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Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises

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Abstract. Upper respiratory tract disease (URTD) caused by *Mycoplasma agassizii* has been hypothesized to contribute to the decline of some wild populations of gopher tortoises (*Gopherus polyphemus*). However, the force of infection (FOI) and the effect of URTD on survival in free-ranging tortoise populations remain unknown. Using four years (2003–2006) of mark–recapture and epidemiological data collected from 10 populations of gopher tortoises in central Florida, USA, we estimated the FOI (probability per year of a susceptible tortoise becoming infected) and the effect of URTD (i.e., seropositivity to *M. agassizii*) on apparent survival rates. Sites with high ($\geq$25%) seroprevalence had substantially higher FOI (0.22 $\pm$ 0.03; mean $\pm$ SE) than low ($<$25%) seroprevalence sites (0.04 $\pm$ 0.01). Our results provide the first quantitative evidence that the rate of transmission of *M. agassizii* is directly related to the seroprevalence of the population. Seropositive tortoises had higher apparent survival (0.99 $\pm$ 0.0001) than seronegatives (0.88 $\pm$ 0.03), possibly because seropositive tortoises represent individuals that survived the initial infection, developed chronic disease, and experienced lower mortality during the four-year span of our study. However, two lines of evidence suggested possible effects of mycoplasmal URTD on tortoise survival. First, one plausible model suggested that susceptible (seronegative) tortoises in high seroprevalence sites had lower apparent survival rates than did susceptible tortoises in low seroprevalence sites, indicating a possible acute effect of infection. Second, the number of dead tortoise remains detected during annual site surveys increased significantly with increasing site seroprevalence, from $\sim$1 to $\sim$5 shell remains per 100 individuals. If (as our results suggest) URTD in fact reduces adult survival, it could adversely influence the population dynamics and persistence of this late-maturing, long-lived species.

Key words: apparent survival; Florida, USA; force of infection; gopher tortoise; Gopherus polyphemus; *multistate mark–recapture models;* Mycoplasma agassizii; *pathogen transmission; upper respiratory tract disease (URTD); wildlife diseases.*

Introduction

Infectious diseases have been widely recognized as an important factor influencing the dynamics and persistence of wildlife populations (Dobson and May 1986, Scott 1988, Cohn 1991, Wolff and Seal 1993, Daszak et al. 2000, Hudson et al. 2002). The impact of disease can be particularly severe in populations experiencing environmental stress caused by anthropogenic activities such as habitat fragmentation and ecosystem perturbation (Hutchins et al. 1991, Nettles 1992, 1996, Viggers et al. 1993, Woodford 1993, Cunningham 1996). Consequently, wildlife diseases have been implicated in the decline or extinction of several wildlife species (e.g., Hawaiian land birds [Vanriper et al. 1986], black-footed ferrets, *Mustela nigripes* [Thorne and Williams 1988], harbor seals, *Phoca vitulina* [Heidejorgensen et al. 1992], marine turtles [Herbst and Klein 1995], Australian rain forest frogs [Laurance et al. 1996], Serengeti lions, *Panthera leo* [Roelke-Parker et al. 1996], African wild dogs, *Lycaon pictus* [Woodroffe 1997], Ethiopian wolves, *Canis simensis* [Randall et al. 2004], Tasmanian devils, *Sarcophilus harrisii* [Lachish et al. 2007]). However, studies investigating demographic effects of infectious diseases on free-ranging wildlife populations, other than those of economic or public health significance, have been rare (but see Spalding and Forrester 1993, Dhondt et al. 2005).

Population declines are occurring in most chelonian species, including the three North American *Gopherus* spp. (Mitchell and Klemens 2000). Although habitat degradation is the most significant threat to wild populations of tortoises (Mitchell and Klemens 2000), disease-related mortality is also considered a significant factor contributing to population declines (Jacobson 1994). Upper respiratory tract disease (URTD), an infectious disease caused by *Mycoplasma agassizii*, has

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been associated with the decline of several tortoise populations (Berry 1997, McLaughlin 1997, Gates et al. 2002). URTD was first described in clinically ill captive and wild desert tortoises (*Gopherus agassizii*) in the southwestern United States (Jacobson et al. 1991). In the 1980s, major declines of desert tortoise populations were documented at several sites in the Mojave Desert, California, USA (Corn 1994). As a result of this decline, desert tortoises in the Mojave Desert were declared threatened (U.S. Fish and Wildlife Service 1990). Since 1989, serological evidence of *M. agassizii* exposure has been detected in wild populations of gopher tortoises (*Gopherus polyphemus*; see Plate 1) in Florida, some of which have experienced significant mortality events (McLaughlin 1997, Berish et al. 2000, Gates et al. 2002). Evidence suggests that URTD may be a factor contributing to the observed population declines in the desert tortoise as well as the gopher tortoise (Jacobson et al. 1991, 1995); however, conclusive evidence of the effects of URTD in wild populations is missing. Monitoring the disease status of wild tortoises throughout their range is crucial for understanding the dynamics of URTD in wild populations and for evaluating its impact on tortoise populations (U.S. Fish and Wildlife Service 1994).

Pathogens may influence the dynamics of host populations by altering vital demographic rates such as survival and reproduction. The spread of pathogens in populations of susceptible hosts is governed by the force of infection (FOI), defined here as the probability per year of a susceptible tortoise becoming infected. However, empirical estimates of the FOI and investigations of the effects of diseases on host demography are rare for free-ranging wildlife populations (but see Pech and Hone 1988, Caley and Hone 2002, Faustino et al. 2004, Heisey et al. 2006, Hosseini et al. 2006). Investigations of the population-level effects of infectious diseases, while challenging, are essential for a better understanding of the host–pathogen dynamics and also for development of effective management tools. This is especially important when the species of interest is a keystone species such as the gopher tortoise, which is critical to the health of upland ecosystems and to persistence of several commensal species that inhabit gopher tortoise burrows (Eisenberg 1983, Diemer 1986).

