are mostly dormant around this time (Harris et al. 2015; McRae et al. 1981). Once all tortoises entered winter dormancy (hereafter "dormancy") we visually confirmed their status using the juvenile burrow scope. To prevent disturbing tortoises, we continued tracking but did not scope burrows again until the following spring when tortoises began to show signs of activity.

Analysis

Tortoises will often leave their burrows to forage before quickly returning (Halstead et al. 2007, Mushinsky et al. 2003) back to their original burrow. Because these quick foraging bouts are ephemeral, we only considered movements between burrows to be ecologically significant moves for evaluating post-release movement. Therefore, all movement analyses are based solely on locations of tortoises when they moved between burrows. We used ArcMAP (ESRI, version 10.1, Redlands, CA, USA) to plot tortoise movements from their release pen. We used the Spherical Law of Cosines to calculate step distances (i.e., linear movements of individuals between successive burrow locations) and linear displacement from release sites (i.e., straight line distance of each burrow from the release pen) to dormancy. From these data, we calculated the number of steps, mean step length, maximum step length, minimum step length, and cumulative step length (i.e., sum of all step lengths) for each tortoise. We also used movement values to document mean displacement, minimum displacement, maximum displacement, and final displacement (i.e., displacement at dormancy) for each tortoise. All movement calculations were averaged across individuals in a release group. However, because tortoise mortality biases movement calculations, we also averaged across individuals that survived to dormancy in each release group. For Treatment 3, we used a Student's T-test to compare movement metrics between HS and DR tortoises.

We documented survivorship to first dormancy for all three release groups based on whether they were dead, alive, or censored (i.e., lost from the study due to transmitter detection failure) as of 15 November in the year they were released. We also documented annual survivorship (i.e., 1 year subsequent to pen removal) and cumulative survivorship to second dormancy for *Release 1*. We estimated survival using the Kaplan-Meier estimator for staggered entry (Pollock et al. 1989) in the *asbio* package in Program R (Aho 2015; http://CRAN.R-project.org/package=asbio). Survival data are presented as mean ± 95% confidence intervals. We used log-rank tests following the methods of Pollock

et al. (1989) to compare survival curves between *Release 1* and *Release 2*, between the two release areas for *Release 2*, and between *HS* and *DR* tortoises in *Release 3*.

We evaluated potential factors influencing whether or not tortoises survived from *Release 1* and *Release 2*, including tortoise size, release area, and movement. Because the amount of movement exhibited by an individual is biased by the length of time it survives, we used the first step length as our movement metric, and only included tortoises that survived long enough to construct at least one burrow. To analyze these variables in relation to survival, we created four univariate models and four additive models and tested them for best fit using Akaike's Information Criterion for small sample sizes (AIC_c). Specifically, the univariate models examined one of the following: 1) mass of tortoises at release (g), 2) release area (northwest or southeast), and 3) first step distance from the release site (m). The additive models examined 1) release area and mass, 2) release area and distance of first step from release, 3) mass and distance of first step from release, and 4) a global model with all variables included. We used Program R (Version 3.1.0; R Development Core Team 2014) for all statistical analyses. All methods followed protocols approved by the University of Georgia Institutional Animal Care and Use Committee (# A2014 08-006-Y1-A0), GADNR (29-WJH-14-93), and GA state parks (#172014).

RESULTS

Release 1: In 2013 we collected 19 eggs from two viable clutches on SCI and 11 hatched (57.9%). A yearling tortoise was brought from the Georgia Sea Turtle Center (GSTC) at Jekyll Island, GA for headstarting, bringing the total number of tortoises to 12. We head-started all 12 at SCI for an average of 249.6 \pm 1.5 days and all survived the head-starting period. While in captivity, tortoises gained an average of 48.5 \pm 4.0 g (initial = 38.1 \pm 0.9 g; final = 86.5 \pm 4.4 g) and reached an average carapace length (CL) of 71.2 \pm 1.2 mm. During the soft-release penning period, no vertebrate predator gained access to tortoises; however, red imported fire ants entered one pen and depredated one of the tortoises.

Because this tortoise was depredated prior to pen removal, it was excluded from analyses, reducing our sample size to 11.

We tracked all 11 tortoises for 486 days until tracking was terminated on 15 November 2015. We mapped the step-wise movement from release to first dormancy (Figure 3.4) and second dormancy locations (Figure 3.5). All movement metrics are summarized in Table 3.2. Eight tortoises survived to first dormancy (72.7%; Figure 3.6A). All tortoises survived through the first dormancy until mid-April when they began moving again (i.e., 100% dormancy survivorship). One head-start died before the second dormancy and two were censored (i.e., only transmitters found with no signs of predation), resulting in an annual survivorship of 60.6% (95% CI: 27.2-93.9%) for *Release 1* cohort for the first year post-release. One other tortoise was predated before the second dormancy period (i.e., 15 November 2015) resulting in a final survivorship for the study (i.e., 486 days) of 48.4% (95% CI: 14.4-82.6).

