
THE EFFECTS OF ISOLATION ON THE DEMOGRAPHY AND GENETIC DIVERSITY OF LONG-LIVED SPECIES: IMPLICATIONS FOR CONSERVATION AND MANAGEMENT OF THE GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*)

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Abstract.—In the southeastern United States, habitat loss has fragmented the landscape and isolated many populations of this region's flora and fauna, which has presumably resulted in smaller population sizes and reduced levels of genetic diversity. For example, forestry practices and anthropogenic disturbances are both cited as factors fragmenting the once extensive range of *Gopherus polyphemus*. One localized, but extreme, source of fragmentation was the impoundment of the Chattahoochee River in 1963 to form Walter F. George Reservoir along the border of Georgia and Alabama. The formation of this reservoir isolated populations of *G. polyphemus* on two newly created islands providing a natural laboratory to explore the demographics and genetic effects of fragmentation on a long-lived species. These populations were first surveyed in 1984 and, 21 years later, we revisited them to collect demographic data and tissue samples for genetic analysis. We genotyped all individuals for 10 microsatellite loci, and we tested these data for bottlenecks and compared them to levels of genetic diversity for populations from other portions of the range. We found 45 and two individuals on the larger and smaller islands, respectively. On the large island, however, the population size was identical to the 1984 survey. Only the population structure based on estimated age differed between the 1984 and 2004 surveys, while population size structure based on carapace length, sex ratio, and sex-specific growth rates did not differ. The population of the large island showed genetic evidence of a past bottleneck. The genetic diversity indices from the population of the large island, however, were comparable to or greater than those found at mainland sites, in particular from western populations.

Key Words.—conservation, demography, fragmentation, genetic diversity, *Gopherus polyphemus*, Gopher Tortoise, isolation, USA

INTRODUCTION

Species experience the landscape as a heterogeneous mixture of suitable habitats within a matrix of non-suitable habitats separating them (Hanski and Simberloff 1997). Therefore, the distribution of a species is not commonly continuous or uniform through the landscape (Stacey et al. 1997). The major causes of fragmentation today, however, are not natural but anthropogenic (e.g., Ranney et al. 1981). Habitat loss and fragmentation were identified as the leading cause of extinction of wildlife (Diamond 1984; Groombridge 1992), including turtle species (Mitchell and Klemens 2000). Small, isolated populations have a greater probability of extirpation than larger populations (Gilpin and Hanski 1991) caused by an increased sensitivity to demographic and environmental stochasticity (Lande 1993; Amos and Balmford 2001). In addition, smaller populations usually exhibit lower genetic diversity, which reduces the evolutionary potential of a population (Frankham

1996) and may lead to inbreeding depression (Lande and Barrowclough 1987; Lynch et al. 1993; Lande 1994). Both stochasticity (environmental and demographic) and low genetic diversity can also increase the probability of extirpation.

Certain species and populations are inherently more sensitive to fragmentation, depending on their life-history traits and ecology (Ewers and Didham 2006). In particular, species possessing low reproductive potential and population growth rates, specialized habitat requirements, poor dispersal ability, and/or low abundance are likely to be negatively influenced by fragmentation (reviewed by Henle et al. 2004; Ewers and Didham 2006). The geographic location of a population within a species' range (i.e., the periphery of the range) also alters the influence of fragmentation (Swihart et al. 2003). Long-lived vertebrates possess a suite of life-history traits, such as delayed sexual maturity and low annual reproductive output (Holgate 1967; Murphy

1968), which tend to make them particularly sensitive to the demographic changes (Congdon et al. 1993).

Even though fragmentation can reduce population size and genetic diversity via genetic drift, the severity (i.e., population reduction) and temporal aspects of the fragmentation play important roles in determining to what extent a population maintains genetic diversity. For example, loss of genetic diversity is more rapid in smaller populations (Lande 1993; Amos and Balmford 2001) because of their sensitivity to demographic and environmental stochasticity and drift. However, a population's maintenance of genetic diversity post-fragmentation is highly influenced by the species' suite of life-history traits (Dinerstein and McCracken 1989; Kuo and Janzen 2004; Hailer et al. 2006; Lippé et al. 2006) as well. Although possession of certain life-history traits makes demographic traits of a species sensitive to fragmentation, other life-history traits, in particular longevity (i.e., long-generation time), provide an "intrinsic buffer" to loss of genetic diversity by increasing generation time (Hailer et al. 2006), thus slowing genetic drift.