Like most mycoplasmal respiratory tract infections, the dynamics of URTD within host populations are typically characterized by an acute phase with overt clinical signs and high seroconversion rates, a chronic phase with limited clinical signs and high seroprevalence, and intermittent recrudescent phases with mild to severe clinical signs and high seroprevalence (Brown et al. 1999, Wendland 2007). Although acute mortality may occur, many tortoises likely develop chronic disease, with intermittent expression of clinical signs. The bacteria persist in the nasal cavity and damage the mucosal tissue of the upper respiratory tract, resulting in increased susceptibility to secondary infections (Homer et al. 1998, McLaughlin et al. 2000). In tortoises with chronic mycoplasmosis, mortality is presumed to be the result of severe debilitation and multisystemic disease (Homer et al. 1998, Jacobson et al. 1991, McLaughlin et al. 2000). However, the influence of URTD on demography of wild tortoise populations remains unknown, in large part because of the limited long-term monitoring of infected tortoises in the wild, the chronic nature of URTD, and the long lifespan of the tortoise.

The goal of this study was to quantify the FOI and to investigate population-level effects of URTD on free-ranging gopher tortoises. We conducted intensive mark–recapture and epidemiological studies of 10 discrete populations of gopher tortoises in central Florida, USA, for four consecutive years. Using a multistate mark–recapture model, we estimated the FOI (the probability of transition from susceptible or seronegative state to exposed or seropositive state) and apparent survival rates, and evaluated the effects of environmental factors on the FOI and survival rates. We tested for possible effects of sex, site, and seroprevalence to *M. agassizii* on the FOI and apparent survival of tortoises, and evaluated the effect of mycoplasma serological status on apparent survival of tortoises. In addition, we tested for potential influence of environmental factors hypothesized to influence the FOI and tortoise survival. Finally, we used shell remains as biological markers of mortality and evaluated the relationship between shell remains and seroprevalence to *M. agassizii*.

Materials and Methods

Study species

The gopher tortoise is a terrestrial, burrowing reptile that plays an important ecological role in upland ecosystems (Eisenberg 1983, Jackson and Milstrey 1989, Moler 1992). It is the only North American tortoise species found east of the Mississippi River, where it occurs in Florida and portions of the coastal plain of South Carolina, Georgia, Alabama, Mississippi, and Louisiana (Auffenberg and Franz 1982). The gopher tortoise is currently state listed as threatened in Florida (Florida Fish and Wildlife Conservation Commission 2007).

Study area

In 2002, we conducted a preliminary study of tortoise populations in central Florida to collect relevant data on population and habitat conditions and to assess the status of mycoplasmal upper respiratory tract disease (URTD). Based on the findings of the preliminary study, we selected 10 sites that differed in *Mycoplasma agassizii* seroprevalence for in-depth studies (Fig. 1). The selected sites were Big Shoals State Park (BS), Camp Blanding Wildlife Management Area (CB), Cecil Field/Branan Field Wildlife and Environmental Area (CF), a privately-owned property in central Florida (CE), Fort Cooper State Park (FC), Flying Eagle Wildlife Management Area (FE), Gold Head Branch State Park (GH), Perry Oldenburg Wildlife and Environmental Area (OL),

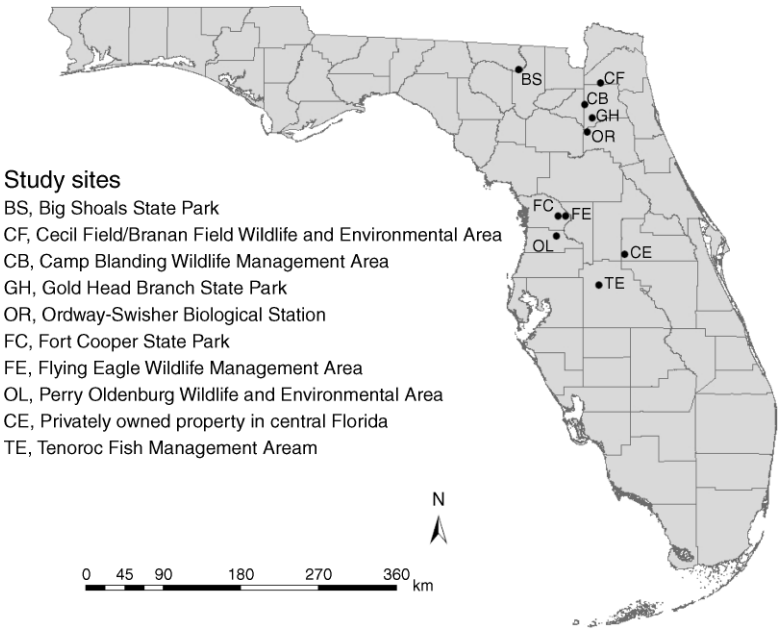

FIG. 1. Location of study sites in Florida, USA.

Ordway-Swisher Biological Station (OR), and Tenoroc Fish Management Area (TE), all in Florida, USA. Habitat quality and type varied substantially among sites. The ground cover at BS, CB, and FE is dominated by Bahia grass (*Paspalum notatum*); BS and CB have been planted with pine trees (*Pinus* spp.). CF contains a mixture of sandhill and pine flatwoods. CE and TE are previously disturbed sites that are dominated by non-native, ruderal vegetation. GH, OR, FC, and OL are sandhill habitats with varying habitat quality and degrees of management.

Field methods

The fieldwork was conducted between 2003 and 2006, during late spring/summer (May–September). Systematic surveys were conducted to locate tortoise burrows and shell remains from deceased tortoises at study sites and consisted of a line of four to eight observers spaced 10 m apart walking parallel transects across the study area. Dead tortoises were photo-documented, georeferenced, labeled, and collected for examination. Approximate time since death was estimated using previously described methods (Dodd 1995).

Mark–recapture protocol was standardized for all the sites. At each of the 10 sites, tortoises were captured opportunistically or with pitfall traps at known active burrows. Each captured tortoise was marked for individual identification on the first capture by drilling holes in the marginal scutes using an established scheme (Cagle 1939), and the identity of previously marked tortoises was recorded. Morphometric measurements were taken, and photographic documentation was made of the face, plastron, carapace, and any lesions observed. Complete health assessments (Berry 1997), standard hematological and serum biochemical profiles (Christopher et al. 2003), and diagnostic tests for the presence of *Mycoplasma agassizii* (Brown et al. 1995, Wendland et al. 2007) were performed.