Release 2: In 2014, we collected 157 eggs from 22 clutches at SCI, RBSP, and the YWMA and 144 hatched (91.2%; see Quinn et al. 2016 for details on hatching success by site). Two hatchlings had severe deformities and were humanely euthanized. An additional egg was collected from an injured female undergoing rehabilitation at the GSTC and brought to SCI, bringing the 2014 cohort total to 143 tortoises. We head-started 45 tortoises at SCI and 98 at SREL. Tortoises were in captivity for an average of 273.2 \pm 0.9 days and all survived the head-starting period. While in captivity, tortoises gained an average of 70.1 g (initial = 32.6 \pm 0.4 g; final = 102.8 \pm 2.5 g) and reached an average CL of 76.0 \pm 0.7 mm. Of these, 133 were used for soft-release (i.e., *Release* 2) with the remaining 10 used later in *Release* 3. Despite treating soft-release pens with AMDRO[®], three transmittered tortoises were depredated by fire ants while in their pens at the southeast release area and were excluded in the post-release results. Transmitters from these deceased tortoises were placed on other tortoises prior to removal of pens to maintain a sample size of 30. Although we initially planned for all head-started tortoises to remain in their pens for approximately 30-50 days, we removed the pens from the

southeast release area after only four days in 2015 to help prevent further predation by fire ants. We also staggered the release at the northwest area to avoid increased predation by mesopredators.

We tracked tortoises for an average of 126 days (See table 3.1 for release date ranges) until tracking was terminated on 15 November 2015. We mapped the step-wise movement of tortoises from their release to first dormancy location (Figure 3.7). All movement metrics are summarized in Table 3.2. Of the 30 transmittered tortoises, 13 (43.3%) survived until first dormancy. However, due to the staggered release of tortoises, the Kaplan-Meier survivorship estimate to first dormancy was only 16.1% (95% CI: 8.1-24.1%). Tortoises suffered far fewer causalities at the northwest release area (n = 4; 25%) compared to the southeast release area (n = 13; 92.9%) and survivorship estimates were significantly higher at the northwest area (70.0% survivorship; 95% CI: 48.3-91.7%; Figure 3.6B) compared to the southeast area (7.1% survivorship; 95% CI: 0.0 - 20.6%; χ^2 = 10.3, df = 1, *P* = 0.006; Figure 3.6C).

Release 1 and Release 2

We combined data between *Release 1* and *Release 2* for an overview of head-start tortoise movement and survival. We radio-tracked tortoises 915 times (i.e., 915 tracking events) until first dormancy. Step length and mortality were highest soon after release (Figure 3.8). Despite steps over 40 m making up only a small portion (9.8%) of all tortoise step lengths, most (88%) occurred in the first 30 days post-release. The majority of mortality also occurred within the first 30 days post-release (68.2%). Tortoises constructed a total of 63 burrows and tortoises that survived to first dormancy constructed an average of 2.04 burrows (range: 0-7). Two head-starts (i.e., one from *Release 1* and one from *Release 2*) moved to an adult burrow after release, but they both eventually constructed their own. Mean step length was 18.5 ± 1.9 m. The majority of movements between successive burrows was under 20 m (72.8%), and most were 10 m or less (57.1%; Figure 3.9). The shortest single movement between successive burrows was 0.1 m and the largest was 119.1 m. Two tortoises from *Release 2* never moved from the release pen and so the minimum displacement from release was 0.0 m. The maximum displacement of any tortoise from its release pen was 122.0 m.

No censoring was required for *Release* 1 or *Release* 2 between release and first dormancy. The mean known survival (i.e., not including censors) to first dormancy of both releases combined was 58.2%. However, the Kaplan-Meier survivorship to first dormancy was significantly higher for tortoises in *Release* 1 (72.7% survivorship; 95% CI: 46.4-99.0%) compared to tortoises from *Release* 2 (16.1% survivorship; 95% CI: 8.1-24.1%; $\chi^2 = 9.1$, df = 1, *P* < 0.005). *Release* 1 tortoise survival was also significantly higher than *Release* 2 tortoises at the southeast release area ($\chi^2 = 10.6$, df = 1, *P* = 0.005) but was not significantly different between the *Release* 2 tortoises at the northwest area ($\chi^2 = 0.08$, df = 2, *P* = 0.96). Of the eight models used to evaluate factors associated with survival, seven were within the top 0.95 cumulative weight (Table 3.4). While the release area candidate model was the best fit model, it was within 2.0 Δ_i values of the null model, suggesting that there were other biological factors associated with survival. First step distance candidate models all fell below the null model and were therefore not strongly related to survival.

Release 3

In 2015, we collected 108 eggs from 15 clutches at SCI, RBSP, and the YWMA and 81 hatched (75%). We used 10 of these hatchlings, hatched in 2015, for our *DR* group, retaining the remaining 71 for head-starting (i.e., a future release; data not presented here). After spending an average of 369.9 ± 3.3 days in captivity, the *HS* had gained an average of 94.6 ± 12.1 g (initial = 35.4 ± 0.6 g; final = 130.0 ± 11.9 g) and reached an average CL of 83.9 ± 1.4 mm. The *DR* tortoises spent an average of 10.6 ± 1.9 days in captivity before release and were an average CL of 48.5 ± 0.6 mm and an average mass of 31.4 ± 0.8 g.

We radio-tracked *HS* and *DR* tortoises from their release on 14 September 2015 to the last tracking event on 15 Nov 2015 (62 days; Figure 3.10) for a total of 90 and 62 tracking events for *HS* and

DR tortoises, respectively. Surviving *HS* constructed an average of 1.25 (range: 1-2) burrows per tortoise and surviving *DR* tortoises constructed an average of 0.75 (range: 0-1) burrows but the difference was not significant ($\chi^2 = 1.8$, df = 1, *P* = 0.17). Movement metrics for *HS* and *DR* tortoises are summarized in Table 3.3. Student's T-tests showed that none of the movement metrics were significantly different between surviving *HS* and *DR* tortoises ($P \ge 0.75$).

