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Disease dynamics during wildlife translocations: disruptions to the host population and potential consequences for transmission in desert tortoise contact networks

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Abstract

Wildlife managers consider animal translocation a means of increasing the viability of a local population. However, augmentation may disrupt existing resident disease dynamics and initiate an outbreak that would effectively offset any advantages the translocation may have achieved. This paper examines fundamental concepts of disease ecology and identifies the conditions that will increase the likelihood of a disease outbreak following translocation. We highlight the importance of susceptibility to infection, population size and population connectivity – a characteristic likely affected by translocation but not often considered in risk assessments – in estimating outbreak risk due to translocation. We then explore these features in a species of conservation concern often translocated in the presence of infectious disease, the Mojave Desert tortoise, and use data from experimental tortoise translocations to detect changes in population connectivity that may influence pathogen transmission. Preliminary analyses comparing contact networks inferred from spatial data at control and translocation plots and infection simulation results through these networks suggest increased outbreak risk following translocation due to dispersal-driven changes in contact frequency and network structure. We outline future research goals to test these concepts and aid managers in designing effective risk assessment and intervention strategies that will improve translocation success.

Introduction

Wildlife translocation has developed into a widely used tool to either reintroduce or supplement existing populations in response to the growing needs of wildlife management and conservation. The frequency and objectives of translocations worldwide are increasing in an attempt to reduce the impacts of fragmentation, habitat loss and climate change (Fischer & Lindenmayer, 2000; Thomas, 2011; Weeks *et al*., 2011). Regardless of specific project goals, the challenge for wildlife managers is: How can wildlife translocations be executed in a manner that simultaneously minimizes risk to natural populations and to the translocated individuals?

One major risk in translocation is the threat of infectious disease to the recipient population, the translocated animals and the larger potential host community. An infectious disease is any abnormal function or change in structure of an organ or organ systems in a host due to colonization by a pathogen (any disease-causing parasite including bacteria, viruses, protozoa, fungi, helminths and ectoparasites). Unforeseen disease outbreaks can result in significant mortality or reduced fitness, and hence reduce rather than augment the population (Cunningham, 1996; Deem, Karesh & Weisman, 2001; Kock, Woodford & Rossiter, 2010; Sainsbury & Vaughan-Higgins, 2012). Many early translocations either failed or exhibited complications due to disease (reviewed in Cunningham, 1996; Kock *et al*., 2010), necessitating the development of methods to identify and reduce disease threats (Leighton, 2002; Armstrong, Jakob-Hoff & Seal, 2003; Miller, 2007; Hartley & Gill, 2010).

During preliminary disease risk assessments, managers may attempt to prioritize pathogens based on characteristics

that signify the greatest threat to a translocation. Such ranking is often based on a pathogen's current presence or likelihood of introduction into the translocated or resident host population, the virulence or severity of disease caused in infected individuals, and anticipated transmission rates (Miller, 2007). The latter requires further knowledge of the duration and frequency of infectiousness, contact rates between infected and susceptible hosts, and host susceptibility to infection given pathogen exposure – parameters that are often unknown in wild populations and highly variable among individual hosts and environmental conditions (Anderson, 2009).

In addition to the uncertainty associated with natural disease dynamics, these assessments should consider translocation as a potentially disruptive event that may influence the parameters that define disease risk and must therefore ask: Will susceptibility to infection and disease be affected by translocation? Will the frequency of contact between hosts and, thus, pathogen exposure change? Will translocation disrupt the present structure of the population and the spatial extent of pathogen transmission? Without further investigation of potential translocation effects on key components of the host – parasite system, managers may underestimate the disease risks associated with a translocation.

Published translocations that cite post-release mortality due to disease are commonly associated with resident pathogens encountered at the release site (Ewen *et al.*, 2012). In reinforcement translocations, individuals are released into an existing population of conspecifics with a natural parasite community. When this occurs, several population characteristics may change as relocated hosts move across the landscape and interact with established residents and their parasites. Many of these changes may increase the risk of, spread, and magnitude of disease outbreaks even if all relocated animals are healthy at the time of release. In this paper, we will discuss common features observed following translocations that can affect transmission and illustrate potential consequences with preliminary data on Mojave Desert tortoises *Gopherus agassizii*. We propose future research on multi-scale processes relevant to population disease dynamics and accurate translocation risk assessments.

Dynamics of disease invasion

Disease dynamics in wild populations rely on several processes that begin with the transmission of a pathogenic parasite from an infectious host to a susceptible host, resulting in infection. This infection may or may not progress to clinical disease, which can cause symptoms that either lead to mortality or the clearance of the infection through the actions of the immune system. In some instances, the pathogen is not cleared and the infection is persistent. During infection, there are often one or more periods of infectiousness during which the host can transmit the infective stages of the parasite to other hosts or to intermediate vectors.