ELISA test

URTD is a chronic disease that is intermittently expressed. Tortoises without clinical signs of URTD can still be infected and may transmit the pathogen under appropriate conditions (Jacobson et al. 1995). Therefore, we used an immunoassay, rather than visual assessment of clinical signs, to measure exposure to *M. agassizii* in tortoises. An enzyme-linked immunosorbent assay (ELISA) that has been validated for both desert and gopher tortoises, and has been shown to be a reliable and reproducible assay, was used to determine the serological status of captured tortoises (Schumacher et al. 1993, Wendland et al. 2007). Animals were classified as seronegative or seropositive based on the ELISA results. Seronegative tortoises were considered as susceptible animals for the analyses described next.

Capture–mark–recapture (CMR) analysis

Estimation of apparent survival and force of infection.—We used four years of capture–mark–recapture (CMR) data from 340 adult male (carapace length $\geq$ 180 mm) and 329 adult female (carapace length $\geq$ 220 mm) tortoises (Table 1). We considered two serologic states based on results of the ELISA test: (1) susceptible (seronegative, s) and (2) exposed (seropositive, p) states. We used the multistate CMR model (Hestbeck et al. 1991, Brownie et al. 1993, Williams et al. 2001, Fujiwara and Caswell 2002) implemented using the RMark interface (Laake and Rexstad 2007) for Program

TABLE 1. Numbers of seronegative (female/male) and seropositive (female/male) tortoises captured and number of shells recovered at each site during each year of the study, and the total number of seroconverted individuals at each site throughout the study.

Site	2003			2004			2005			2006			Total no. seroconverts
	Sero−	Sero+	Shell	Sero−	Sero+	Shell	Sero−	Sero+	Shell	Sero−	Sero+	Shell	
BS	10/13	0/0		18/19	1/0	0	25/21	0/1	0	21/23	0/1	0	3
CB	5/6	2/0		9/12	0/1	1	10/13	2/0	0	7/8	1/3	0	1
CF	2/1	8/10	6	3/4	16/16	9	8/11	21/31	6	5/4	17/19	8	5
CE	3/12	1/1		11/10	4/4	0	7/3	7/9	1	10/9	2/1	1	8
FC	8/12	0/0		17/11	0/0	0	13/15	1/0	0	8/5	4/9	6	2
FE	8/11	0/0		9/20	0/1	5	11/14	0/0	1	15/12	0/0	1	0
GH	6/6	2/6	1	13/8	3/8	2	12/11	6/9	1	9/14	6/6	4	3
OL	16/5	1/0		20/14	3/3	1	15/8	4/4	1	3/3	12/15	3	6
OR	15/7	0/0		22/12	0/0	4	29/16	0/0	1	30/22	1/1	2	1
TE						0			0			1	
Total	73/73	14/17	7	122/110	27/33	22	130/112	41/54	11	108/100	43/55	26	29

Notes: Study sites were Big Shoals (BS), Camp Blanding (CB), Cecil Field (CF), a privately owned property in central Florida (CE), Fort Cooper (FC), Flying Eagle (FE), Goldhead Branch State Park (GH), Oldenburg (OL), Ordway (OR), and Teneroc (TE). Data from TE were included only in the analysis of shell remains. Empty cells indicate that data are not applicable.

MARK (White and Burnham 1999) to estimate serologic state-specific recapture (ρ_s, ρ_p) and survival (S_s, S_p) rates, and also the force of infection (FOI). The FOI (ψ) quantifies the probability that a susceptible tortoise becomes infected the following year, conditional on surviving the period in the susceptible state. Given the chronic nature of mycoplasmal URTD and limited evidence for clearance of the microbe from the respiratory tract (Wendland 2007, Wendland et al. 2007), we assumed that once infected, tortoises remained in the seropositive state and were presumed to be colonized by *M. agassizii*. Therefore, the probability of transition from seropositive to susceptible state (i.e., recovery rate) was fixed at zero. CMR data collected from TE were not included in the analyses due to very low recapture rates of adult tortoises at this site, but this site was included in the analysis of tortoise remains.

We used Program UCARE V2.02 (Choquet et al. 2003) to assess the goodness-of-fit of the general multistate model. We used Akaike's Information Criterion, corrected for small sample size (AIC_c), for model comparison and for the identification of the most parsimonious model from a candidate model set (Burnham and Anderson 2002). Model comparison was based on the differences in AIC_c values (ΔAIC_c). We used AIC_c weight as a measure of relative support for each model. We tested for the effects of sex, carapace length (an index of age), site, serologic status, and seroprevalence (i.e., proportion of seropositive individuals in the population) on apparent survival, and for the effects of sex and *M. agassizii* seroprevalence on the FOI.

Effects of URTD on apparent survival.—Using the most parsimonious model, we tested for the effects of serologic state and *M. agassizii* seroprevalence on apparent survival rates and evaluated the hypothesis that mycoplasmal URTD reduces apparent survival. We evaluated these potential effects using three different approaches. First, we tested for the effect of the serologic state (seropositive vs. susceptible or seronegative) on survival of tortoises. Second, we tested for the difference in survival of all tortoises (i.e., regardless of serologic state) in sites with high ($\geq$25%) vs. low ($<$25%) seroprevalence. We chose the 25% cutoff point based on the distribution of seroprevalence values. Most of the sites characterized as low seroprevalence are believed to be mycoplasma negative based on serology, culture/polymerase chain reaction (PCR) analysis of nasal flushes (Brown et al. 1995), and absence of clinical signs of URTD among tortoises. Occasional seropositive results from these sites likely represent false-positive results given the predictive values of the assay at low prevalence (Wendland et al. 2007); however, because these results could not be confirmed, we classified the sites as low seroprevalence. Finally, we compared apparent survival of susceptible tortoises from high seroprevalence sites to those from low seroprevalence sites. This last analysis was conducted to test for possible acute effects of the disease on tortoise survival (i.e., newly infected tortoises dying within weeks or months post-infection). Because tortoises were sampled once annually, within-year seroconversion and death was unlikely to be detected. The majority of individuals captured as seropositive were likely to be those that survived the initial acute phase and transitioned into the chronic form of the disease. Thus, we tested for potential acute effects by comparing the survival of susceptible individuals in high prevalence sites to those in low prevalence sites. In all cases, we appropriately accounted for factors that could influence capture probability, FOI, or survival rate before testing for the effect of mycoplasma serological status on the survival rate.