Eight *HS* tortoises survived to the end of the study, one was depredated, and one was censored. Four *DR* tortoises survived, four were depredated, and two were censored. Although transmitters were still active, all three censored tortoises never moved from the adult burrows they were released in. Scoping did not reveal any sign of these tortoises and so their status is unknown (i.e., left censored). Kaplan-Meier survivorship estimates from release to first dormancy was 88.9% (CI: 68.4-100%) for *HS* and 50.0% (CI: 15.4-84.6%) for *DR* (Figure 3.11). Despite a 39% difference in means, the survival estimates were not significantly different, although they were approaching significance ($\chi^2 = 2.49$, df = 1, *P* = 0.11).

Causes of Mortality

All tortoises in all three releases appeared to be clinically healthy prior to release (but see chapter 2 for a more detailed analysis of health and growth metrics) and it appeared that none of our tortoises during this study died due to anything other than predation. A total of 27 tortoises (44.3%) were predated among all three releases. Mortality during this study was most likely caused by fire ants and mammalian predators. We determined that 16 (59.3%) were predated by mammals, nine were predated by fire ants (33.3%) and two could not be determined conclusively. All mortality occurring while animals were in pens (n=4) were due to fire ants and occurred at the southeast release area. While the majority of post-release mortality also occurred at the southeast release area (n = 18), fire ant and mammalian-caused mortality occurred at the northwest release area as well (n = 9). Coyote (*Canis*

latrans) scat was found near the release areas and wildlife cameras detected several raccoons (*Procyon lotor*) and stray dogs (*Canis familiaris*) near the release sites soon after release. These three species were the most likely mammalian predators of released tortoises.

DISCUSSION

Survival and Growth during Head-starting- Head-start tortoise survivorship in captivity was high (100%) and all tortoises grew throughout the head-starting process. After 8-9 months in captivity, mean mass increased by more than 300% and mean carapace length was comparable to two to three year old free-ranging tortoises (i.e., ranges 66-87 mm CL, Aresco and Guyer 1999). While prior studies have head-started desert tortoises (*Gopherus agassizii*) for longer than one year (Michell and Michell 2015; Nagy et al. 2015), understanding the potential effects of head-starting for a minimal time frame is important given the time, energy, and financial resources required to head-start tortoises. Furthermore, captive settings are often quite different from the natural environment experienced by wild conspecifics. Captive settings may lead to health issues such as metabolic bone disease or shell pyramiding (see Jackson et al. 1976) or behavioral issues including loss of fear of predators (Griffin et al. 2000). Increasing head-starting time may therefore increase the likelihood of these issues arising. However, we saw no signs of metabolic bone disease, shell pyramiding, or any other clinical illness prior to release.

Site fidelity of soft-released head-starts- After release, head-started tortoises demonstrated remarkably high site fidelity. The largest movements occurred soon after release but decreased after the first few weeks post-release. Mean movement distances between burrows for surviving head-starts ranged from 24.4 m in *Release 1* to 19.6 m in our *Release 2* and no head-start tortoise traveled further than 119.1 m in a single movement bout between burrows. Prior studies on hatchling and yearling gopher tortoises have documented mean movement between burrows that were slightly smaller for hatchlings (8.0 – 17.1 m; Butler et al. 1995; Pike 2006). However, these same studies also documented

maximum movements between burrows that were even higher than our head-starts (139.4 – 150 m). Furthermore, hatchlings from these previous studies moved > 70 m away on average from their natal nests. By contrast, we documented the mean movement from release sites of surviving head-starts in this study to be 52.7 m for *Release 1* and 11.5 m for *Release 2*. Therefore, head-start movements typically fell well within normal ranges for young, wild tortoises based on the current literature. Furthermore, head-start movements were relatively small compared to those seen in adult tortoises translocated to the same study site at Yuchi WMA. The furthest displacement from a release site for our head-starts was only 122 m. By comparison, Bauder et al. (2012 and 2014) documented > 1km moves for five translocated adult tortoises soft-released at YWMA in 2012. Even resident tortoises at YWMA moved upwards of 272 m from the burrow where they were first captured. Therefore, head-start site fidelity from release was not only adequate, but exceptional even when compared to wild conspecifics.

Survivorship of soft-released head-starts- Survivorship estimates to first dormancy was variable between *Release 1* and *Release 2* (Range: 7.1%-72.7%) but was higher (70.0%-72.7%) when we were able to follow our soft-release methodology. Although the study duration precluded annual estimates of our *Release 2* tortoises, our *Release 1* head-starts displayed 60.1% annual survival, over four times the estimated annual survival of hatchlings (i.e., 12.8%; Perez-Heydrich et al. 2012). Indeed, many studies on hatchling tortoises end with few, if any, left alive (Butler and Sowell 1996; Epperson and Heise 2003; Pike and Seigel 2006).

Initial survival variability for *Release 2* can likely be explained by the differences in release times between the northwest (70.0% survival; 30+ day penning) and southeast (7.1%., 4 days of penning) release areas. Unfortunately, fire ant predation in pens at the southeast site forced us to remove pens prematurely, allowing tortoises a chance to escape. Subsequently, tortoises had less time to construct sufficient burrows and were likely more vulnerable to mammalian mesopredators. Our survivorship analysis (i.e., Kaplan-Meier procedure with staggered entry) assumed that the release areas were not

statistically independent of each other. This assumption was likely violated because penning duration is presumably linked with survival. Therefore, we analyzed the two sites separately.

