

Effects on Survival of Desert Tortoises (*Gopherus agassizii*) Urinating During Handling

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ABSTRACT. – Desert tortoises (*Gopherus agassizii*) mitigate their exposure to arid conditions by using their urinary bladders as water reserves. I investigated whether tortoises that urinated and lost water during handling by field researchers had lower survival compared to tortoises that did not urinate. I analyzed data from 6 years at 3 sites in the Sonoran Desert of Arizona. Survival varied by site, increased with body size, and decreased if tortoises urinated during handling. Mean annual differences in survival of average-sized tortoises between individuals that urinated during handling and those that did not ranged from approximately 5 to 13% among the 3 sites. Recapture rates also varied by site and increased with body size, but urinating had no detectable effect. These results highlight the importance of developing well-defined study objectives and procedures that minimize the probability of desert tortoises urinating during processing, not only to avoid compromised data due to reduced survival (or other dehydration-induced behavioral, reproductive, or physiological responses) but also to ensure that research activities do not compromise the status of study populations themselves.

KEY WORDS. – Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; survival; Program MARK; water loss; handling; urination; management; Sonoran Desert; USA; Arizona

The desert tortoise (*Gopherus agassizii*) occurs within an arid environment throughout most of its distribution, with average annual rainfall ranging from as little as 101 mm in the Mojave Desert to 324 mm in the Sonoran Desert (but up to 664 mm in Sinaloa thornscrub; Germano, 1994). In addition, desert tortoises consume a diet that is at various times osmotically stressful or dehydrating (Nagy and Medica, 1986; Peterson, 1996a). Desert tortoises mitigate against these conditions by using their urinary bladders as water reservoirs, in which nitrogenous wastes are diluted and from which water may be reabsorbed during drought conditions (Dantzler and Schmidt-Nielsen, 1966; Minnich, 1977; Nagy and Medica, 1986; Peterson, 1996a). Desert tortoises conserve water extremely well, as demonstrated by low rates of water flux, even during drought; low water use relative to energy metabolism; and low water influx rates relative to changes in body mass (Peterson, 1996a). Due to this ability to conserve water and tolerate temporary physiological imbalances, and by remaining inactive in burrows, tortoises can tolerate drought and opportunistically exploit unpredictable and ephemeral resources (Peterson, 1996a). Even so, chronic drought has been directly implicated, through physiological effects of dehydration and starvation, in the decline of at least one tortoise population in California (Peterson, 1994).

Desert tortoises lose water primarily through evaporation (Minnich, 1977), but studies in which tortoises are handled often result in the side effect of some individuals urinating during processing (Jacobson et al., 1993; pers. obs.). This water loss could result in serious health threats or compromise normal behavior or physiology, especially during hot, dry summer months. Therefore, researchers might be compromising tortoise survival with “standard” research

and management activities. I addressed this question by studying 3 tortoise populations in the Sonoran Desert of Arizona, but my results are also relevant in the Mojave Desert, where the tortoise is listed by the U.S. Fish and Wildlife Service as threatened (USFWS, 1990).

METHODS

Study Sites and Tortoise Processing. — I examined recapture data for desert tortoises that did or did not urinate when handled during annual surveys (1990–94, 1998) of 3 populations in Arizona: the Eagletail Mountains (ET; 33°22'N, 113°17'W), Maricopa County; Granite Hills (GH; 32°50'N, 111°21'W), Pinal County; and Little Shipp Wash (LS; 34°33'N, 113°05'W), Yavapai County (Fig. 1). Surveys took place between early August and late October each year. Field workers pulled tortoises from burrows when necessary and handled them carefully. Individual tortoises were encountered up to 10 times per annual survey, but field workers typically handled each individual a maximum of 3–4 times during a survey. Processing generally included weighing with a sling and spring scale, recording several morphological measurements with calipers and a metal rule, notching marginal scutes to mark unmarked individuals, a brief health assessment, and photo documentation of the carapace and plastron.