The Gopher Tortoise (*Gopherus polyphemus*) is a long-lived species (i.e., longevity 40–60 years; Landers 1980) and one of the best studied and heavily managed turtle species in North America (Ernst and Lovich 2009). Despite this fact, *G. polyphemus* experienced and continues to experience severe population declines throughout its range (Auffenburg and Franz 1982; McCoy and Mushinsky 1992; Waddle et al. 2006; Hammond 2009) primarily due to habitat loss and degradation (Auffenburg and Franz 1982; Lohoeferner and Lohmeier, unpubl. report; McCoy et al. 2006). Interestingly, *G. polyphemus* exhibits most the characteristics suggested by Henle et al. (2004) and Ewers and Didham (2006) to result in a species being inherently sensitive to fragmentation. In particular, *G. polyphemus* has low annual reproductive potential and population growth rates (Ernst and Lovich 2009), specialized habitat requirements (i.e., open tree canopy, herbaceous understory, and xeric sandy soils; Ernst and Lovich 2009), and poor dispersal ability (i.e., < 1 km; Eubanks et al. 2003). Undoubtedly, habitat loss has severely altered population dynamics in the federally listed western portion of the range, which is west of the Tombigbee and Mobile Rivers, Alabama (U.S. Fish and Wildlife Service 1990), where habitat fragmentation, especially in Mississippi populations, has long been considered a problem (Lohoeferner and Lohmeier 1981). Several Mississippi populations were shown to possess low rates of hatching success and recruitment (Epperson and Heise 2009) and reduced levels of genetic diversity compared to populations in the eastern portion of the range (Ennen et al. 2010).

Although much of the distribution of *G. polyphemus* has become fragmented, limited research is focused on

investigating the effects of isolation on demography and genetic diversity. Here, we present data on two sites inhabited by *G. polyphemus* that were isolated from the mainland in 1963 by the construction of a dam in the Chattahoochee River that formed Walter F. George Reservoir (WFGR). One island is approximately 8.45 ha while the other island is 1.90 ha (Wester 1986). In 1984, about 20 years after isolation, surveys were conducted that found 45 and 27 individuals on the large and small islands, respectively (Wester 1986). Population structures within these sites were skewed toward smaller individuals (i.e., 66.7% of the total population) without secondary sex characteristics (Wester 1986), suggesting reproduction and recruitment were occurring. Because of their locations and known history of isolation, these islands provide an opportunity to study the effects of isolation on a population of a long-lived species, *G. polyphemus*. The goals of this study were to conduct a population survey of the islands to compare demographic and growth data with a survey conducted 21 years prior (Wester 1986) and to investigate the genetic compositions of the populations of *G. polyphemus* on the islands relative to eastern and western portions of the range.

MATERIALS AND METHODS

Study area.—We captured *Gopherus polyphemus* from several sites throughout the range between 2004 and 2008 (Fig. 1). Two island sites, which included the large island (L_island: 31° 42' 49" N, 85° 06' 15" W) and the small island (S_island: 31° 41' 03" N, 85° 04' 51" W), were sampled for tissue collection for genetic comparisons and demographic data. For a genetic comparison to the island sites, we sampled from eight non-island sites, in the western (i.e., threatened under the Endangered Species Act) and eastern (not federally listed) portions of the species' range. The four western sites consisted of localities in Mississippi with known low genetic diversity (Ennen et al. 2010): T44 West (T44W: N 31° 04' 52", W 89° 07' 45"), T44 East (T44E: N 31° 04' 47", W 89° 06' 05"), McLaurin (McL: N 31° 08' 47", W 89° 06' 05"), and Crossroads (Xrd: N 30° 57' 24", W 89° 06' 32"). The four eastern sites were mainland localities in Georgia that were located relatively close to the two island sites: Fort Benning (FtB: 32° 21' 27" N, 84° 57' 22" W), Andrew's Lock and Dam (ALD: 31° 15' 51" N, 85° 06' 29" W), Green Grove (GG: 31° 17' 08" N, 84° 27' 36" W), and Wade Tract (WT: 30° 45' 43" N, 83° 59' 59" W). No demographic data were available for non-island sites; accordingly, they were included only for comparative purposes, where we compared genetic diversity between island and non-island sites.