Effects of environmental covariates.—Using the most parsimonious model identified here, we examined the potential influence of environmental covariates on survival, recapture, and the FOI. Environmental covariates included site-specific estimates of tortoise density, relocation history, extent of spatial sampling, relative abundance of exotic plants, factors related to basking

site availability, and factors related to food availability. Tortoise abundances were estimated at each site by conducting burrow surveys to estimate the abundance of tortoise burrows, and then applying site-specific estimates of burrow occupancy rates as determined with a burrow camera. Relocation history was determined based on relocation permits issued by Florida Fish and Wildlife Commission (FWC) and one instance of documented but non-permitted relocations (0 = no history of relocations, 1 = documented history of relocations; in past wildlife rescue operations, gopher tortoises were relocated to some of the study populations). The covariate "extent of spatial sampling" grouped sites into two categories with respect to the proportion of the available tortoise habitat sampled (0 = relatively open habitats, where immigration and emigration are likely, and 1 = relatively closed habitats, where immigration and emigration are less likely). The covariate "exotic vegetation" divided sites into two groups with respect to the relative abundance of exotic and native species of plants (0 = low relative abundance of exotic plants, 1 = high relative abundance of exotic plants). We used woody stem density, and percent covers for woody vegetation, canopy, and bare ground in a principal components analysis (PCA), and used the first principal component as a measure of basking site availability. Likewise, the first principal component of PCA with percent covers of forbs, legumes, and grass quantified the food availability.

We tested for the influence of the potentially important covariates on survival rate by modeling the logit of each rate as a linear function of the covariates. If the 95% confidence interval for the slope parameter did not include 0, the relationship was considered statistically significant (Williams et al. 2001). Because we only had data on a subset of the factors that could have influenced FOI and survival rates, we considered the influence of each covariate separately.

Analysis of tortoise remains

In addition to the CMR analysis, we analyzed the number of fresh (<40 months old) tortoise shell remains found in each site and year to establish whether either (1) the number of shell remains found was correlated with seroprevalence in a given site and year or (2) the number of shell remains found was greater in high- than in low-prevalence sites. Specifically, we wanted to fit a statistical model that would allow (given the total population on a site) overdispersion in shell counts, possible variation among sites and years, and a positive effect of prevalence on shell counts. Following Elston et al. (2001), we used the model

$$S \sim \text{Poisson}(Te^{a_0+a_1P+\varepsilon_s+\varepsilon_y+\varepsilon_{sy}})$$

where S is the number of shell remains recovered at site s in year y; T is the total population size (estimated as described previously); P is the seroprevalence at a site in a given year; and ε_s, ε_y, and ε_{sy} are random zero-mean normal variates which describe site effects, year effects, and site–year interactions, respectively. Since there is only one observation per site–year combination in the data set, including ε_{sy} is equivalent to a *lognormal-Poisson* model, which allows for overdispersion and has similar characteristics to the more familiar negative binomial model, to describe the data rather than a simple Poisson (Elston et al. 2001). Ignoring random effects, the expected number of shells per individual at a site is $e^{a_0+a_1P}$; thus, a_0 represents the expected log proportion of shells/individual at 0 seroprevalence, while a_1 represents the increase in log proportion per percentage increase in seroprevalence. For small values of a_1, this parameter approximately equals the percentage increase; for example, $a_1 = 0.02$ would correspond to a 2% increase in shells/individual for a 1% increase in seroprevalence.

A similar model allowing only for categorical, rather than continuous, differences in seroprevalence is

$$S \sim \text{Poisson}(Te^{a_0+a_2H+\varepsilon_s+\varepsilon_y+\varepsilon_{sy}})$$

where now H is a dummy variable equal to 1 for high-prevalence (≥25% seropositive) sites and 0 otherwise; a_2 now represents the log ratio of shells/individual in high vs. low sites. We also fitted a null model with a_1 (or a_2) set to zero.

Fitting this model, which is technically a GLMM (generalized linear mixed model), by standard frequentist approaches is challenging because of the small sample size (e.g., only 3 years and 10 sites with which to establish the site and year variances). While various approximations such as penalized quasi-likelihood have been used to fit such models, their accuracy is still debated (Browne and Draper 2006). Thus, we used a Bayesian analysis with weak priors to estimate the effect of prevalence on shell counts. We used Normal priors with standard deviations of 10 for a_0 and a_1, and uniform (0, 100) priors on the inverse variances (i.e., the prior standard deviation ranged from 0.1 to infinity); we also tried U(0, 100) priors on the standard deviations themselves. We ran the model in WinBUGS (version 1.4; Spiegelhalter et al. 2003) via the R2WinBUGS R interface, running 6000 iterations for each of three chains with overdispersed starting points, discarding the first 3000 iterations and thinning the final posterior sample to 1000 values, and checking convergence according to the Gelman-Rubin criterion (Gelman et al. 2003).

We evaluated the results of the models by examining means and 95% credible intervals for the posterior distributions of the parameters. We used the deviance information criterion (DIC; Spiegelhalter et al. 2002) for model comparison.

Results

Apparent survival and force of infection

The goodness-of-fit test did not reveal evidence for lack of fit ($\chi^2_4 = 3.09$, $P = 0.54$). In the first set of

TABLE 2. Mark–recapture analysis of annual recapture and apparent survival rates of adult gopher tortoises.

Model no.	Model name	No. parameters	ΔAIC_c	AIC_c weights	Deviance
1	S(sex) ρ(site) ψ(.)	12	0.0	0.166	1698.5
2	S(.) ρ(site) ψ(.)	11	0.4	0.139	1700.9
3	S(.) ρ(sex + site) ψ(.)	12	0.9	0.104	1699.4
4	S(sex) ρ(site) ψ(sex)	13	1.8	0.067	1698.3
5	S(sex) ρ(sex + site) ψ(.)	13	2.1	0.059	1698.5
6	S(.) ρ(site) ψ(sex)	12	2.2	0.056	1700.7
7	S(site) ρ(site) ψ(.)	19	2.2	0.055	1686.1
8	S(cl) ρ(site) ψ(.)	12	2.4	0.051	1700.9
9	S(cl) ρ(sex + site) ψ(.)	13	2.5	0.048	1698.9
10	S(.) ρ(sex + site) ψ(sex)	13	2.8	0.042	1699.2
11	S(site) ρ(sex + site) ψ(.)	20	2.9	0.040	1684.7
12	S(sex + site) ρ(site) ψ(.)	20	3.2	0.034	1685.0
13	S(sex) ρ(sex + site) ψ(sex)	14	3.9	0.024	1698.3
14	S(site) ρ(site) ψ(sex)	20	4.1	0.021	1685.9
15	S(cl) ρ(site) ψ(sex)	13	4.2	0.020	1700.6
16	S(cl) ρ(sex + site) ψ(sex)	14	4.3	0.019	1698.7
17	S(sex + site) ρ(sex + site) ψ(.)	21	4.7	0.016	1684.4
18	S(site) ρ(sex + site) ψ(sex)	21	4.7	0.016	1684.5
19	S(sex + site) ρ(site) ψ(sex)	21	5.0	0.013	1684.7
20	S(sex + site) ρ(sex + site) ψ(sex)	22	6.6	0.006	1684.2
21	S(.) ρ(sex $\times$ site) ψ(.)	20	10.8	0.001	1692.6