Field workers visually estimated the volume of urine lost during handling, so volume estimates are imprecise and probably suffer from observer biases. Therefore, I reduced the data into the following groups based on tortoise size and estimated fluid lost. I coded juvenile tortoises (< 180 mm midline carapace length [CL] and lacking secondary sexual characteristics) as urinating (= 1) if the estimated volume



Figure 1. Study site locations, Sonoran Desert, Arizona. ET = Eagletail Mountains; GH = Granite Hills; LS = Little Shipp Wash.

recorded was ≥ 1 ml. I coded adult tortoises (≥ 180 mm CL) as urinating (= 1) if the estimated volume was ≥ 5 ml. I coded all tortoises that did not urinate in a given year, and those with estimates below the above cutoffs, as not urinating (= 0). By definition, tortoises not captured in a given year did not urinate that year.

Survival Estimation.—I estimated annual survival (ϕ) and recapture (p) rates as described in Lebreton et al. (1992) and Burnham and Anderson (1998) using Program MARK (Version 2.0, December 2000; White and Burnham, 1999). I divided the data from each of the 3 sites into 3 groups: adult males, adult females, and juveniles. I also included 6 individual covariates: size at first capture (CL) and the binary urination covariate for each year, 1990–94.

I first defined a candidate model set, which included a fully parameterized global model (Burnham and Anderson, 1998). The global model included group (g) and time (t) effects and their interactions for both ϕ and p : model $\{\phi_{g^*t} p_{g^*t}\}$. Model notation follows Lebreton et al. (1992). I tested the goodness-of-fit (GOF) of the global model to the data by using Program MARK's parametric bootstrap procedure, with 500 bootstrap samples, and by examination of χ^2 tests in Program RELEASE (Version 3.0, embedded in

Program MARK). Additional models in the candidate set included reductions of the global model achieved by eliminating parameters. I included models with the covariate, initial CL, usually separated by site. I modeled effects of tortoises urinating in two ways: 1) survival effects of urination varied by year (time effect; e.g., model $\{\phi_{cl(site)+void(t)}\}$), and 2) survival effects of urination were constant across years (constant effect; e.g., model $\{\phi_{cl(site)+void(c)}\}$). The candidate model set included a total of 22 models (Table 1). I estimated parameters in all models with the logit link function.

I based model selection on comparison of the quasi Akaike Information Criterion ($QAIC_c$; Lebreton et al., 1992; Burnham and Anderson, 1998). $QAIC_c$ includes a quasi-likelihood adjustment (\hat{c}) for overdispersion in the data, which I calculated by dividing the \hat{c} calculated directly by Program MARK for the global model by the mean of simulated values of \hat{c} from the 500 bootstrap samples. I accepted the model with the lowest $QAIC_c$ as the most parsimonious model for the data. I made comparisons among models in the candidate set and estimated the relative importance of different parameters by using normalized Akaike weights (w_i ; Burnham and Anderson, 1998). The ratio of w_i between any two models indicates the relative degree to which a particular model is better supported by the data than the other model, and the sum of w_i for all models containing a given parameter indicates the relative importance of that parameter.

RESULTS

Between 1990 and 1994, 67 individual tortoises were marked and released at ET, and 162 individuals were marked each at GH and LS (Table 2). Through the 1998 survey, 44 individuals (66%) were recaptured at least once at ET, 107 (66%) at GH, and 111 (69%) at LS. About half the tortoises at ET (48%) and LS (52%) urinated at least once when handled through 1994, with a lesser proportion at GH (36%; Fig. 2).

The data fit the global model $\{\phi_{g^*t} p_{g^*t}\}$ poorly ($p < 0.002$). Examination of χ^2 tests from Program RELEASE revealed only one significant result, but most individual tests suffered from insufficient data in one or more cells. Most tests lacking sufficient data suffered from low expected values in cells containing new (unmarked) individuals or individuals not seen between two or more surveys. A high proportion of the individuals in each test were captured in each survey, precluding robust comparisons between groups of tortoises captured or not captured in a given survey. All combined GOF tests within groups produced non-significant results ($p > 0.09$). Lack of model fit was apparently due to overdispersion of the data (model $\hat{c} = 5.611$; bootstrap mean $\hat{c} = 3.492 \pm 0.0178$ SE), so I estimated $QAIC_c$ with adjusted $\hat{c} = 1.607 (= 5.611/3.492)$.