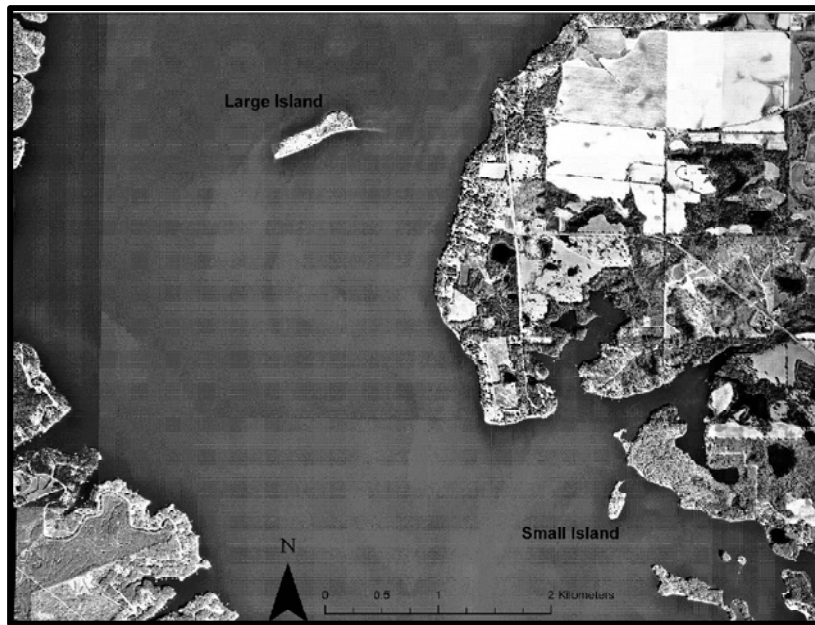
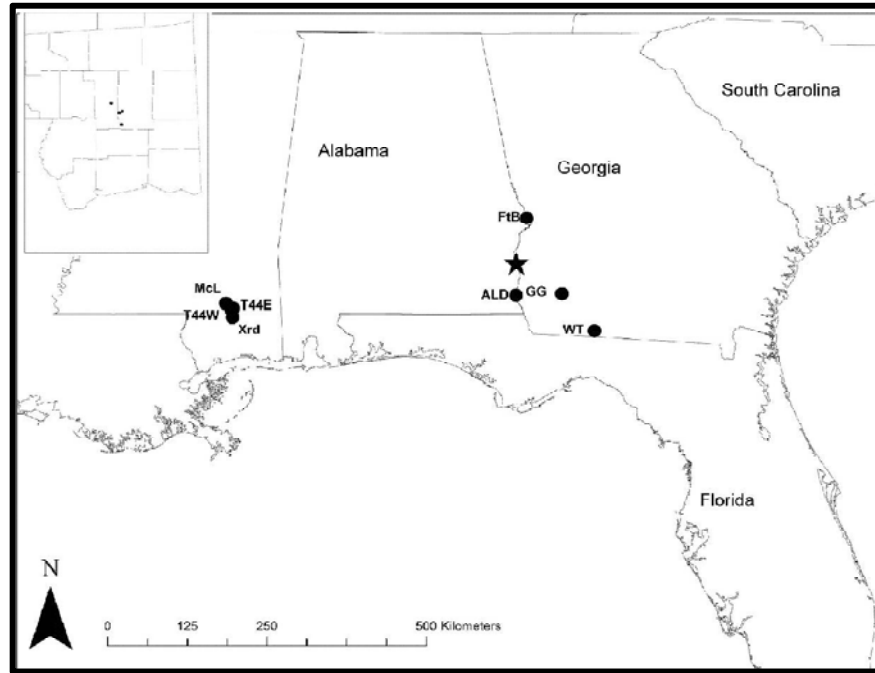


FIGURE 1. Both island populations (indicated by the star) of Gopher Tortoises (*Gopherus polyphemus*) were located in Walter F. George Reservoir, located on the Chattahoochee River on the border of Alabama and Georgia (Top). Four reference sites (circles) were located in the surrounding area of the island populations, and four other sites were located in the western portion of the range for *G. polyphemus*. The map at the bottom shows the relative location of the two islands with respect to the shore of the reservoir.

Capture and collection methods.—Using Tomahawk Live Traps (Tomahawk Live Traps, Tomahawk, Wisconsin, USA), we captured *G. polyphemus* at each of the 10 sites. On the islands, we scoped all located burrows using an infrared camera both before and after the capture of the inhabitant with the exception of hatchling burrows. Therefore, we are confident that we sampled the majority of the tortoise population on the islands. We took tissue samples either as blood or a shell clip at all 10 sites. For blood samples, we collected approximately 0.5 mL of blood from the iliac or femoral veins using a heparinized 23- or 26-gauge needle and 1-mL syringe, and this was placed into a 1.5-mL vial with approximately 0.5 mL of tissue preservation buffer (Seutin et al. 1991). Some tissue samples were collected from shell shavings or clips of the marginal scutes and stored in 95% ethanol. All tissue samples were stored at -20°C .

For the island sites, we recorded standard morphological measurements (i.e., carapace length, plastron length, and mass) and, with the exception of one individual, took a digital photograph of the dorsal and ventral side for each individual. Also, we gave each island individual a unique identification number by notching or drilling the marginal scutes. We determined sex by secondary sex characteristics (i.e., plastron concavity and length of gular projections) reported by McRae et al. (1981). We estimated age by counting scute annuli. Although some question the validity of determining ages of turtles using scute annuli (Wilson et al. 2003), others have validated this relationship within several *Gopherus* species (e.g., *Gopherus agassizii*, Germano 1988, 1998), including *G. polyphemus*, but only to 13–15 y (Mushinsky et al. 1994, Aresco and Guyer 1998). This limitation makes it difficult to determine the age of *G. polyphemus* past sexual maturity, which in southwestern Georgia occurs at an age of about 19–21 y (Landers et al. 1980). Although the best way of calculating growth rates are from data of known ages or validation of the 1:1 ratio between scute annuli and years of age, this type of data was unobtainable for our study. Therefore, we assumed a 1:1 ratio between annuli and age and excluded tortoises more than 21 years old in the growth analysis. We compared our demographic data with those of Wester (1986), which were collected on the islands in 1984.

