Gopher Tortoise Hatching Success from Predator-Excluded Nests at Three Sites in Georgia

Gopher Tortoise (*Gopherus polyphemus*) populations are declining throughout their native range in the southeastern United States (Auffenberg and Franz 1982). Research into recruitment could lead to a better understanding of the dynamics of populations and perhaps identify strategies that would allow depleted populations to increase recruitment. Manipulating nest survival rates is one potential conservation strategy that can be used to bolster recovering populations, provided that sources of adult mortality have already been or are being mitigated (Tuberville et al. 2009). Egg hatching success, one of the primary factors influencing recruitment in oviparous reptiles, is controlled by both extrinsic (i.e., environmental factors that affect an egg's ability to hatch after nest construction) and intrinsic variables that influence egg failure prior to nest construction (i.e., infertility, resource provisioning to the egg or other maternal effects, genetic relatedness or incompatibility of parent pair; Bernardo 1996; Simmons 2005). While intrinsic decreases in recruitment are difficult to counteract, extrinsic threats may be more readily manageable. Perhaps the largest extrinsic threat to Gopher Tortoise hatching success are mammalian meso-predators such as Raccoons (*Procyon lotor*), Gray Foxes (*Urocyon cinereoargenteus*), Striped Skunks (*Mephitis mephitis*), and Nine-banded Armadillos (*Dasypus novemcinctus*) (Douglas and Winegarner 1977; Landers et al. 1980; Wright 1982; Smith et al. 2013). Consequently, many nest protection measures have been used to help conserve tortoises and other chelonians. It is often assumed that hatching success is higher than if predators had not been removed. However, studies from western ranges document relatively low hatching success even of eggs protected from predation (Epperson and Heise 2003; Hurley 1993; Noel et al. 2012). As part of a separate head-starting study involving the collection of eggs for rearing hatchling Gopher Tortoises, we sought to determine hatching success of protected nests comprising our recipient and two donor sites. Our objectives were to: 1) document and contrast hatching success in the absence of predation among our sites, and 2) demonstrate that our method of protecting nests does not significantly decrease hatching success.

Methods

From 27 June–28 July 2014, we searched for 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 Yuchi Wildlife Management Area (YWMA) in Burke County, Georgia (see Bauder et al. 2014). We searched for eggs by excavating soil in and around the entrances of active adult burrows. Burrow activity status was assigned based on presence of tortoise footprints in and around the burrow, absence of leaf litter on the apron and

DANIEL P. QUINN*
TRACEY D. TUBERVILLE
KURT A. BUHLMANN
University of Georgia Savannah River Ecology Laboratory,
Aiken, South Carolina 29802, USA

*Corresponding author: e-mail: dpq695@srel.uga.edu

TABLE 1. Summary of results from nest searching and egg incubation from Gopher Tortoise populations at St. Catherines Island (SCI), Reed Bingham State Park (RBSP), and the Yuchi Wildlife Management Area (YWMA), Georgia.

Site	No. clutches	No. eggs	Clutch size (range)	No. eggs damaged *	No. hatched	Site-level hatching success	Hatching success per clutch (range)
SCI	6	49	8.2 ± 2.7 (6–11)	0	44	89.8%	90.2 ± 8.4% (80.0–100%)
RBSP	9	67	7.4 ± 2.9 (3–12)	4	58	92.1%	92.9 ± 14.2% (57.1–100%)
YWMA	7	47	6.7 ± 1.4 (4–9)	2	42	93.3%	93.2 ± 8.9% (80.0–100%)

*Eggs damaged during the initial searching process were included in clutch size and egg numbers per site but were not included in further analyses.

TABLE 2. Hatching success of Gopher Tortoise (*Gopherus polyphemus*) eggs at different study sites throughout the species' range. Eggs were either incubated in-situ and not protected from nest predators (IS), incubated in-situ and protected from nest predators (ISP), or collected and incubated in laboratory incubators (INC). Dashed line delineates western and eastern populations.