The most parsimonious model in the candidate set was model $\{\phi_{cl(site)+void(c)} p_{cl(site)}\}$ (Table 1). Survival varied by site, increased with CL, and depended on a constant urination

Table 1. Summary of model testing for desert tortoises marked at 3 sites in Arizona. Models are sorted by increasing $QAIC_c$ value. Models with $QAIC_c$ weights (w_i) > 0.01 are listed. The global and constant models are included below the dashed line for reference. Subscripts reflect different factors in the model (cl = straight midline carapace length in mm, cl(site) = variation by cl and between sites, void(c) = constant urination effects, void(t) = temporal urination effects, g = discrete group, t = time, . = constant). K = number of parameters.

Model	$QAIC_c$	$\Delta QAIC_c$	w_i	K	Deviance
$\phi_{cl(site)+void(c)} p_{cl(site)}$	1041.686	0.000	0.3298	9	1644.773
$\phi_{cl(site)+void(c)} p_{cl(site)+void(c)}$	1042.278	0.592	0.2453	10	1642.445
$\phi_{cl+void(c)} p_{cl(site)}$	1042.816	1.130	0.1875	7	1653.127
$\phi_{cl+void(c)} p_{cl(site)+void(c)}$	1043.973	2.287	0.1051	8	1651.719
$\phi_{cl(site)} p_{cl(site)}$	1045.350	3.664	0.0528	8	1653.933
$\phi_{cl(site)+void(t)} p_{cl(site)}$	1046.794	5.108	0.0256	13	1639.823
$\phi_{cl+void(t)} p_{cl(site)}$	1047.313	5.627	0.0198	11	1647.250
$\phi_{cl} p_{cl(site)}$	1047.325	5.639	0.0197	6	1663.632
$\phi_{g^*t} p_{g^*t}$	1130.958	89.272	0.0000	70	1575.312
$\phi . p .$	1144.015	102.329	0.0000	2	1831.984

Table 2. Reduced m -array of desert tortoise captures, 1990–94 and 1998, pooled across groups within sites. R_i = number of marked individuals released in year (i), including both newly marked and previously marked individuals; annual values are the number from a given release cohort first recaptured in that year; r_i = the total number of individuals from a release cohort recaptured at least once in a subsequent survey; m_j = the total number of recaptures in a given year.

Site	Year	R_i	1991	1992	1993	1994	1998	r_i
Eagletail Mountains	1990	32	28	2	2	0	0	32
	1991	36		20	10	4	0	34
	1992	28			15	5	3	23
	1993	36				26	1	27
	1994	47					24	24
	m_j		28	22	27	35	28	
Granite Hills	1990	45	29	4	6	1	0	40
	1991	70		42	13	4	2	61
	1992	74			46	18	0	64
	1993	94				67	9	76
	1994	109					46	46
	m_j		29	46	65	90	57	
Little Shipp	1990	83	52	13	4	2	1	72
	1991	83		58	13	1	1	73
	1992	89			62	9	1	72
	1993	100				55	12	67
	1994	76					36	36
	m_j		52	71	79	67	51	

effect (Fig. 3). Overall tortoise survival rates tended to increase from LS to GH to ET (Table 3). Survival of tortoises that did not urinate during handling varied little during the study, with a maximum annual difference of only 0.004 at LS (for tortoises of average size; Table 3). Differences ($\bar{x} \pm SD$) in survival between tortoises that did or did not urinate increased from ET (0.046 ± 0.0089) to GH (0.091 ± 0.0171) to LS (0.128 ± 0.0224).

Recapture rates also varied by site and increased with CL, but urination had no detectable effect (Fig. 4). Recapture rates were constant between years ($w_i < 0.01$ for all models containing a temporal effect on p). Ranges of recapture rates (60–300 mm CL) at each site were 0.251–0.845 at ET, 0.262–0.852 at LS, and 0.356–0.900 at GH (Fig. 4); the largest tortoise at GH measured 259 mm CL ($p \approx 0.850$).