We tested (Shapiro-Wilk) all morphological data for normality prior to statistical testing. We conducted statistical analyses on each sex separately to account for sexual dimorphism. To compare our morphological data (i.e., carapace and plastron length and mass) to that of Wester (1986), we conducted several one-way analyses of variance (ANOVA) using mass, carapace length, and plastron length as dependent variables, and sampling year as the independent variable. In the ANOVA, the “year” variable represented 2005 (i.e., this study) and

1984 (i.e., Wester 1986). However, we could not compare the mass of individuals between surveys because of the small sample size of males weighed in 2005. To determine if size and age structure changed on the islands from 1984 to 2005, we conducted nonparametric Kolmogorov-Smirnov tests using carapace length and estimated age as the dependent variables. Similarly, we conducted a Chi-square (Yates correction for continuity) test to determine if the sex ratio differed between 1984 and 2005.

Because spatial or temporal differences in growth rates can influence demographic parameters of turtle populations (Lovich and Gibbons 1990; Lovich et al. 2010), we compared growth in the population of the large island between 1984 and 2005. Past studies of growth in *Gopherus polyphemus* used non-linear equations based on known age or growth-interval forms of the von Bertalanffy equation (Mushinsky et al. 1994; Aresco and Guyer 1998). We used tortoises less than 22 y of age and fit our data using linear regression, a technique previously used by Germano (1992) for *G. agassizii*. Males and females were analyzed separately because of sexual size dimorphism (Mushinsky et al. 1994). We included juveniles of unknown gender in the growth analyses for each sex (Lovich et al. 1998; Germano and Bury 2009; Germano 2010). All statistical analyses were performed using JMP 7.0.1 (SAS Institute, Cary, North Carolina, USA) and Systat 13 (Systat Software, Evanston, Illinois, USA) software using an alpha of 0.05.

Genetic methods.—We extracted total genomic DNA from the samples using the blood or tissue protocols from the Qiagen DNeasy extraction kit (QIAGEN Inc., Valencia, California, USA). We genotyped each individual for 10 microsatellite loci: A003, A006, A110, B004, B011, B102, B110, B118, D004, and D006 (Kreiser et al., pers. comm.). The polymerase chain reaction (PCR) amplifications were conducted in a total volume of 12.5 μL using 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 1.5–2.0 mM MgCl_2 , 200 μM dNTPs, 0.1875 units of *Taq* polymerase (New England Biolabs), 0.3 μM of the M13 tailed forward primer (Boutin-Ganache et al. 2001), 0.3 μM of the reverse primer, 0.1 μM of the M13 labeled primer (LI-COR, Lincoln, Nebraska, USA), 20–100 ng of template DNA and water to the final volume. Thermal cycling conditions consisted of an initial denaturing step of 94°C for two min followed by 35 cycles of 30 sec at 94°C , one min at $56\text{--}60^{\circ}\text{C}$, and one min at 72°C . A final elongation step of 10 min at 72°C ended the cycle. Alleles were visualized using a LI-COR 4300 DNA Analysis system and genotypes were scored using Gene Image IR v. 3.55 (LI-COR, Lincoln, Nebraska, USA).

We assessed the presence of null alleles and scoring errors using MICRO-CHECKER v. 2.2.3 (van

TABLE 1. The change in population size and density of Gopher Tortoises (*Gopherus polyphemus*) on the small island (S_Island) and the large island (L_Island) from 1984 to 2005. Population density is reported in the number of tortoises per hectare, and ha refers to the size of the island in hectares.

Site	ha	Population Size		Population Density	
		1984	2005	1984	2005
L_Island	8.45	45	45	5.33	5.33
S_Island	1.9	27	2	14.21	1.05

Oosterhout et al. 2004). We conducted exact tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) in GenePop v. 3.4 (Raymond and Rousset 1995) with the significance of these tests adjusted using a sequential Bonferroni correction (Rice 1989). For each of the 10 sites, we measured the number of alleles (N_A), percentage of polymorphic loci (%P), and expected heterozygosity (H_E) values using GenePop v. 3.4 (Raymond and Rousset 1995). To test for a genetic signature of a population bottleneck, we used BOTTLENECK ver 1.2.02 software (Cornuet and Luikart 1996) and the M ratio of Garza and Williamson (2001). For BOTTLENECK, we used the two-phased model of microsatellite evolution with a 30% stepwise mutation model and a 70% infinite allele model run with 1,000 permutations. Parameters for M ratio included a proportion of one-step mutations of 90% and an average size non one-step mutations (Δg) of 3.5. We determined the significance of each value of M by comparison to the critical value obtained from 95% threshold of 10,000 simulations of an equilibrium population.

Because we only found two individuals at the smaller island site, we could not statistically test for differences in levels of genetic diversity between the island and non-island sites. Therefore, the comparisons between sites were descriptive only. Because our sites had varying sample sizes, we conducted several Spearman's correlations (the data were not normal) to determine if sample size influenced genetic diversity, which it did not (N_A : $r_s = 0.41$, $P = 0.27$; H_E : $r_s = 0.23$, $P = 0.55$; %P: $r_s = 0.04$, $P = 0.91$). Therefore, we did not need to use rarefaction to adjust for sample size, and we use raw numbers in our descriptive statistics. We used BOTTLESIM (version 2.6; Kuo and Janzen 2003) to investigate the retention of genetic diversity in the large island population over 200 years. We ran the simulations with completely overlapping generations, dioecious reproduction, random mating, and 1,000 iterations. We kept populations constant (i.e., 45 individuals) throughout the simulations with life span set at 40. Number of females in the population was held constant at 15 with sexual maturity at age 15 y.