None of our survivorship candidate models were significantly better than the null for Release 1 and *Release 2* tortoises that survived to make at least one movement from the release site. Interestingly, models that included first movement distance were the worst fit, suggesting that initial movement distances from the release site were not strongly related to survivorship in this study. One of the primary concerns of releasing juvenile tortoises that are still susceptible to predation is that they will fail to acclimate to their new surroundings, wander away from the release area, and eventually die due to predation or exposure. However, movement distance was not strongly correlated with whether or not a head-start survived to their first dormancy period. Rather, other biological factors are likely responsible for the predation we documented. Predation pressures can vary at small scales and behavioral variation between individuals may have also made some tortoises more prone to predation. For example, tortoises depredated by mesopredators may have simply been too close to the entrance of their burrows to escape or spent more time out of their burrows, making them more susceptible to predation than the survivors. Furthermore, it was difficult to predict which sites at YWMA might have increased predation pressure. It's possible that tortoises depredated by fire ants post-release were simply more likely to encounter fire ants based on densities and spatial arrangement of fire ant colonies at our sites.

Site Fidelity and Survivorship of Hard-released Head-starts and Hatchlings- Survival estimates for *HS* were 39% higher than *DR* tortoises. Surprisingly, the statistical results of the *HS* and *DR* tortoises suggest that head-start tortoise movement and survival were not different from hatchlings. However, these results may be due partly to small sample sizes (i.e., n = 10 each) resulting in large confidence intervals for both groups. Sample size was reduced further by three animals censored at the onset of the study (i.e., one *HS* and two *DR*). However, although not statistically significant, the ecological

significance of such disparity may indicate that head-starting can increase survival of released tortoises. Larger sample sizes and longer tracking time intervals should be used in the future to determine if survival is consistently and significantly higher in head-started tortoises.

Without long-term penning (i.e., 9-12 months; Tuberville et al. 2005), adult gopher tortoises have high desertion rates from release sites (Diemer 1989). We used soft-release pens for *Release 1* and *Release 2* tortoises based off of these findings because we wanted to promote site fidelity in headstarts. However, in our comparative study of hard-released *HS* and *DR* tortoises, *HS* tortoises showed no more movement or dispersal than soft-released head-starts from previous releases (i.e. maximum step length and displacement were both 43.8 m for hard-released *HS* compared to maximum step length of 119.1 m and displacement of 122.0 m for *Release 1* and *Release 2*.). It's possible that *HS* tortoises simply had less time to move before dormancy compared to *Release 1* and *Release 2* head-starts. However, 91% of all *HS* movement over 1 m occurred in the first two days post-release suggesting that *HS* tortoises had sufficient time to move across the landscape, but chose not to. The high site fidelity and survival of the hard-released *HS* tortoises may suggest that soft-release penning is unnecessary for promoting high site fidelity in head-starts. However, soft-release penning may still be necessary to allow naïve tortoises time to construct adequate burrows and begin foraging on native plants prior to full release.

Predation - It is unknown why fire ants invaded pens at the southeast release area but not the northwest release area. However, the southeast release area has a higher degree of herbaceous ground cover which could be more conducive to fire ant colonization (Lubertazzi and Tschinkel 2003). We never detected any large fire ant mounds at our site, but rather, fire ants seemed to be distributed diffusely throughout the landscape. Although we attempted to treat release areas with AMDRO[®], treatment did not prevent predation by fire ants. Pre-release treatment of sites for fire ants may not have been

successful because the lack of obvious centralized mounds prevented detection of fire ant activity and effective administration of bait.

Mammalian mesopredators (i.e., raccoons, skunks, armadillos) are perhaps the most common predator of hatchling and juvenile tortoises (Smith et al. 2013). Although we initially saw little evidence of mammalian predation (i.e., only one predation of the tortoises in *Release 1* prior to first dormancy), they were a major factor during *Release 2*, especially at the southeast release area. Motion sensitive wildlife cameras documented at least three individual raccoons in the southeast area post-release and we found raccoon footprints around the burrow entrances inside the penning area. Wildlife cameras also captured feral dogs around the release area. By releasing tortoises from pens at the southeast site early to mitigate fire ant mortality, we may have simply exchanged fire ants for mammalian predators. Interestingly, the northwest release area did not receive the same attention from mammals immediately after their release. However, on 28 August 15 we noted several tortoise burrows at the northwest release area that were clearly dug into partially by a raccoon based on foot prints around the burrow aprons. Only one tortoise was depredated at this time, suggesting that many tortoises were able to escape into their burrows. We feel this further justifies our hypothesis that tortoises at the southeast area were not given ample time to construct burrows long enough to avoid predation. Without substantial knowledge of site-specific predator habits or their population densities, it can be difficult to predict the outcome of head-start releases. By placing all of our tortoises into only two areas, we may have increased the chances of predators learning where head-starts were located. Even a small number of predators can learn where and how to access head-starts (i.e., "habit depredation"; Leopold 1933), thereby wreaking havoc on management efforts. Future head-starting initiatives should consider increasing their releases spatially and temporally to help prevent large scale predation issues like we saw at our southeast site for *Release 2*.

Conclusions- Our initial results have demonstrated that head-starting gopher tortoises could be a useful tool for population augmentation. No head-start moved further than 122 m from the release sites, and none left the two managed areas, much less the YWMA boundaries. Our annual head-start survival (60.8%) was high compared to the literature estimates for hatchlings. Although the difference in survivorship for head-starts and hatchlings at our site was not statistically significant, which may be due to small sample sizes, a 39% increase in survival may very well be biologically significant. Small size is often thought to be the cause of high predation rates in young turtles. However, the size thresholds at which predation significantly declines are generally not well understood and change depending on the species and the site-specific predator base. While our head-starts were small enough to be susceptible to predation, their increased survivorship rates compared to hatchlings may indicate that head-starting is an effective management tool for boosting population numbers.