Notes: Differences in Akaike's Information Criterion corrected for small sample size (ΔAIC_c), AIC_c weights, and model deviances (−2 log[Likelihood]) are given for each model. Symbols are: S, apparent survival rate; ρ, recapture rate; ψ, force of infection; sex, sex effect; site, site effect; and cl, linear effect of carapace length. A period (.) indicates constant value of the parameter. A plus sign (+) indicates additive effect, and $\times$ indicates interactive effect. Only the models with AIC weights $\geq$0.001 are shown.

analyses, we ignored the serologic state-specific differences in survival and recapture rates and tested for (1) main effects of sex, site, and carapace length, and additive and interactive effects of sex and site on annual recapture and apparent survival rates, and (2) effect of sex on the force of infection (FOI). The most parsimonious model included the effect of sex on apparent survival rate, the effect of site on recapture rates, and constant FOI (Table 2, model 1). However, a reduced model with $\Delta AIC_c < 1$ indicated that the sex effect on apparent survival rate was not substantial (Table 2, model 2). The overall FOI (mean ± SE) was estimated as 0.10 ± 0.01; sex-specific estimates were males, 0.11 ± 0.02, and females, 0.10 ± 0.02. The apparent survival rate was 0.95 ± 0.04 for females and 0.89 ± 0.04 for males. Recapture rates varied among sites, ranging from 0.31 ± 0.05 in Perry Oldenburg Wildlife and Environmental Area (OL) to 0.69 ± 0.05 in Big Shoals State Park (BS). Carapace length did not have a substantial influence on either apparent survival or recapture rate. The effect of sex on the serologic state-specific survival rates was retested later in our analyses.

Next, we evaluated the effect of *Mycoplasma agassizii* serologic status on tortoise survival. First, we tested for the effects of serologic state (of tortoises) and *M. agassizii* seroprevalence (at each site) on apparent survival rates (Table 2). For these analyses, we ignored the sex effect and used the model that included constant survival rate as the base model (Table 2, model 2). We used *M. agassizii* seroprevalence both as a continuous site covariate and as a discrete variable (<25% = low seroprevalence and ≥25% = high seroprevalence sites). We also tested for the interactive effect of serologic state and seroprevalence on survival to distinguish between apparent survival rates of susceptible individuals in high and low prevalence sites. As would be expected, the survival rate of seropositive tortoises in low prevalence sites was inestimable due to the small sample sizes. Therefore, we pooled the seropositive tortoises from low and high prevalence sites together and estimated one survival rate for seropositive tortoises. This modified survival model will be referred to as S(*sl-sh-p*). For these analyses, we tested three alternative models of FOI (ψ), where ψ was (1) constant, (2) a function of *M. agassizii* seroprevalence at a given site, or (3) differed between high and low seroprevalence sites. Because only a small number of individuals seroconverted from negative to positive during this study, we could not test for the effect of site on ψ.

The most parsimonious model included the effect of serologic state on survival rate, the effect of site on recapture rate, and the effect of *M. agassizii* seroprevalence on FOI (Table 3, model 1). Not surprisingly, the FOI was significantly higher at sites with higher seroprevalence (odds ratio = 203.5, 95% CI: 34.8, 1188.8). Using seroprevalence as a discrete variable, ψ was estimated as 0.23 ± 0.04 in high prevalence sites, and 0.04 ± 0.01 in low prevalence or mycoplasma-negative sites (Table 3, model 6). Apparent survival rate for susceptible tortoises was estimated as 0.88 ± 0.03. The mean apparent survival of seropositive tortoises was not lower than that of susceptible (seronegative) individuals as one might expect; the survival rate of seropositive tortoises was very close to one (0.99 ± 0.0001). Apparent survival of all tortoises in high prevalence sites (0.92 ± 0.05) was similar to that in low prevalence sites (0.91 ± 0.04). However, there was some evidence for a difference in the survival of

TABLE 3. Multistate mark–recapture analysis of the effect of mycoplasma serological status on the survival rate of adult gopher tortoises.

Model no.	Model name	No. parameters	ΔAIC_c	AIC_c weights	Deviance
1	S(serology) ρ(site) ψ($prev_{cov}$)	13	0.0	0.530	1651.7
2	S(sl-sh-p) ρ(site) ψ($prev_{cov}$)	14	1.6	0.240	1651.2
3	S(.) ρ(site) ψ($prev_{cov}$)	12	2.8	0.128	1656.6
4	S($prev_{cov}$) ρ(site) ψ($prev_{cov}$)	13	4.6	0.053	1656.3
5	S($prev_{gr}$) ρ(site) ψ($prev_{cov}$)	13	4.9	0.046	1656.6
6	S(serology) ρ(site) ψ($prev_{gr}$)	13	11.3	0.002	1663.0
7	S(sl-sh-p) ρ(site) ψ($prev_{gr}$)	14	12.9	0.001	1662.6
8	S(.) ρ(site) ψ($prev_{gr}$)	12	14.0	0.000	1667.8
9	S($prev_{cov}$) ρ(site) ψ($prev_{gr}$)	13	15.8	0.000	1667.5
10	S($prev_{gr}$) ρ(site) ψ($prev_{gr}$)	13	16.1	0.000	1667.8
11	S(serology) ρ(site) ψ(.)	12	42.1	0.000	1695.9
12	S(sl-sh-p) ρ(site) ψ(.)	13	43.5	0.000	1695.2
13	S(.) ρ(site) ψ(.)	11	45.1	0.000	1700.9
14	S($prev_{cov}$) ρ(site) ψ(.)	12	46.8	0.000	1700.6
15	S($prev_{gr}$) ρ(site) ψ(.)	12	47.1	0.000	1700.9

Notes: The base model used for these analyses was S(.) ρ(site) ψ(.) (Table 2, model 2). Abbreviations are: serology, serologic state effect (susceptible and seropositive); $prev_{cov}$, effect of seroprevalence as site covariate; $prev_{gr}$, effect of seroprevalence as a grouping factor (high and low seroprevalence sites); and sl-sh-p, grouping factor based on serologic state and seroprevalence (susceptible in low seroprevalence sites, susceptible in high seroprevalence sites, and seropositive). Other abbreviations are defined in Table 2.

susceptible tortoises between high and low prevalence sites (model 2, $\Delta AIC_c < 2$): survival of susceptible tortoises in low prevalence sites (0.90 ± 0.04) was slightly higher than that in high prevalence sites (0.86 ± 0.05).