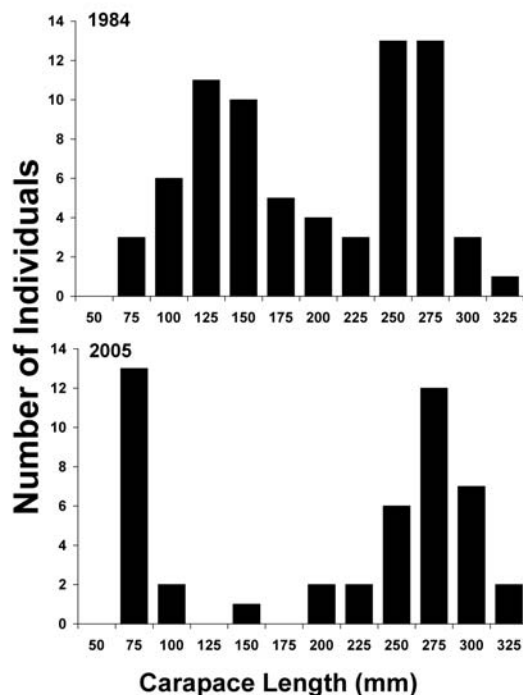


FIGURE 2. A histogram of the size-class distribution of 45 *G. polyphemus* individuals in 2005 and 1984 (Wester 1986) on the large island in Walter F. George Reservoir, located on the Chattahoochee River on the border of Alabama and Georgia.

RESULTS

Island demography and growth.—Trapping yielded 45 individuals from the large island and two individuals from the small island (Table 1), none of which were marked by Wester (1986). The small island supported a lower density of tortoises (1.05/ha) than the large island (5.33/ha; Table 1). The large island had a sex ratio of 0.53:1 (male to female); while we found one male and one female on the small island. The sex ratios between 1984 (1.14:1) and 2005 did not differ significantly ($\chi^2 = 0.63$, $df = 1$, $P = 0.43$) on the large island. Most age classes were represented within the large island population, and juveniles (i.e., all immature individuals, including putative hatchlings) made up 48.9% of the population. We found that the population structure based on carapace length had not shifted on the large island between 1984 and 2005 ($D = 0.244$, $P = 0.12$; Fig. 2). However, population structure based on estimated age had shifted more toward older individuals on the large island between 1984 and 2005 ($D = 0.375$, $P = 0.01$). Linear growth equations provided a close fit to the data, as shown by high coefficients of determination (Table 2). Growth rates between the sexes were significantly different only in 2005 when considering the overlap between the 95% confidence intervals based on

TABLE 2. Growth analysis results for Gopher Tortoises (*Gopherus polyphemus*) on the large island, comparing slopes of the regression lines coupled with standard error and 95% confidence intervals among the sexes and between years.

Sex/Year	Slope (SE)	95% Confidence Interval	R ²
Female			
1984	11.36±0.60	10.76-11.96	0.96
2005	11.41±0.41	11.00-11.82	0.98
Male			
1984	10.90±0.65	10.25-11.54	0.96
2005	10.46±0.47	9.99-10.93	0.97

standard errors of the slopes (Table 2); however, differences were not significantly different for the sexes between years. For females, there was no difference in carapace length ($F_{(1, 20)} = 0.02, P = 0.88$), plastron length ($F_{(1, 20)} = 0.21, P = 0.65$), and mass ($F_{(1, 11)} = 0.01, P = 0.93$) between years. For males, there was no difference in carapace ($F_{(1, 14)} = 0.36, P = 0.56$) and plastron ($F_{(1, 15)} = 0.37, P = 0.55$) lengths between years (Table 3).

Genetic analyses.—We genotyped 204 individuals from nine sites, which had varying sample sizes (Table 4). There was no evidence of null alleles or LD at any site, and HWE expectations were only violated at one locus (D004) in two populations (T44E and T44W). Because the small island only had two individuals in 2005, we are only reporting genetic results from the large island and the other non-island sites (Table 4). The island site was polymorphic at all 10 loci as were all of the eastern populations. Also, the island site had similar levels of genetic diversity ($N_A = 4.7 \pm 2.2, H_E = 0.61 \pm$

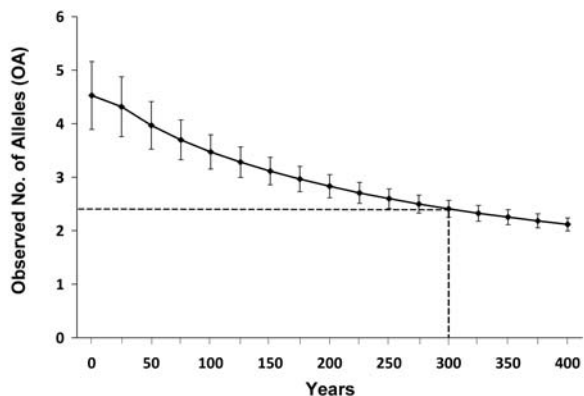


FIGURE 3. The results of the BOTTLESIM analysis showing the loss of genetic diversity in the population of the large island in the form of allelic richness (N_A). Vertical bars indicate standard errors over 400 years or 10 generations, assuming the population size remained constant. The intersection of the dashed lines represents the mean number of alleles for all western populations.