In order to truly boost populations, head-starts ultimately need to assimilate with the native population to increase the breeding stock of adults. Therefore, regardless of species, individuals used for augmenting depleted populations need to demonstrate both high site fidelity to their release site and high survivorship to maturity. Sexual maturity is dictated largely by body size in tortoises (lverson 1980; Landers et al. 1982) and one of the benefits of head-starting tortoises to larger size classes is that they likely reach sexual maturity sooner than their wild counterparts. Therefore, head-starts may also increase the reproductive stock of populations more quickly. However, while non-head-started tortoises take 15-20 years to reach reproductive maturity (Ashton and Ashton 2008), it should still take head-starts 10+ years depending on growth in captivity and release habitat. Such a large time span makes studying one-year old head-starts through to maturity impractical. Instead, this study was designed to evaluate initial head-start performance just after release, likely the most critical time period for a translocated tortoise. Our study design allowed us to quickly assess the potential that head-starts could play in restoring populations to sustainable levels. However, future research will need to re-evaluate the

status of head-starts once they have had time to mature in order to make any truly conclusive statements on head-starting efficacy.

Management Recommendations- In this study, head-start survival depended largely on presence of predators at release sites affecting our ability to follow our intended release methodology. Therefore, potential release sites and areas need to be evaluated before large releases occur and appropriate steps should be taken to mitigate unnaturally high predation levels. Future head-starting efforts should consider releasing tortoises at larger spatial and temporal scales to reduce the likelihood of predation in two ways. First, it reduces the likelihood that a large number of tortoises are being released in a place or time when survivorship would be lower (e.g., in locations with more fire ants or when they are more active). Second, high densities of tortoises could in itself also increase predation rates (e.g., learned Raccoons returning to the same site to search for tortoises). Furthermore, soft-release penning may not be necessary for juvenile tortoises. Although our study was not designed to test penning vs. nonpenning treatments, we were surprised to see so little movement and relatively high survivorship for our head-starts that were hard-released. It may be beneficial, especially in areas where fire ants are a concern, to forgo soft-release pens and instead evaluate hard-releases of head-starts at adult burrows, again spreading out releases in space and time. This could help prevent excess predation caused by fire ants in pens, while also preventing increased predation by mammalian mesopredators, thus increasing the initial efficacy of head-starting in gopher tortoises.

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Table 3.1. Summary of gopher tortoise (*Gopherus polyphemus*) releases and known fates as of 15 November 2015 at Yuchi WMA, Georgia, broken down by release method and release groups. Numbers and letters in italics represent release groups. Soft-release date is the date tortoises were placed in pens; release date is the date when soft-release pens were removed or when hard-releases occurred; number of days represents the amount of time (in days) between release. NW = northwest, SE = southeast release area. HS = head-start; DR = directly released hatchling.

	Soft-Release			Hard-Release		
	Release 1	Release 1	Release 2	Release 2	Release 3 (HS)	Release 3 (DR)
Cohort	2013	2013	2014	2014	2014	2015
Release Year	2014	2014	2015	2015	2015	2015
Release Area	NW	SE	NW	SE	NW	NW
Soft Release Date	30-May-14	30-May-14	5-Jun-15	5-Jun-15	N/A	N/A
Release Date	16-Jul-14	16-Jul-14	Staggered*	9-Jun-15	14-Sep-15	14-Sep-15
No. Released	6	6	72	71	10	10
No. Tracked	5	6	16	14	10	10
No. Days	486	486	125.5*	159	62	62
No. Survived	3	1	12	1	8	4
No. Deceased	2	3	4	13	1	4
No. Censored	0	2	0	0	1	2

* Staggered releases from 6-Jul-15 through 22-Jul-15, mean number of days in pens = 39 days

Table 3.2. *Release 1* and *Release 2* movement metrics of head-started gopher tortoises (*Gopherus polyphemus*) radio-tracked from release through 15 November 2015 (i.e., estimated dormancy) at Yuchi WMA, Georgia. Values are averaged among individuals by release group and are calculated for all individuals released (i.e., including deceased using subscript 'all') and for only the tortoises that were alive at the end of the study (subscript 'surv'). "Steps" represent movements between successive burrows and cumulative step is the sum of all step lengths between burrows. Displacement is the linear distance from the release site with final displacement indicating the distance from release. Data are presented as means with ranges presented in parentheses.

Motrice	Release Group					
wietrics	Release 1 _{all}	Release 1 _{surv}	Release 2 _{all}	Release 2 surv		
Ν	11	4	30	13		
Days in Study (d)	301.7	486	69.4	128.1		
	(1-486)	(NA)	(3-159)	(NA)		
Number of Steps	5.9	8.8	1.4	2.7		
Number of Steps	(1-14)	(6-10)	(0-7)	(0-7)		
Mean Sten (m)	24.4	19.6	10.6	9.9		
Mean Step (III)	(10.9-41.5)	(15.1-24.4)	(0-119.1)	(0-21.2)		
Min Stop(m)	14.5	6.6	8.1	6		
wini. Step (iii)	(0.1-41.5)	(4.8-8.7)	(0-119.1)	(0-14.4)		
Max Stop (m)	51.4	58.0	13.7	14.6		
Max. Step (III)	(22.1-109.5)	(22.1-84.4)	(0-119.1)	(0-46.1)		
Cumulative Step (m)	120.7	165.9	22.4	33.8		
Cumulative Step (III)	(22.3-220.0)	(117.7-218.6)	(0-119.1)	(0-96)		
Aug Displacement (m)	50.2	52.7	12.5	11.5		
Avg. Displacement (m)	(15.3-114.5)	(20.3-91.2)	(0-119.1)	(0-43.2)		
Min Displacement (m)	41.8	38.9	10.9	8.08		
	(8.2-109.5)	(8.2-76.5)	(0-119.1)	(0-39.2)		
Max Displacement (m)	59.5	65.9	14.3	15.2		
Max. Displacement (III)	(22.3-122.0)	(31.5-111.2)	(0-119.1)	(0-46.1)		
Final Displacement (m)	53.1	53.2	13.2	12.7		
rinal Displacement (III)	(16.8-122.0)	(16.8-111.2)	(0-119.1)	(0-43)		