In epidemiological studies, the basic reproduction number, R_0, is used to quantify the transmission potential of a disease. R_0 can be defined as the number of secondary infections caused by a single infected individual introduced into a population made up entirely of susceptible individuals. In a population of N individuals with a transmission rate (β), each infective individual can, on average, give rise to βN new infections during an infectious period of $1/\gamma$, where γ is the average rate of recovery, and thus R_0 can be estimated as

$$R_0 = \beta N / \gamma$$

If R_0 is less than 1, then on average an insufficient number of hosts are infected for continued transmission and the outbreak fails to establish. Larger transmission rates (which can stem from higher contact rates or higher susceptibility to infection), long infectious periods and greater population sizes will all facilitate an outbreak. There are also some potential nonlinearities in the system that may increase the likelihood of an outbreak, particularly when there is variation in susceptibility and infectiousness between individual hosts. For example, if highly susceptible hosts are also more infectious, the likelihood of an epidemic will increase although duration of the epidemic may be shorter (Keeling *et al.*, 2002; Hudson *et al.*, 2008).

When host populations exhibit heterogeneity, mean field models may fail to capture the dynamics and network models can be used to integrate individual level variation in contact, susceptibility and transmission (Keeling & Eames, 2005; Bansal, Grenfell & Meyers, 2007). These models represent hosts as nodes in a network with connections between nodes signifying unique contacts or transmission pathways. The structure of the network influences the rate of spread and the likelihood distribution of a disease outbreak, and hence the basic reproduction number (R_0) (Cross *et al.*, 2004; Bansal *et al.*, 2007; Porphyre *et al.*, 2008). An individual's position in the network not only influences his infection risk but also his role in transmission (Fig. 1) (Christley *et al.*, 2005; Drewe, 2010). Contact networks are rarely incorporated into risk assessments, but could provide a useful tool for identifying risk at several scales. We will use these models in our pilot study to illustrate their applicability to translocation risk assessments.

Dynamics of infectious disease associated with host translocations

Stress, virulence and susceptibility

Capturing and releasing animals can result in increased stress. In addition to invasive procedures such as handling, veterinary examination, captivity and transport, translocation also contributes several subtle stressors such as an increase in population size that may intensify conspecific competition, disturbance via repeated monitoring,

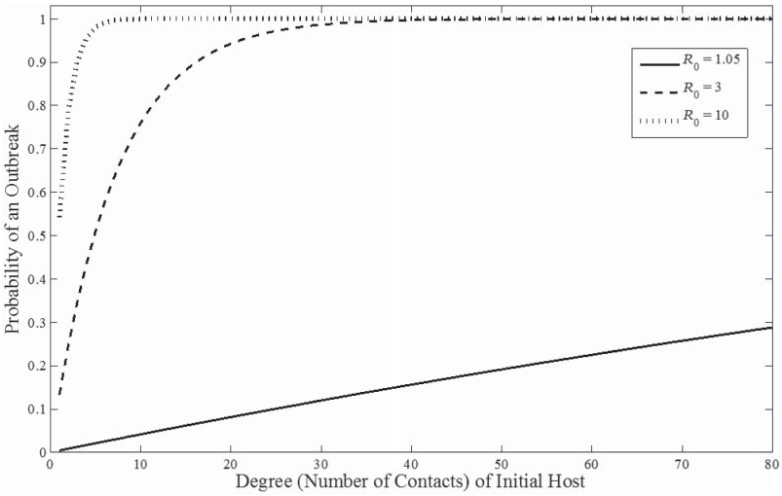

Figure 1 The probability of a disease outbreak in relation to the number of contacts made by the initial invading host with respect to the average value of the basic reproduction number R_0.

interruption of social bonds and introduction to a novel environment (see Teixeira *et al*., 2007, Dickens, Delehanty & Romero, 2010, and Parker *et al*., 2012 for a thorough review of translocation-related stress research). Repeated or prolonged exposure to multiple stressors may contribute to chronic stress where sustained physiological changes trigger immunosuppression that can increase susceptibility to infections and consequently increase pathogen virulence, both of which influence R_0 and the likelihood of an outbreak (Lafferty & Holt, 2003; Dickens *et al*., 2010).

Sudden outbreaks of disease following translocation may not involve the introduction of a novel parasite as is often assumed, but instead may result from the alteration of an existing host – parasite relationship due to stress and increased susceptibility. Stress-induced physiological changes may increase transmission rates of endemic pathogens or result in normally nonpathogenic parasites eliciting disease, which can increase disease prevalence and possibly mortality. For example, Coccidia are typically commensal microparasites in the Eurasian crane *Grus grus*, but intensity of infection can increase when host densities are high and will cause disease if immature birds are stressed (Sainsbury & Vaughan-Higgins, 2012). The behavioral and physiological changes that occur in response to acute and chronic stressors have been linked to higher rates of disease, suggesting preexisting host – parasite relationships change in the presence of stress (Dickens *et al*., 2010).