0.2) as eastern sites (N_A ranging from 3.4–4.9, H_E ranging from 0.50–0.61). Both the M ratio and BOTTLENECK software detected evidence of bottlenecks at several of our sites. For example, bottlenecks were detected on the large island and the ALD site through heterozygosity excess or deficiency using the BOTTLENECK software. The M ratio software detected bottlenecks only within the FtB site.

Frankham et al. (2002; but also see Soulé et al. 1986) proposed that one goal of conservation is the preservation of 90% of the initial genetic diversity for 100 years. In our simulations for the large island population, both the observed number of alleles (OA)

TABLE 3. The means and standard deviation of the three morphological measurements recorded for *Gopherus polyphemus* inhabiting the large (L_Island) and small islands (S_Island) in Walter F. George Reservoir in 1984 and 2005. Abbreviations are defined in the text.

Year	Site/Sex	N	CL (mm)	PL (mm)	Mass (g)
2005					
L_Island					
	Female	15	279.1±29.7	267.9±27.2	3885.0±993.6
	Male	8	253.3±16.9	245.5±19.8	3730.0±452.6
	Juvenile	22	111.1±72.4	106.6±70.7	665.6±1009.5
S_Island					
	Female	1	275.00	263.00	NA
	Male	1	261.00	250.00	2900.00
1984					
L_Island					
	Female	7	277.2±18.8	273.4±23.1	3936.3±1130.1
	Male	8	249.2±8.4	250.0±8.0	2931.0±332.1
	Juvenile	30	145.3±57.1	144.5±57.5	826.8±892.0
S_Island					
	Female	5	268.4±12.9	264.6±14.6	3623.2±740.6
	Male	3	247.3±8.7	253.0±12.1	2860.0±433.0
	Juvenile	19	147.9±48.9	148.1±50.5	854.2±770.2

TABLE 4. A comparison of genetic diversity levels of Gopher Tortoises (*Gopherus polyphemus*) among nine sites evaluated in this study. Site and genetic diversity abbreviations are provided in the text, and N represents sample sizes. The values reported for N_A and H_E are averaged (with standard errors) across 10 microsatellite loci.

Site	N	N_A	H_E	% P
Western				
T44W	40	2.9±1.8	0.39±0.2	90.0%
T44E	20	2.4±1.0	0.37±0.2	90.0%
McL	8	2.1±0.6	0.37±0.2	90.0%
Xrd	16	2.3±1.0	0.39±0.2	90.0%
L_Island	44	4.7±2.2	0.61±0.2	100.0%
Eastern				
ALD	10	4.4±2.6	0.61±0.2	100.0%
WT	15	3.6±1.0	0.50±0.2	100.0%
GG	16	3.4±1.1	0.51±0.2	100.0%
FtB	35	4.9±2.3	0.60±0.2	100.0%

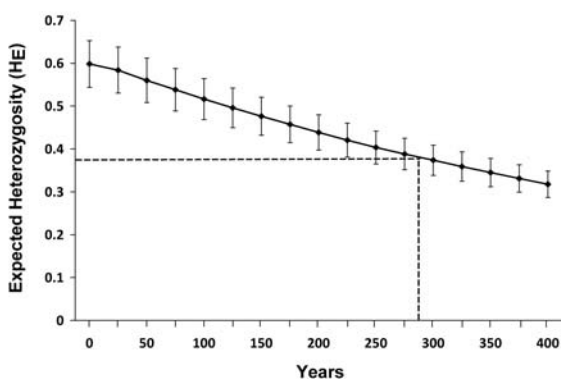


FIGURE 4. The population of the large island gradually lost genetic diversity in the form of expected heterozygosity (H_E). Vertical bars indicate standard error over 400 years or 10 generations, assuming the population size remained constant. The intersection of the dashed lines represents the mean H_E for all western populations.

and expected heterozygosity (H_E) declined steadily over time (Figs. 3 and 4), with 90% of the initial genetic diversity being retained for only 40 and 74 years for OA and H_E , respectively. In approximately 300 years (i.e., 7.5 generations), genetic diversity levels of the large island would be similar to federally listed western populations (Figs. 3 and 4).