Table 3.3. *Release 3* movement metrics of radio-tracked head-started (*HS*) and directly released hatchling (*DR*) gopher tortoises (*Gopherus polyphemus*) at Yuchi WMA (GA) from 14 September 2015 (i.e., release date) through 15 November 2015 (i.e., estimated dormancy). Values are averaged among individuals by release group and are calculated for all individuals released (i.e., including deceased using subscript 'all') and for only the tortoises that were alive at the end of the study (subscript 'surv'). "Steps" represent movements between successive burrows and cumulative step is the sum of all step lengths between burrows. Displacement is the linear distance from the release site with final displacement indicating the distance from release. Data are presented as means with ranges presented in parentheses.

Motrice	Release Group					
Wietrics	HS _{all}	HS _{surv}	DR _{all}	DR _{surv}		
Ν	10	8	10	4		
Days in Study (d)	56.0	62	40.1	62		
	(2-62)	(NA)	(2-62)	(NA)		
Number of Steps	1.1	1.38	0.6	1.5		
Number of Steps	(0-3)	(1-3)	(0-3)	(1-3)		
Mean Sten (m)	11.6	13.8	4.6	11.5		
	(0-43.8)	(2.3-43.8)	(0-29.3)	(3-29.3)		
Min Stop (m)	10.1	12.7	4.3	10.7		
wini. step (iii)	(0-43.8)	(1-43.8)	(0-29.3)	(3-29.3)		
Max Stop (m)	12.1	15.2	5.2	13		
Max. Step (III)	(0-43.8)	(5-43.8)	(0-29.3)	(3-29.3)		
Cumulativo Stop (m)	12.7	15.9	6.2	15.5		
cumulative step (III)	(0-43.8)	(6.9-43.8)	(0-29.3)	(3-29.3)		
Avg Displacement (m)	11.4	14.3	5.1	12.8		
Avg. Displacement (III)	(0-43.8)	(5.2-43.8)	(0-29.3)	(3-29.3)		
Min Displacement (m)	10.5	13.2	4.9	12.4		
wini. Displacement (m)	(0-43.8)	(3.9-43.8)	(0-29.3)	(3-29.3)		
Max Displacement (m)	12.4	15.4	5.2	13		
Max. Displacement (III)	(0-43.8)	(5.7-43.8)	(0-29.3)	(3-29.3)		
Final Displacement (m)	12.3	15.4	5.2	13		
Final Displacement (m)	(0-43.8)	(5-43.8)	(0-29.3)	(3-29.3)		

Table 3.4. Models created to determine factors associated with mortality of *Release 1* and *Release 2* head-started gopher tortoises (*Gopherus polyphemus*) at the Yuchi WMA, Georgia to first dormancy. Tortoises that did not move away from the pen before predation were excluded from the models. Akaike's Information Criterion for small sample size (AIC_c) was used to evaluate the models. 1 = null model, Release Area = side of road released (i.e., northwest or southeast), M = tortoise mass at time of release, First Step= first step distance (m) from the release site, Global = SOR+M+FMD.

Model	К	AIC _c	Δ _i	Wi	Cum. Wt
Release Area	3	44.52	0.00	0.29	0.29
Release Area + Mass	4	45.36	0.85	0.19	0.48
Null	2	45.78	1.26	0.15	0.63
Mass	3	46.60	2.08	0.10	0.73
First Step	3	46.88	2.37	0.09	0.82
Release Area + First Step	4	46.90	2.39	0.09	0.91
Global	5	48.10	3.58	0.05	0.95
Mass + First Step	4	48.14	3.62	0.05	1.00



Figure 3.1. Soft-release pen locations used within two 1-hectare release areas (northwest and southeast) for *Release 1*(Summer 2014) and *Release 2* (Summer 2015) head-started gopher tortoises (*Gopherus polyphemus*) at the Yuchi WMA, Georgia. Due to red imported fire ant (*Solenopsis invicta*) invasion during the 2015 release, the pens in the southeast release area were removed after four days. All other pens were left in place for 30-50 days.



Figure 3.2. Hatchling (left) and head-started (right) gopher tortoises (*Gopherus polyphemus*) just prior to hard release at Yuchi WMA, Georgia on 14 September 2015. Transmitters were affixed to the fourth vertebral scute.



Figure 3.3. Soft-release pens for head-started gopher tortoises (*Gopherus polyphemus*) at the Yuchi WMA, Georgia: (A) Portable chain link pens used for *Release 1* soft-release, (B) Hardware cloth pens used for *Release 2* soft-release.



Figure 3.4. *Release 1m*ovement paths of gopher tortoises (*Gopherus polyphemus*) at Yuchi WMA, Georgia from location of soft-release pens (white boxes) to first dormancy locations. Small red dots indicate burrow locations used by tortoises. Large red dots with an "X" indicate locations of deceased tortoises.