Model $\{\phi_{cl(site)+void(c)}p_{cl(site)}\}$ fit the data 34% better than the next best model, which included a constant urination effect in the recapture rate (model $\{\phi_{cl(site)+void(c)}p_{cl(site)+void(c)}\}$), and 76% better than the next model, in which survival was

assumed to be the same between sites (model $\{\phi_{cl+void(c)}p_{cl(site)}\}$) (Table 1). Examination of Akaike weights further illustrated the relative importance on model selection of tortoises urinating. Constant urination effects were very important to survival estimation ($\sum w_{void(c)} = 0.86768$; $n = 4$), while temporal effects and a lack of modeled urination effects generally resulted in less parsimonious model fit for survival ($\sum w_{void(t)} = 0.05634$, $n = 6$; $\sum w_{no\ void\ effect} = 0.07599$, $n = 12$). Urination effects were unimportant to modeling recapture rates ($\sum w_{no\ void\ effect} = 0.64801$, $n = 18$). Including neither constant nor temporal effects improved model fit ($\sum w_{void(c)} = 0.35042$, $n = 2$; $\sum w_{void(t)} = 0.00158$, $n = 2$).

DISCUSSION

Desert tortoises rarely achieve resource balances (e.g., water, energy, protein) synchronously. Instead, they temporarily relinquish homeostasis (Nagy and Medica, 1986; Peterson, 1996a), or they may be regarded as simply having

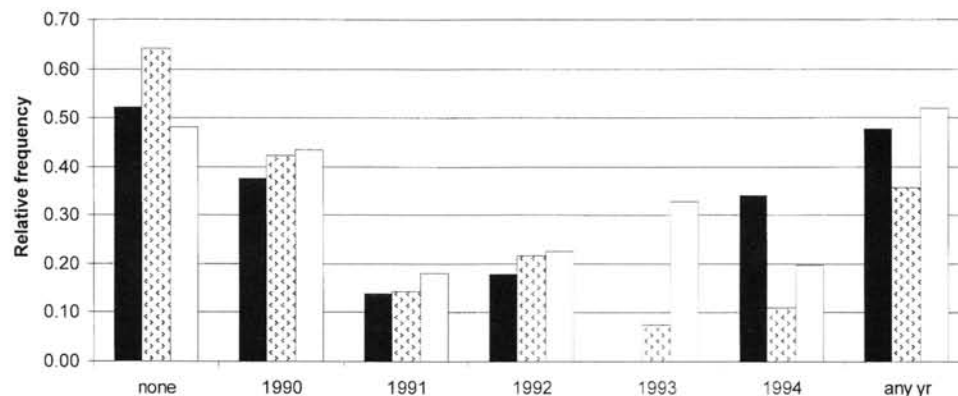


Figure 2. Relative frequency of desert tortoises urinating during handling at 3 sites in the Sonoran Desert, Arizona. Urination histories are shown for each year, 1990–94. By definition, tortoises not captured in a given year did not urinate that year. Solid black bars = Eagletail Mountains; stippled bars = Granite Hills; white bars = Little Shipp Wash.

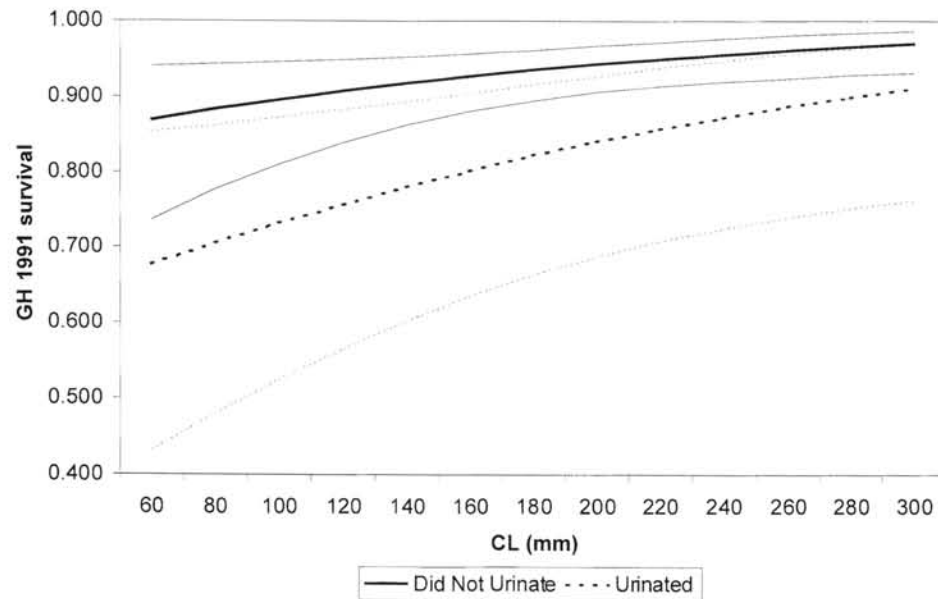


Figure 3. Representative graph of size-specific annual survival and 95% confidence intervals for desert tortoises that urinated or not during handling in the Sonoran Desert, Arizona, at Granite Hills (GH).