We examined the effect of serologic state and seroprevalence on sex-specific survival of tortoises. We used the three most parsimonious models identified above (Table 3, models 1–3) and tested for additive and interactive effects of sex on apparent survival and FOI. The most parsimonious model included additive effects of sex and serologic state on survival (Table 4, model 1). The apparent survival of susceptible females (0.93 ± 0.04) was slightly higher than that of susceptible males (0.85 ± 0.04). However, a reduced model with $\Delta AIC_c < 1$ indicated that the sex effect on survival was not substantial (model 2). A model with a similar support indicated additive effect of sex and interactive effects of serologic state and seroprevalence on survival rates (model 3). Based on this model, apparent survival rates of susceptible females were slightly higher than that of susceptible males. Furthermore, apparent survival of susceptible tortoises in high-seroprevalence sites was less than that in low-seroprevalence sites. This effect was more pronounced in susceptible males (low, 0.89 ± 0.05 vs. high, 0.79 ± 0.07) than in females (low, 0.96 ± 0.04 vs. high, 0.91 ± 0.05; Fig. 2). In both models, survival rates of seropositive males and females were very close to one with very small standard errors.

Effects of environmental covariates

Using model 1 in Table 3 as the base model, we examined the influence of environmental covariates on apparent survival and recapture rates, and FOI. We first tested for the effects of percent ground cover, population density, and extent of spatial sampling on recapture

TABLE 4. Multistate mark–recapture analysis of the additive and interactive effects of sex and mycoplasma serological status on adult survival, recapture, and force of infection of adult tortoises.

Model no.	Model name	No. parameters	ΔAIC_c	AIC_c weights	Deviance
1	S(serology + sex) ρ(site) ψ($prev_{cov}$)	14	0.0	0.182	1649.1
2	S(serology) ρ(site) ψ($prev_{cov}$)	13	0.5	0.141	1651.7
3	S(sl-sh-p + sex) ρ(site) ψ($prev_{cov}$)	15	0.7	0.131	1647.7
4	S(serology + sex) ρ(site) ψ($prev_{cov}$ + sex)	15	1.5	0.085	1648.6
5	S(serology) ρ(site) ψ($prev_{cov}$ + sex)	14	2.0	0.067	1651.1
6	S(serology × sex) ρ(site) ψ($prev_{cov}$)	15	2.1	0.064	1649.1
7	S(sl-sh-p) ρ(site) ψ($prev_{cov}$)	14	2.1	0.064	1651.2
8	S(sl-sh-p + sex) ρ(site) ψ($prev_{cov}$ + *sex*)	16	2.2	0.061	1647.2
9	S(sex) ρ(site) ψ($prev_{cov}$)	13	3.0	0.041	1654.2
10	S(.) ρ(site) ψ($prev_{cov}$)	12	3.4	0.034	1656.6
11	S(sl-sh-p) ρ(site) ψ($prev_{cov}$ + sex)	15	3.6	0.030	1650.6
12	S(serology × sex) ρ(site) ψ($prev_{cov}$ + sex)	16	3.6	0.030	1648.6
13	S(sl-sh-p × sex) ρ(site) ψ($prev_{cov}$)	17	4.2	0.022	1647.1
14	S(sex) ρ(site) ψ($prev_{cov}$ + sex)	14	4.4	0.020	1653.5
15	S(.) ρ(site) ψ($prev_{cov}$ + sex)	13	4.8	0.017	1656.0
16	S(sl-sh-p × sex) ρ(site) ψ($prev_{cov}$ + sex)	18	5.7	0.010	1646.5

Notes: A plus sign (+) indicates an additive effect, and × indicates an interactive effect. Other symbols are defined in Tables 2 and 3.

rate. Among these covariates, only the extent of spatial sampling had a significant effect on recapture rate (odds ratio = 0.37, 95% CI: 0.25, 0.55). As expected, recapture rates were higher in sites where most of the available tortoise habitat was sampled (0.66 ± 0.05) than in sites where only a fraction of the tortoise habitat was sampled (0.42 ± 0.03). However, this model was less parsimonious than the general model with site effect on recapture rate ($\Delta AIC_c \gg 2$); therefore, we used the site effect on recapture rate for further analyses. Next, we tested for the effects of tortoise density, exotic vegetation, relocation history, extent of spatial sampling, and factors related to food and basking-site availability on the apparent adult survival rate. Tortoises in sites with a known relocation history had lower survival rates (0.79 ± 0.05) than those in sites with no relocation history (0.95 ± 0.03). Also, tortoises in sites with more basking-site availability had higher survival rates than those in sites with lower basking-site availability (odds ratio = 5.26, 95% CI: 1.19, 23.1). Finally, we tested for the effects of tortoise density, exotic vegetation, relocation history, and habitat-related factors on the FOI. None of these factors substantially influenced the FOI.

Analysis of tortoise remains

The model easily converged (Gelman-Rubin statistics were very close to 1), and the general conclusion was that seroprevalence had a weak but detectable effect on the number of shell remains located per individual present at the site. Specifically, the posterior mean of a_0 was −4.8 and of a_1 was 0.02 [illegible] corresponding to a 2% increase in shells/individual per percentage point increase in seroprevalence, or equivalently to an increase from $120 \times e^{-4.8} = 0.99$ to $120 \times e^{-4.8+70\times0.02} = 4.0$ shells with an increase from 0% to 70% seroprevalence on a site with 120 tortoises. The 95% credible interval of a_1 for the model with a uniform prior on the inverse variance was (0.003, 0.036), suggesting that the data would support conclusions ranging from a marginally positive effect to a 12-fold increase in shell density for a 70% increase in seroprevalence.