Figure 3.5. *Release 1* movement paths of gopher tortoises (*Gopherus polyphemus*) at Yuchi WMA, Georgia from soft-release pens on 16 July 2014 to second year dormancy locations. Small red dots indicate burrow locations used by tortoises. Large red dots with an "X" indicate locations of deceased tortoises.



Figure 3.6. Post-release Kaplan-Meier survivorship curves (large dashes with open circles) with 95% confidence intervals (small dashes) for head-started gopher tortoises (*Gopherus polyphemus*) at Yuchi WMA, Georgia, for the A) *Release 1* (n=11); B) *Release 2* in the northwest release area (n=16); and C) *Release 2* in the southeast release area (n=14). Note: Because survival curves can only be compared over similar time intervals we shortened graphs B and C (which took 18 and 22 weeks to reach dormancy period respectively) to only cover the first 17 weeks post-release. However, survivorship for B and C did not change after week 17 to dormancy and so final survival on graphs ultimately represent survival to first dormancy as well.



Figure 3.7. *Release 2* total movement paths of gopher tortoises (*Gopherus polyphemus*) at Yuchi WMA, Georgia from soft-release pens from time of release (9 June – 22 July 2015) to first dormancy location. Small green dots indicate burrow locations used by tortoises. Large green dots with an "X" indicate locations of deceased tortoises.



Figure 3.8. Movement distance and survival to first dormancy for *Release 1* and *Release 2* head-start gopher tortoise (*Gopherus polyphemus*) at Yuchi WMA, Georgia. Blue dots represent all non-zero meter movements between burrows to first dormancy for both cohorts. Mortality and movement both decrease over time.



Figure 3.9. *Release 1* and *Release 2* head-started gopher tortoise (*Gopherus polyphemus*) movement distances between burrow locations at Yuchi WMA, Georgia.



Figure 3.10. *Release 3* movement paths of gopher tortoises (*Gopherus polyphemus*) at Yuchi WMA, Georgia from hard release sites (i.e., adult burrows) on 14 September 2015 to dormancy. Small yellow and green dots indicate *HS* and *DR* burrow locations respectively. Large yellow and green dots with an "X" indicate locations of deceased *HS* and *DR* tortoises respectively.



Figure 3.11. *Release 3* Kaplan-Meier survivorship curves with 95% confidence intervals (small dashes) for gopher tortoises (*Gopherus polyphemus*) hard-released on 14 September 2015 at the mouth of adult burrows at the Yuchi WMA, Georgia. Tortoises were either (A) head-started (*HS*; n=10); or (B) direct released hatchlings (*DR*; n=10).

CHAPTER 4

CONCLUSION

Turtle populations are declining worldwide and hands-on manipulative conservation strategies have been increasingly employed to aid population recovery. Turtles are long-lived, late to mature (i.e., typically 10-20 years), and produce few offspring that survive to adulthood, making populations particularly susceptible to changes in adult survivorship (Heppell et al. 1996). Once threats to adult mortality have been mitigated, increasing the number of adults in a depleted population should dramatically increase population viability. Logistically though, large numbers of adult turtles are not usually readily available for planned population augmentation efforts. Rather, head-starting- the practice of protecting especially vulnerable life stages of a species to increase the likelihood of survivorship for conservation purposes (Burke 2015), may be the most viable augmentation strategy for conservation efforts of depleted turtle populations. That is, hatchlings can be readily acquired for headstarting without reducing donor population viability relative to adults. The gopher tortoise (Gopherus polyphemus) is commonly used for population augmentation initiatives, making it an ideal candidate for head-starting research. Clearly head-starting projects need to focus on post-release performance of animals; however, evaluating head-starts while in captivity is often overlooked. Therefore, the goal of my thesis was to evaluate the efficacy of head-starting gopher tortoises, both during captivity and after release.

In Chapter 2, I evaluated plasma chemistry and size metrics of head-started gopher tortoises reared in captivity for approximately 9 months. I compared tortoise size and plasma chemistry values among three diet supplement treatments: 1) a control diet, 2) the control diet plus a sod grass mat, and

3) the control diet plus natural gopher tortoise forage grown in pallets in a greenhouse. I compared growth and plasma chemistry values using multivariate and univariate analyses. All tortoises grew during the head-starting period from an average mass of 32.4 g at hatching to a final mass just prior to release of 88.1 g. Diet treatments had little effect on growth metrics and no effect on plasma chemistry values. The lack of differences between treatments may be explained by the fact that the tortoises in our study did not seem to find grasses palatable. Head-starts did not appear to ingest the sod grass supplement or the grasses in the natural food plot supplement, reducing the potential effects of both diets. Furthermore, natural plots of vegetation proved to be logistically challenging to grow in a timely manner; therefore, natural plots may not have offered tortoises the opportunity to forage enough to result in differences in plasma chemistry or growth among treatments. Regardless, this study demonstrated that our control diet of commercially-available leafy vegetables mixed with Zoo Med[®] Grassland Tortoise Feed was suitable for rearing clinically healthy tortoises that grew to the size of two or three year old wild conspecifics.