a highly variable “internal milieu” (Henen, 1997). Water loss during times of drought could have direct negative impacts on individual tortoises (see Peterson, 1994), but water retention may be equally important during wetter conditions in order to maintain osmotic balance and to prepare for the upcoming dry season.

Desert tortoises experience negative water balance, even when eating succulent forage, if drinking water is unavailable (Nagy and Medica, 1986). Osmolality of bladder urine and blood plasma increases seasonally as tortoises consume spring forage, which is high in potassium (Minnich, 1977; Nagy and Medica, 1986). Initially, water is reabsorbed from the bladder so that plasma osmolality remains constant while urine osmolality increases; when urine osmolality reaches about 300 mOsmol, osmolalities of both fluids increase together (Peterson, 1996a). Tortoises eventually cease feeding and become inactive in the absence of summer rainfall and plant production (Minnich, 1977; Nagy and Medica, 1986). Only when tortoises are able to drink free water can they flush their bladders and return to water balance and normal osmolality (Minnich, 1977; Nagy and Medica, 1986). Metabolic rate also increases after tor-

toises are able to drink; after flushing their bladders, tortoises resume feeding and obtain energy for digestion and growth (Peterson, 1996b). Decreases in water stored in the bladder could affect the ability of tortoises to feed by limiting the amount of solutes that can also be stored in the bladder.

Increases in body water volume due to water retention in the bladder also reduce the effects of evaporative water loss (Nagy and Medica, 1986) and are important for egg production (Henen, 1997). Reproductive females have higher total body water than non-reproductive females and forfeit this water to produce eggs; non-reproductive females conserve body water and maintain water balance, allowing them to return to a physiological condition capable of reproducing the following year (Henen, 1997). Water lost during handling could therefore affect reproductive output in individual females. Finally, crystallized urate precipitates (bladder stones) may form in dehydrated tortoises and may damage, even puncture, the bladder epithelium (Minnich, 1977). Otherwise healthy captive tortoises have been observed with bladder stones after having died from no visible outward cause, and observers have found

Table 3. Annual survival rates for desert tortoises of average size (180 mm CL) at 3 sites in Arizona, based on whether they urinated during handling or not (95% confidence intervals in parentheses).

Year	Eagletail Mountains		Granite Hills		Little Shipp Wash	
	Did Not Urinate	Urinated	Did Not Urinate	Urinated	Did Not Urinate	Urinated
1990	0.972 (0.868–0.994)	0.939 (0.745–0.988)	0.938 (0.898–0.964)	0.872 (0.797–0.922)	0.905 (0.845–0.943)	0.810 (0.711–0.881)
1991	0.970 (0.863–0.994)	0.912 (0.649–0.983)	0.936 (0.894–0.961)	0.822 (0.688–0.906)	0.901 (0.842–0.940)	0.743 (0.580–0.858)
1992	0.971 (0.864–0.994)	0.924 (0.694–0.985)	0.937 (0.896–0.962)	0.845 (0.740–0.912)	0.903 (0.843–0.941)	0.773 (0.641–0.867)
1993	0.971 (0.864–0.994)	0.924 (0.691–0.985)	0.937 (0.895–0.962)	0.843 (0.737–0.912)	0.902 (0.843–0.941)	0.771 (0.637–0.866)
1994	0.971 (0.865–0.994)	0.926 (0.700–0.985)	0.937 (0.896–0.962)	0.848 (0.747–0.913)	0.903 (0.843–0.941)	0.777 (0.649–0.868)