DISCUSSION

The effects of fragmentation on certain species' populations have a temporal element associated with the population response to the disturbance (e.g., Ewers and Didham 2006). For long-lived species, the signature of a population decline and the effects of fragmentation sometimes do not immediately manifest themselves but occur slowly over an extended period of time after the disturbance (Congdon et al. 1994; Mortimer 1995;

Ewers and Didham 2006). This could be the case for the *G. polyphemus* population on the large island but not the small island. For example, the population of the small island decreased over 21 years from 27 to two individuals, while the population of the large island seemingly remained stable. Interestingly, the small and large island sites were smaller in area (i.e., 1.90 and 8.45 ha, respectively) than what Cox et al. (1987) and Eubanks et al. (2002) considered as the minimum patch size for a population of 50 individuals of *G. polyphemus*; 10–20 ha and 19–41 ha respectively. Therefore, the decrease in population size on the smaller island was expected and probably linked to the small size of this island (i.e., decrease in availability of habitat and resources). However, the population size stability on the large island, where a small patch of 8.45 ha supported a population of 45 *G. polyphemus* since the mid-1960s, was contrary to the findings of Cox et al. (1987) and Eubanks et al. (2002).

Besides the area difference between the islands, there are potential habitat differences attributing to the disparities between population sizes. Before completion of the dam and creation of the reservoir, the large island was an upland ridge with deep sandy soils and an open canopy, which is considered good habitat for *G. polyphemus* (Ed. Wester, pers. obs.). Conversely, the small island does not possess these characteristics. Since the creation of the reservoir, the vegetation changed because of the lack of fire, which caused both islands to have a more closed canopy (Roger Birkhead, pers. obs.). The large island, however, still contains more relatively open areas than the small island.

The initially high density on the small island followed by a decline over time matches the general trend exhibited by other species experiencing fragmentation (e.g., Debinski and Holt 2000). In a very similar scenario, this phenomenon was also reported in a Red-footed Amazonian Tortoise (*Geochelone carbonaria*) population that was isolated from the mainland by a reservoir in Venezuela (Aponte et al. 2003). The 1984 *G. polyphemus* density of the small island was high (14.21 tortoises/ha) compared to most other studies: 6.9 tortoises/ha (Auffenberg and Iverson 1979), 10.23 tortoises/ha (Auffenberg and Franz 1982), 3.3–15.8 tortoises/ha (Landers and Speake 1980), 11.3 tortoises/ha (Kushlan and Mazzotti 1984), and 5.33 tortoises/ha on L_Island (this study), but the high density was followed by a population decline to a much lower density of 1.05 tortoises/ha in 2005.

Although population size on the large island was identical (i.e., 45 individuals) in 1984 and 2005, even after an anthropogenic perturbation (i.e., the formation of WFGR in 1963), we found no individuals marked by Wester (1986). This suggests that within 21 years, all individuals had been replaced. Even if the earlier survey missed several tortoises, 40% of the population was

young enough (i.e., estimated to be one to 10 y old [Wester 1986]) for us to recapture them 21 years later. The lack of recaptures could be explained by growth of the marginal scutes that potentially obscured the notches of individuals, in particular hatchlings and juveniles, leading us to consider individuals as unmarked. However, in 2005, the population of the large island had six individuals large enough (CL: 283–334 mm) to be an estimated 40 years or older (i.e., using Landers et al. [1982] estimations based on carapace length), which equates to six individuals of approximately 20 years old on the island in 1984. Because annual growth of *G. polyphemus* is negligible (< 1 mm; Landers et al. 1982) after 19 years of age, the probability of misidentifying notched scutes on adult *G. polyphemus* in the population of the large island is extremely low, especially because notches in adult and sub-adult individuals in 1984 were at least 6.35 mm in depth (Ed Wester, pers. comm.). Although shell notches in turtles validated by long-term studies were near permanent (Gibbons 1990), we recognize that there is a potential for missing previously marked individuals, especially hatchlings and juveniles, because of growth of the marginal scutes. Given that growth rates and sex ratios were not appreciably different between 1984 and 2005, demographic differences must be due to changes in recruitment or mortality. An increase in mortality would also explain not finding any marked individuals. Interestingly, the longevity for *G. polyphemus* was estimated between 40–60 years (Landers 1980), which would suggest that the island population has a much lower longevity (or higher emigration potential) than previously reported.

Another alternative to explain the appearance of population turnover on the large island could be that tortoises immigrated from the surrounding mainland populations, either by floating, or intervening human activities. Several tortoise species (i.e., *Testudo gigantea* and *Geochelone elephantopus*), including *Gopherus polyphemus*, have the ability to float and swim (Patterson 1973), making immigration to the large island feasible. The large island is approximately one and 1.9 km from the eastern and western shores of the lake, respectively. Also, there is the potential for anthropogenically assisted immigration events to the large island. Humans are known to frequent the island via boat access and could have transported individuals to the island. Conversations with U.S. Army Corps of Engineers personnel confirmed that they have not released tortoises on either island over the last 40 years (Bill Smallwood, pers. comm.). Although both immigration scenarios could be possible, we did not witness any individuals swimming or floating within WFGR or see humans placing tortoises on the island.