Although plasma chemistry reference values exist for free-ranging adults, the plasma chemistry values documented in the second chapter are among the first for juvenile gopher tortoises (but see Erickson 2015). I found that there was substantial overlap in values between head-start juveniles and wild adults and wild juveniles, suggesting that plasma values for head-starts were not outside of the range for wild tortoises. Only total protein did not overlap between head-starts and adult gopher tortoises. However, head-starts generally had low total protein (TP) values. Low TP values could be caused by the lack of some natural forage or even the lack of appropriate hind-gut microbial communities that aid digestion. Although I would recommend a base diet of commercial leafy greens, which are logistically easy to procure, future head-starting efforts may want to include some forbs and legumes (MacDonald and Mushinsky 1988; Mushinsky et al. 2003) and/or inoculate tortoises with natural microbes involved in digestion. Inoculation could be accomplished by using feces from healthy
wild adults (Lance and Morafka, 2001). However, it is possible that young juveniles simply have naturally low TP values compared to older individuals (Kakizoe et al. 2007; Delgado et al. 2011)

In Chapter 3, I monitored post-release performance (i.e., movement and survivorship) of headstarted gopher tortoises soft-released at the Yuchi WMA in Burke County, Georgia (n = 51). The first release of head-starts occurred in the summer of 2014 and tortoises were tracked until November of 2015. The second release occurred in summer of 2015 and tortoises were also tracked until November of 2015. To their respective first dormancy period, the first release had 72.7% survivorship and the second release had 70.0% survivorship at the northwest release area and 7.1% survivorship at the southeast release area. Variation in survivorship for the second release was caused by early removal of soft-release pens at the southeast site due to red imported fire ants (Solenopsis invicta) gaining access to three pens and depredating head-starts. Fire ants were common across the landscape at Yuchi WMA, but population densities were difficult to discern. We opted to remove the pens to allow tortoises a chance to escape fire ants because tortoises had already begun constructing burrows in the pens and we had seen little evidence from the first release that mammalian mesopredators were potentially problematic. However, the early removal of pens at the southeast release area may have enabled mammalian mesopredators, namely raccoons (Procyon lotor), to prey on tortoises before they were able to build burrows extensive enough to protect them. All tortoises from the first release that survived to dormancy also survived over the winter to the following spring. Annual survivorship was 60.6% and survivorship to second dormancy was 48.4%. By contrast, Perez-Heydrich et al. (2012) estimated that hatchling gopher tortoise annual survivorship was only 12.8%, suggesting that head-starting results in more animals surviving to larger size classes.

Overall movement distances between subsequent burrows were small (0.1 - 119.1 m) and no tortoise ever moved further than 122.0 m from their release site. Even the largest movements in this study were smaller than some of the largest movements of wild hatchling and adult tortoises (Butler et

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al. 1995, Tuberville et al. 2005, Bauder et al. 2014) suggesting that head-starts demonstrated extremely high site fidelity, staying well within the recipient population. Movement distances were greatest the first 2-3 weeks after release but quickly decreased, which coincided with our highest mortality period (i.e., the first month post-release). Although movement distance itself was not associated with mortality, it is likely that tortoises are more susceptible to predation soon after release because they are leaving a centralized penning area which may be easier for predators to detect and building new burrows that may take several days to adequately construct.

Finally, I compared movement and survivorship for head-starts and hatchlings (n = 10 of each) hard-released simultaneously at adult tortoise burrows in the fall of 2015. Over the course of two months (i.e., until tortoises were considered dormant), two hatchlings and one head-start were censored because their signal never left the adult burrows where they were released. None of our movement metrics were significantly different between head-starts and hatchlings suggesting that head-starts and hatchlings move across the landscape similarly. However, head-start survivorship was higher (88.9%) compared to hatchlings (50.0%). Although this difference was not statistically significant, it was approaching significance at P = 0.1. The lack of statistical significance between head-starts and hatchlings may be due to the small sample sizes, the wide confidence intervals created by censoring, and the short time-frame of the study. Future research should focus on increasing the sample size of hatchlings and head-starts to help determine if there are in fact any significant differences between head-starts and hatchlings, especially regarding survival.

The data from this thesis suggests that head-starting may be a promising population recovery tool so long as predation can be mitigated soon after release when tortoises may be more susceptible to predation. Of course, different populations may face unknown challenges regarding predators and release sites (e.g., predatory species and predator densities). Perhaps the best way of mitigating mortality soon after release would be to conduct releases at different temporal and spatial scales,

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thereby reducing the likelihood of large mortality events due to an inappropriate site. Another consideration may be to head-start tortoises for longer time periods as in Nagy et al. (2015). Once tortoises reach a size threshold past a predator's ability to prey upon them, post-release survival will likely increase. While head-starting for longer periods could help post-release survival, it is important to also note that increased time in captivity may also have behavioral and/or physiological drawbacks (e.g., decreased site fidelity, inappropriate response to predators, growth related illnesses, increased susceptibility to pathogens, etc.). Head-starting can also be a time-intensive and costly endeavor. Management organizations need to weigh the cost of head-starting with the benefit it offers to depleted populations. However, tortoises are arguably one of the easiest taxonomic groups to raise in captivity. Head-starts had high hatching rates, 100% survival in captivity, and I have demonstrated that suitable diets can be readily acquired. Furthermore, many husbandry materials (i.e., rearing tubs, light fixtures, food plates, etc.) are reusable. While an actual cost analysis should be performed, the head-starting process for gopher tortoises is reasonably inexpensive compared to other species that need specialized resources in order to thrive.

Perhaps the most important aspect for management organizations to consider is the long-term efficacy of head-starting. Currently, no research has been conducted on populations with head-started gopher tortoises to determine the population-level benefit once they have had time to reach maturity (but see Green 2015 for long term head-start survivorship in Blanding's turtles; *Emydoidea blandingii*). Once more time has passed (i.e., 10-15 years), future research should focus on re-evaluating head-started tortoise survivorship and reproductive activity at Yuchi WMA. If head-starts stay on the managed site, survive to reproduce (i.e., increasing recruitment into the population), and are cost effective, then head-starting should be considered a viable management tool to help gopher tortoise populations persist into the future. Furthermore, if head-starting can aid gopher tortoise species as well.

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