Author	Incubation method	No. eggs	Hatch success	Location
Hurley (1993)	INC	14	50.0%	Ben's Creek WMA (LA)
	ISP	17	64.7%	Ben's Creek WMA (LA)
	IS	15*	0%	Ben's Creek WMA (LA)
Epperson and Heise (2003)	ISP	381	16.1–41.5%	Desoto National Forest (MS)
Noel et al. (2012)	INC	34	58.8%	Desoto National Forest (MS)
	ISP	53	16.7%	Desoto National Forest (MS)
Arata (1958)	IS	13	92.3%	Alachua County (FL)
Butler and Hull (1996)	ISP	103	80.6%	University of North Florida Campus (FL)
Demuth (2001)	INC	162	4.2–63.6%	Merritt Island NWR and Canaveral National Seashore (FL)
	ISP	12	66.7–83.3%	Merritt Island NWR and Canaveral National Seashore (FL)
Smith (1995)	ISP	75/92	67–97%	Katharine Ordway Preserve (FL)
Landers et al. (1980)	ISP	179	86.0%	Silver Lake Station, Decatur County (GA)
Rostal and Jones (2002)	INC	45	84.5%	George L. Smith State Park and Fort Stewart Army Reservation (GA)
Smith et al. (2013)	ISP	78*	66.4%	Joseph W. Jones Ecological Research Center (GA)
	IS	73*	34.9%	Joseph W. Jones Ecological Research Center (GA)
Burke et al. (1996)	INC	26	63–89%	Tillman Sand Ridge, Jasper County (SC)

*Number of eggs not reported. Number of clutches reported instead with nest survival percentage substituted for hatch success.

in the burrow, and smooth soil leading down the burrow (Smith et al. 2005). We used shovels to carefully shave the first 10–15 cm of soil in thin layers. We then removed deeper layers of soil to a depth of approximately 30 cm using small trowels and our hands. We searched as far into the burrow tunnel as possible and approximately one meter out from the entrance onto the burrow apron. When eggs were found, we placed flagging tape in the nest chamber to help locate eggs at a later date. We then recovered nests with the displaced soil so that eggs were not visible but flagging tape was. To protect eggs in-situ, we covered each nest with a ~0.5 m wide x 0.75 m long rectangle of ¼" (0.64 cm) hardware cloth and staked down the edges with landscaping stakes. We then covered the hardware cloth with the remaining displaced soil until the soil overburden was flush with the rest of the burrow apron. This ensured that all parts of the nest-protecting equipment were covered and that nest depth remained unaltered.

Because nest deposition dates (and therefore expected hatching dates) were unknown and because the distances between study sites precluded daily checks of nests left in-situ, we returned to each site to collect eggs 11–18 August before any eggs were expected to hatch (i.e., the earliest hatching dates in the literature; Landers et al. 1980). We carefully excavated soil and removed nest protection equipment. We then extracted each egg individually and marked the top of the egg with a soft lead pencil. Because turtle eggs can be sensitive to rotation and movement (Bustard 1972), we maintained all eggs in a stable, upright position throughout manipulation. Using tap water and a light towel, we carefully rinsed eggs and dried them off. We then placed eggs by clutch into separate plastic bins, burying them to approximately half their diameter in moistened perlite (1:1 water/perlite mass ratio). We incubated eggs at approximately 30°C based on hatching success in previous studies (Burke et al. 1996; Demuth 2001) and inspected them daily for hatching. We incubated eggs

from YWMA and RBSP in two Avey RCAB200 Reptile Cabinet Incubators at University of Georgia's Savannah River Ecology Lab in Aiken, South Carolina. We incubated eggs collected on SCI in two Georgia Quail Farm 1202 (110V, 225W) incubators at SCI. As pipping occurred, we removed eggs from the substrate and placed them individually in separate bins to allow hatchlings to absorb their external yolk sac. We designated eggs as non-viable if we smelled decomposition or if three weeks had passed since last clutch-mate had hatched. We opened all non-viable eggs to detect signs of embryogenesis. If an embryo was present or there were signs of vascularization, we documented the egg as fertile but failed. If not present, we designated the egg as infertile. We used ANOVA in program R (Version 3.1.0; R Development Core Team 2014) to compare clutch size and hatching success per clutch among sites and a χ^2 contingency test to compare survival had all fertile eggs hatched.

Results

SCI.—In late June we excavated 87 burrow aprons and found six nests (6.9%) comprising 49 intact eggs. On 10 Aug we collected eggs, which began hatching in the lab on 21 Aug and finished hatching 10 Sep. Mean clutch size was 8.2 eggs (range 6–11). Of the 49 eggs (Table 1), 44 hatched (89.8%). Three of the five unhatched eggs showed signs of late stage development, and two were designated as infertile.

RBSP.—In early July we excavated 66 burrow aprons and found nine nests (13.6%) comprising 67 eggs. Four eggs were accidentally damaged during nest searching, discarded, and were not included in further data analysis. On 11 Aug, we collected eggs, which began hatching on 18 Aug and finished hatching 20 Sep. Mean clutch size was 7.4 eggs (range 3–12). Of the 63 intact eggs collected (Table 1), 58 hatched (92.1%). Two of the unhatched eggs showed signs of late stage development, and three were designated as infertile.