Although population size can remain constant long after a perturbation event, age-class structure of long-lived species can shift relatively quickly (Holmes and

York 2003). For example, the age-class structure of a population of *Geochelone carbonaria* was skewed more toward juveniles approximately 20 years after isolation from the mainland (Aponte et al. 2003). In the case of our *G. polyphemus* population that was isolated from the mainland for approximately 40 years, age structure on the large island statistically changed over a 21-y period but was not skewed towards juveniles. This incongruence between Aponte et al. (2003) and our 2005 age-structure data were probably associated with differences in time since isolation. For example, when comparing age structure 20 years after the isolation events (i.e., Wester [1986] vs. Aponte et al. [2003]), the population inhabiting the large island was skewed toward juveniles (66.7% of the population), similar to the island population of *G. carbonaria*. Alternatively, the low proportion of juveniles on the large island in 2005 is potentially associated with habitat quality. For example, McCoy and Mushinsky (2007) suggested that minimum patch size is complicated by habitat quality and that low proportions of observed juveniles were potentially associated with populations with high densities and poor habitat quality. Although habitat quality undoubtedly decreased due to lack of fire on the large island, population density on this island in 2005 was relatively low compared to known population densities of 3.3–15.8 tortoises/ha for *G. polyphemus* (Auffenberg and Iverson 1979; Landers and Speake 1980; Auffenberg and Franz 1982; Kushlan and Mazzotti 1984).

Although age-class structure shifted between 1984 and 2005, population structure based on carapace length was not different between years but still was more skewed toward larger individuals. Because of issues with using annuli to estimate age without validation of the 1:1 ratio (Wilson et al. 2003), the population shift based on age structure could be due to differences between our and Wester's (1986) ability to accurately estimate age from annuli. It is worthwhile to note, however, that size structure and age structure do not always correspond in the wild. For example, Bury et al. (2010) reported that age and size structure did not match in half of their populations of *Actinemys marmorata*. Therefore, our incongruent results could be a natural phenomenon and not a reflection of technique. Similar to population structure based on size, the sex ratio shifted from a slight male bias (i.e., 1.14:1) to a female bias (i.e., 0.53:1), although this was not statistically different between years.

After 40 years of isolation, the population of tortoises the large island has shown genetic signatures of past bottlenecks. Undoubtedly associated with the isolation and fragmentation event (i.e., formation of the reservoir), the population has definitely experienced an overall reduction in population size as the island site was isolated from the rest of the landscape. Although we

know the temporal extent of the bottleneck, the severity of the bottleneck (i.e., in terms of population reduction) is unknown because of the lack of demographic data before the island's formation. Despite the genetic signature of a bottleneck, the island population has maintained higher or comparable levels of genetic diversity compared to western and eastern sites. The maintenance of comparable levels of genetic diversity on the island could be explained by the severity of the bottleneck. If the severity of the bottleneck was minimal, then genetic diversity could be maintained at or near pre-bottleneck levels. Another factor is that the loss of genetic diversity in a long-lived species is slower because the long generation time slows the process of genetic drift (Dinerstein and McCracken 1989; Kuo and Janzen 2004; Hailer et al. 2006). In the case of the large island, only 1–2 generations have passed since isolation. This limits the opportunity for genetic drift to reduce genetic diversity and could likely explain the retention of genetic diversity in this population.

Alternatively, the isolation from the mainland did not necessarily have to translate into a population bottleneck that led to a reduction in genetic diversity. In fact, the formation of the island may have led to a temporary increase in density as tortoises in the area sought refuge on higher ground as the lake began filling. This phenomenon was shown in other species (e.g., Debinski and Holt 2000). Therefore, the island potentially possessed a much larger than normal pool of alleles within the population than would be expected in a comparable mainland area immediately prior to the isolation event. Another alternative explanation of the retention of genetic diversity on the large island could be that tortoises immigrated from the mainland.

The BOTTLESIM software predicted that the reduction of genetic diversity in the island population would occur rapidly. For example, the island population would only retain 90% of the original genetic diversity for 40–70 years depending on which genetic index was modeled. Eventually, the population will lose up to 47.4% of its original genetic diversity in 200 years. Even though long-generation times provide an “intrinsic buffer” to loss of genetic diversity by genetic drift (Hailer et al. 2006), the population of the large island will likely lose genetic diversity rapidly because of its relatively small size. The impact of the loss of genetic diversity on the island population remains to be seen. Interestingly, western populations have lower levels of genetic diversity than eastern populations, and this phenomenon was correlated with reproductive problems (Ennen et al. 2010). The general perception is that gopher tortoises in the western portion of the range have experienced substantial fragmentation (U.S. Fish and Wildlife Service 1990). Given the low genetic diversity within the western populations, it appears that enough generations of gopher tortoises have existed in isolation

since fragmentation to reduce the benefits of the “intrinsic buffer” associated with long-generation time. By way of comparison, the BOTTLESIM analysis of the large-island site predicted a comparable level of genetic diversity to that of western populations in approximately 300 years or 7.5 generations. However, we note that another factor that may also be influencing the level of genetic diversity in the western sites is the fact that these populations are located on the periphery of the range (Lewontin 1974).

Habitat loss and the subsequent fragmentation and isolation of populations are major problems for the world's fauna and flora. For long-lived species, isolation and fragmentation appear to influence demographic traits (i.e., size-structure) faster than genetic diversity. However, the buffer against genetic drift associated with long-generation times may only provide several generations worth of protection when population size is low. These results may provide some insight into the appropriate strategies for managing tortoises and other long-lived organisms.

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