The random effects of year, site, and their interaction were relatively small: the estimated posterior means for the standard deviations of these effects were 0.94 (site), 0.41 (year), and 0.33 (individual), and the 95% credible intervals of individual site and year deviations all included zero, indicating that no sites or years were detected as significant outliers from the overall pattern.

The alternative categorical model for the effect of seroprevalence gave similar, although slightly weaker, results: the mean of a_2 was 0.87 (corresponding to an $e^{0.87} = 2.4$-fold increase in shell density on high-prevalence sites), although the 95% credible interval (−0.22, 2.34) included zero. Both models including seroprevalence as a predictor had very similar DIC values (93.9 for the continuous model vs. 94.2 for the categorical model), lower than the DIC of the model without prevalence (96.2), although not substantially lower.

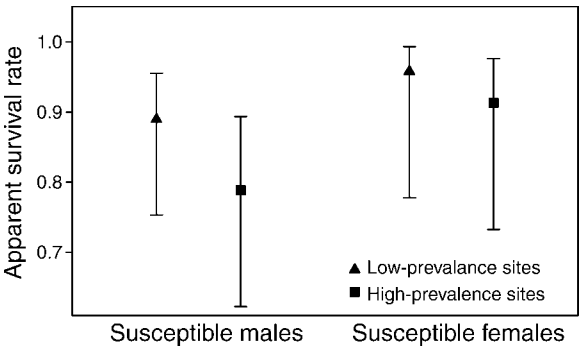

FIG. 2. Estimates of annual apparent survival rates for adult male and female tortoises in susceptible state in sites with low (<25%) and high (≥25%) seroprevalence to *Mycoplasma agassizii*. Mean values and 95% credible intervals were estimated using model 3 in Table 4. Survival rates for seropositive male and female tortoises were estimated as 1.00 with inestimable credible intervals due to very small standard errors (<0.0001).

DISCUSSION

Emerging infectious diseases (EIDs) have played an important role in shaping human history, and their roles in influencing global biodiversity are being increasingly recognized in recent years (Anderson and May 1991, Daszak et al. 2000, Hudson et al. 2002, Collinge and Ray 2006). EIDs have been implicated in population declines or local extinction of a number of species (reviewed in Daszak et al. 2000). Moreover, evidence suggests that incidences of EIDs in wildlife populations have increased substantially in recent years (Daszak et al. 2000).

The gopher tortoise is a threatened species which is critically important for the maintenance of biodiversity and ecological communities in upland habitats in Florida (Eisenberg 1983, Diemer 1986). Populations of gopher tortoises have been declining for several decades (Auffenberg and Franz 1982, McCoy et al. 2006). Although loss, fragmentation, and degradation of habitat have been the main causes of tortoise population declines, mycoplasmal upper respiratory tract disease (URTD) has been suggested to have contributed to these declines, at least in recent years (Enge et al. 2005). The rates at which susceptible individuals become exposed and infected are important determinants of the impact of a pathogen on the dynamics of host populations, but little is known about the force of infection (FOI) or the effect of *Mycoplasma agassizii* on the survival of free-ranging tortoises. Similarly, little quantitative information is available that examines the impacts of environmental factors on host survival and pathogen transmission dynamics.

We have provided, for the first time, estimates of mycoplasmal FOI in free-ranging populations of gopher tortoises. The FOI was five to six times higher at sites

PLATE 1. The gopher tortoise, *Gopherus polyphemus*. Photo credit: U.S. Geological Survey.

with high (≥25%) *M. agassizii* seroprevalence (0.22 ± 0.04) than at sites with low (<25%) seroprevalence (0.04 ± 0.01), in accordance with theoretically predicted interplay between the FOI and disease prevalence (Anderson and May 1991). Also, our results indicate that the spread of *M. agassizii* was rapid at sites characterized by high seroprevalence. When seroprevalence was low, the FOI remained low and clinical signs of URTD were rare.

Although some gopher tortoise populations with high *M. agassizii* seroprevalence have experienced recent or historical die-offs and increased clinical disease expression (Berish et al. 2000, Gates et al. 2002), the specific role of mycoplasmal URTD as a primary or an exacerbating factor in the die-offs remains unknown. Given the best available evidence about this pathogen, we expected to find a negative effect on survival. Contrary to our initial expectations, apparent survival rate of seropositive tortoises was not lower than that of seronegative tortoises. In fact, the apparent survival rate of seropositive tortoises approached one and was higher than that of seronegative tortoises. This result was surprising, given our understanding of the dynamics of chronic mycoplasmal respiratory disease in mammals and birds (Simecka et al. 1992, Cartner et al. 1996, Vicca et al. 2002, Faustino et al. 2004, Lesnoff et al. 2004). One possible explanation for this finding is that seropositive tortoises captured in our study represent individuals within the population that survived the initial infection and then developed chronic disease. Detecting low-level chronic effects of URTD will likely require monitoring tortoise populations over a substantially longer time frame. For example, population declines in the desert tortoise occurred 10–15 years after the initial observation of clinical signs of URTD (Berry 1997); short-term studies would not have detected these effects. Also, chronically infected tortoises may be less likely to emigrate from a given site when compared to healthy conspecifics, which could lead to a higher apparent survival of infected tortoises as observed in our study.

Because tortoises were sampled only annually, it was not possible to directly measure within-year seroconversion or acute effects of the disease leading to death of tortoises if they occurred (Simecka et al. 1992). Therefore, we tested for potential acute effects of mycoplasmal URTD indirectly by evaluating apparent survival in susceptible (i.e., seronegative) tortoises. Survival of susceptible adult males in high seroprevalence sites was slightly lower than that in low seroprevalence sites, providing evidence that URTD may have an acute effect on tortoise survival. However, only two of the populations studied were actually in the acute phase of disease during the 5-year duration of the study. One population, Cecil Field/Branan Field Wildlife and Environmental Area (CF), was transitioning from acute to chronic disease during the study, and the other, Fort Cooper State Park (FC), had a number of newly seroconverted tortoises during the last year of the study. Thus, our ability to detect the influence of URTD on survival of tortoises was limited by the timing and short duration of the study and by annual sampling intervals used in the present study. Detection of acute effects would be possible only with a fine-scale sampling protocol (e.g., weekly or monthly), which is difficult to implement under field conditions.