YWMA.— In mid-late July, we excavated 61 burrow aprons and found seven nests (11.5%) comprising 47 eggs. Two eggs were accidentally damaged during nest searching, discarded, and were not included in further data analysis. We collected eggs in mid-August; they began hatching on 23 Aug and finished hatching on 18 Sep. Mean clutch size was 6.7 eggs (range 4–9). Of the 45 intact eggs (Table 1), 42 hatched (93.3%), and three were designated as infertile.

Comparisons among sites.—Among the three sites, we excavated 214 burrow aprons for nests and found 22 clutches (approx. one nest for every 10 burrows excavated). Six eggs of 163 (3.7%) were accidentally damaged during nest searching, but no eggs were damaged when placing the hardware cloth over nests, during extraction from the nest, or during transport from the field to incubators. Although eggs were not counted when nests were initially detected, we found no evidence of egg damage or depredation during extraction including presence of Red Imported Fire Ants (*Solenopsis invicta*). Hatching success averaged 91.7% among the three sites, and mean hatching success per clutch did not differ significantly among sites ($F_{2, 19}$ = 0.128, P = 0.881). Clutch size averaged 7.4 eggs overall (range = 3–12), and clutch size did not differ significantly among sites ($F_{2, 19}$ = 0.567, P = 0.576). Six unhatched eggs were designated as infertile, however seven showed signs of late stage development. Even if all seven of these eggs had successfully hatched, the resulting hatching success would not be significantly higher than the hatching success we observed in intact eggs (χ^2= 0.75, d.f. = 1, P = 0.10)

Discussion

Hatching success was not significantly different among the three sites and was relatively high compared to other studies that manipulated nests (Table 2). Of the 157 eggs incubated from all three study sites, only 13 failed to hatch (i.e., 91.7% hatching success), 6 of which were designated as infertile. We cannot account for what caused mortality of the remaining seven fertile eggs but even if all had successfully hatched, the resulting hatching success would not be significantly higher than the hatching success we observed. Therefore, in addition to documenting relatively high egg viability and hatching success, our data also indicate that the nest protection techniques we used did not significantly decrease hatching success. Though other studies of eastern tortoise populations reported egg hatching success in protected or lab-incubated nests as high as we report here, our hatching rates are a stark contrast to the rates reported for western populations (i.e., Mississippi and Louisiana), which range from 0%–64.7% (Table 2).

Although innate hatching success appears to be high in most eastern populations for which data are available, extrinsic threats may still significantly reduce recruitment when nests are not protected. For example, we did not detect any evidence that eggs were depredated at our protected nests, but we did observe nest predators (e.g., Raccoons, Nine-banded Armadillos) and their tracks, as well as eggs depredated from nearby unprotected nests. A study in southwest Georgia found that unprotected nests can experience upwards of 65% nest predation (Smith et al. 2013). Nest protection has also been shown to be one of the most effective methods for reducing predation on sea turtle nests by Raccoons and presumably other mesopredators (Ratnaswamy et al. 1997). Although in-situ nest protection serves to prevent vertebrate nest predation, it does not protect pipping and hatchling turtles from invertebrate predation (upwards of 70% predation; Allen et al. 2001). Our protocol of transferring late-stage eggs to the laboratory prior to pipping reduces this risk. While this study was not designed to determine predation rates between protected and unprotected nests, the literature suggests that our mitigation of nest and post-hatching predators very likely increased the number of hatchlings. Therefore, protecting nests may be a potential management option for increasing recruitment in Gopher Tortoise populations.

Because adult survival is the most significant parameter affecting population viability in turtles, increasing recruitment is unlikely to help recover depleted populations on its own (Heppell 1998). Conservation efforts must first start with mitigating adult mortality. However, even once adult mortality has been mitigated, populations may still fall well below a minimum viable population size. The Gopher Tortoise Council MVP Report (2013) set a minimum threshold for Gopher Tortoise populations at 250 adult animals. Unfortunately, adult tortoises are not readily obtainable for planned conservation efforts aimed at bolstering population sizes. Other options such as head-starting hatchling tortoises may be a more useful tool for planned conservation measures aimed at augmenting these depleted populations. One of the primary concerns associated with population manipulations is ensuring that source populations remain viable. By reducing nest predation in the source population, conservationists and resource managers could increase recruitment closer to the intrinsic rate for that population and use surplus offspring to increase population sizes in depleted populations elsewhere (Buhlmann et al.

2015). Although protecting nests to increase recruitment may not necessarily be an efficient conservation strategy by itself, it could be used as a tool to enable other strategies such as head-starting that rely on increasing recruitment in order to augment population sizes.

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