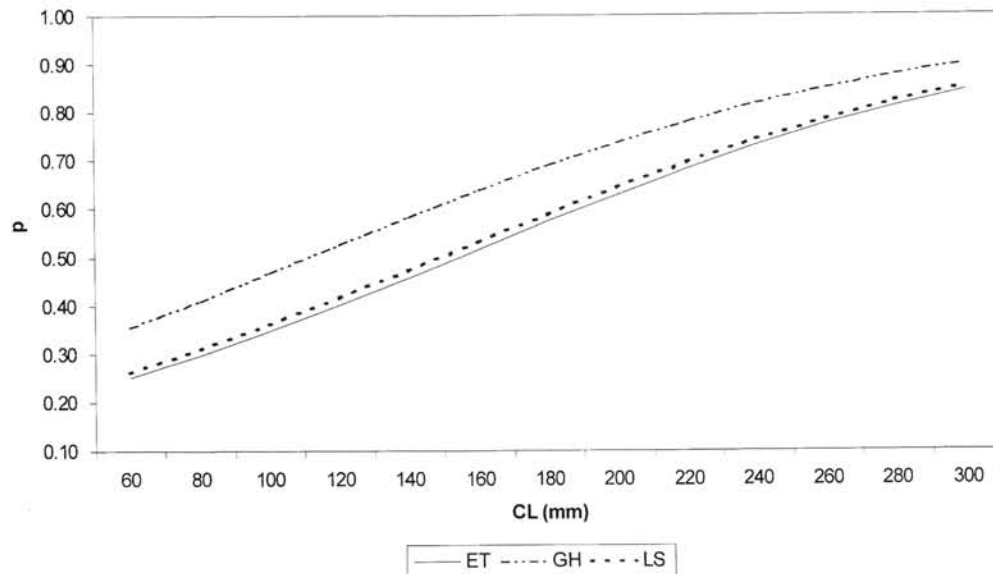


Figure 4. Recapture rates (p) for desert tortoises at 3 sites in the Sonoran Desert, Arizona. Confidence intervals broadly overlap, so only the outermost limits (ET lower and GH upper) are shown. ET = Eagletail Mountains; GH = Granite Hills; LS = Little Shipp Wash.

such stones in carcasses in the field (Miller, 1932; Peterson, 1994).

Even though data used in this analysis included much variation (e.g., equal weighting for individual tortoises that urinated approximately 5 ml to 100 ml), an overall effect still surfaced. Tortoises that urinated during handling had lower survival than those that did not. A decreasing trend in overall survival, even for tortoises that did not urinate, existed from ET to LS. This, combined with an increasing trend in urination effects on survival from ET to LS, suggests that LS tortoises may be more sensitive to dehydration than tortoises in the other populations. These patterns are counter-intuitive based on rainfall patterns: average annual rainfall from each site's nearest weather stations increases from 157–188 mm/yr (ET) to 252 mm/yr (GH) to 349 mm/yr (LS) (Shields et al., 1990). Mountain lions appear to prey on tortoises more at LS than at other sites (Averill-Murray et al., 2002), but how this would relate to urination effects is unclear. Some other factor must exist to explain survival differences between these sites.

Recapture rates were not affected by urination history, indicating that if a tortoise urinated during handling and survived, it was as likely to be recaptured in a subsequent survey as a tortoise that did not urinate. Temporal effects were relatively unsupported by the data, even though rainfall varied by site and year (Shields et al., 1990). Had urine been accurately measured, more detailed models might have quantified effects of the actual amount of fluid lost on survival or recapture rates relative to annual rainfall and available water.

Obviously, most research projects on desert tortoises (and other arid-environment tortoises) will require some degree of handling, so it is important that researchers develop well-defined study objectives and procedures that minimize the probability of animals urinating. Even though body mass is often correlated with hydration state, caution

must also be exercised in using body mass as a measure of condition (Jacobson et al., 1993) when evaluating potential effects on study results due to water loss. Tortoises with full bladders may still be osmotically stressed with high concentrations of metabolic wastes in the bladder and blood plasma (Peterson, 1996a). Depending on the particular study, rehydrating tortoises with an intra-peritoneal injection of an electrolyte/dextrose solution may be appropriate, but such measures or alternatives must also be evaluated. Researchers must always consider how their data collection and handling techniques might affect their study results and the subject animals themselves, especially when working with rare or sensitive species.

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