Faustino et al. (2004) used a similar capture–mark–recapture (CMR) analysis and found that *M. gallisepticum* infection significantly reduced apparent survival

rates in house finches. In that study, data on clinical signs were collected on weekly intervals; consequently, acute effects of the disease on survival of finches were likely to be detected. Our study was based on the serological status of individuals sampled annually and could not detect within-year seroconversion and ensuing mortality if they occurred. Thus, differences in study design and sampling frequency could partially explain the difference in results between the two studies. Further, the manifestation of clinical disease and life-histories of these two hosts are vastly different; differences in findings of these two studies, therefore, are hardly surprising.

The analysis of shell remains, which did find an effect of seroprevalence on density of shell remains, suggests a slightly stronger conclusion. However, this analysis has the following caveats: (1) like the site-dependent CMR analysis, it ties effects of *M. agassizii* to sites and not to individual infection status, so that the effects could be caused by environmental correlates of disease or by other population-level effects associated with disease; (2) unlike CMR, we have no explicit estimate of detectability or differences in detectability among sites.

Can we reconcile the differences among the analyses? The CMR estimates approximate annual apparent survival rates (averaged across sexes) to 0.90 in low-prevalence vs. 0.86 in high-prevalence sites, equivalent to a 40% decrease in annual mortality. The shell remains analysis estimates that we recover ~1 shell per 100 individuals at low prevalence ($e^{a_0} = 0.008$), so only ~1 in 10 mortality events predicted by the CMR analysis leaves a detectable shell. Provided that the ratio of shell counts to population size is proportional to the mortality probability, the proportional changes in shell densities should match the proportional increase in mortality. The estimated four-fold change in shell densities for a change of 70% in mycoplasmal seroprevalence (from 0.99 to 4.0 shells per 120 tortoises) is higher than the estimated 2.5-fold increase in apparent mortality of susceptible tortoises in low- vs. high-prevalence sites, but these values are not statistically distinguishable. Nonetheless, the credible intervals on the change in shell densities exclude 1.0 (indicating a significant increase in shell densities), whereas the confidence limits on the change in apparent mortality include 1.0 (indicating no significant effect; Fig. 3).

The analysis of tortoise remains detected a difference in shell recovery between high- and low-prevalence sites, whereas CMR failed to detect a strong effect of mycoplasmal serological status on tortoise survival. As the shell analysis is less rigorous than CMR, its effects could be biased by differences that CMR controls for (such as sex or detectability differences). On the other hand, the analysis of shells does use a different kind of data; shells record dead individuals (although an unknown, possibly nonrandom, sample), whereas CMR detects only the *absence* of individuals. For both analyses, the actual cause of the mortality is unknown.

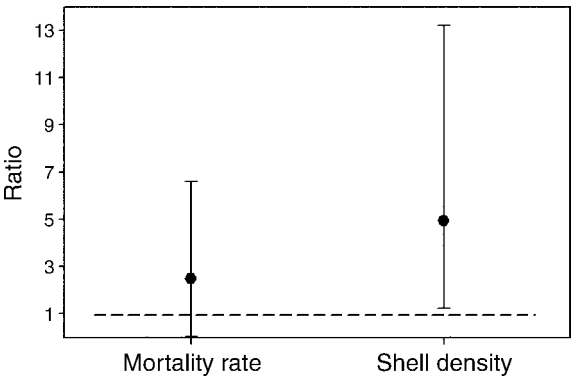

FIG. 3. Point estimates and confidence intervals for the proportional change in overall apparent mortality rate and in shell remains between a site with 0 seroprevalence and a site with 70% seroprevalence. In each case, the ratio is the predicted value (mortality or shell-remains density) for a high-prevalence site divided by the predicted value for a low-prevalence site. Error bars represent 95% credible or confidence intervals. Dashed line at ratio = 1.0 shows the null hypothesis of no change in mortality or shell remains.

Nevertheless, even with shell analysis, the effects (although consistent across a variety of analyses) are just detectable. In the case of a chronic disease in a long-lived species, actually quantifying low-level impacts of an infectious disease on an annual basis can be a daunting task. As with many wildlife populations, it is difficult to tease the evidence of ecological and epidemiological processes apart from the noise of environmental and sampling variability (Fieberg and Ellner 2000). Nonetheless, such studies are crucial for making quantitative assessments with regards to the relative health of different populations.

Two covariates that have management implications influenced apparent survival in our study. First, tortoises in sites with low basking-site availability had reduced apparent survival rates. Our findings provide quantitative evidence that habitat parameters, and therefore management activities, may significantly impact vital demographic rates. Secondly, we found that tortoises on sites with a history of relocation had lower apparent survival rates than those on sites with no documented relocation. Management strategies and federal recovery plans for gopher tortoises include relocation or restocking of tortoises as an option for mitigating impacts of habitat loss due to anthropogenic activities. It is important to note that we did not test for interactions among the covariates, and therefore, further research is required to determine underlying factors that may influence these results. However, the well-intentioned practice of tortoise relocation may negatively impact populations, particularly with regard to the spread of pathogens.

Chronic diseases have potentially severe long-term consequences for populations (Spalding and Forrester 1993, Hess 1996). Population growth rates of species characterized by delayed maturity, low recruitment, and

long lifespans are highly sensitive to changes in adult survival (Crowder et al. 1994, Doak et al. 1994, Oli and Dobson 2003). Thus, even a small decline in the survival of adult gopher tortoises can significantly decrease the population growth rate and the long-term persistence of populations (Doak et al. 1994, Miller 2001). However, statistical detection of small-scale increases in mortality is exceedingly difficult. A concern regarding mycoplasmal URTD is that low-level, long-term increases in mortality may be occurring within populations experiencing chronic disease. Our findings that susceptible tortoises in high seroprevalence populations have decreased apparent survival, coupled with the increase in shell remains at high seroprevalence sites, would suggest a low-level effect in the initial stages of disease. Long-term sampling of well-characterized populations and marked individuals over a 10–20-year period will be required to fully understand the long-term population level effects of the chronic disease state. Alternate approaches may also be required to assess the effects of URTD in populations where the pathogen is already endemic. Finally, estimates of the FOI and survival rates reported here, when incorporated into quantitative demographic models, will provide a basis for predicting the spread and impact of disease on the dynamics and persistence of tortoise populations under different management strategies.

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