Release strategies, host density thresholds and contact rate

A common goal of translocations is to bolster population numbers and establish self-sustaining populations, which is often achieved through the release of large numbers of individuals (Griffith *et al*., 1989; Fischer & Lindenmayer, 2000; IUCN/SSC, 2013). In this way, even if some individuals succumb to mortality or disperse from the intended site, an adequate number of individuals may remain to establish and reproduce. Fischer & Lindenmayer (2000) reported that past translocations generally had higher success when more than 100 animals were released. However, recent studies show that while one-time high number releases positively effect some species, others benefit from repeated low number releases. (Shier, 2006; Linklater & Swaisgood, 2008; Faria, van Oosterhout & Cable, 2010; Shier & Swaisgood, 2012). Despite inconclusive experimental evidence, translocation guidelines recommend releasing large numbers to increase success and that multiple releases and simultaneous releases at multiple sites may have added benefit (IUCN/SSC, 2013).

Current translocation recommendations for high-number releases result in an instantaneous increase in the number of susceptible individuals effectively increasing N and so R_0, potentially fueling an epizootic that previously could not establish or that had spread through the population and died off due to an inadequate supply of susceptible hosts (Lloyd-Smith *et al*., 2005). Such a situation may be produced under natural conditions as part of an established host – parasite relationship. For example, seasonal breeding in the house finch *Carpodacus mexicanus* results in an influx of susceptible hosts, which corresponds with increased R_0 and infections by *Mycoplasma gallisepticum* (Hosseini, Dhondt & Dobson, 2004). Therefore, in populations that typically have stable numbers and low recruitment, sudden increases in host availability caused by translocation may result in significantly altered disease dynamics.

The release of large numbers may also increase contact rates between animals (Linklater & Swaisgood, 2008). High contact rates may not be uniformly distributed in the population; a few individuals have many contacts and whether these individuals are infected early in the invasion can greatly increase the likelihood of an outbreak (Fig. 1). In effect, a few highly connected hosts can be considered 'super spreaders' (Lloyd-Smith *et al*., 2005; Bansal *et al*., 2007; Hudson *et al*., 2008).

Dispersal and contact network structure

Following translocation, many species (e.g. mammals, birds, reptiles, amphibians) experience a period of rapid movement and a tendency to disperse away from the release site (Germano & Bishop, 2008; Kesler *et al*., 2012; Le Gouar, Mihoub & Sarrazin, 2012). This response may be exploratory in nature, or tied to habitat quality, competition, social behavior, homing attempts or stress (Burns, 2005; Letty, Marchandeau & Aubineau, 2007; Dickens *et al*., 2010; Tsoar *et al*., 2011; Gedeon *et al*., 2012). Exploratory or dispersing behavior varies in duration, with normal movement patterns resuming as soon as 1 day after release to as long as multiple years (Heidinger *et al*., 2009; Nussear *et al*., 2012). Dispersal is often viewed as a negative outcome because it can expose animals to risk of mortality from natural enemies and abiotic causes and can lead to establishment outside of the intended settlement area (Miller *et al*., 1999; Germano & Bishop, 2008). Less appreciated is the threat dispersal presents to the population by changing disease transmission risks. Moving over large areas can result in greater overlap with conspecifics. If translocated animals have disproportionately higher contact opportunities and increase the connectivity of animals across the landscape, they could rapidly facilitate disease spread if infected. Keeling & Eames (2005) note that, 'rare long-range connections have a surprisingly large effect' on the magnitude of an infectious outbreak and highlight the importance of long-distance contacts in transmitting disease to otherwise disconnected groups (Eames, 2008).

Many animal populations have spatially clustered distributions, whether in response to a clustered resource, territoriality, or structured social or family groups (Sasaki, 1997; Grear & Schmitz, 2005; Chamaillé-Jammes, Valeix & Fritz, 2007). Meta-population structures can protect populations from epizootics, localizing outbreaks within subgroups and reducing the probability of rapid spread through the entire population (Altizer *et al*., 2003; Lopez, Gallinot & Wade, 2005). Simulations suggest that increased connectivity between subgroups results in increased vulnerability to outbreaks, predominantly with highly infectious, low-severity diseases as high host survival will allow more time for infected individuals to move between groups (Hess, 1994, 1996; Cross *et al*., 2004; Griffin & Nunn, 2011). Dispersal of translocated animals is likely to increase connectivity, and therefore, resident population structure and post-release movements should be incorporated into models of disease spread in risk assessments.

An example of disease risk in translocations: the Mojave Desert tortoise

Introduction to the host – parasite system

Gopherus agassizii is a long-lived, terrestrial tortoise that occurs throughout the Mojave Desert north and west of the Colorado River. The species was listed as threatened under the U.S. Endangered Species Act in 1990, largely due to declines in populations throughout their range, loss of habitat and concerns regarding an upper respiratory tract disease (USFWS, 1990, 1994, 2011). Currently, there are a number of solar energy facilities being developed across the Mojave Desert and plans for several future facilities that will result in loss of habitat and risk of direct harm to tortoises if left on site (Lovich & Ennen, 2011). Consequently, tortoises are being translocated to neighboring occupied habitat.

Disease risk assessments for this species present many challenges: information on existing diseases and how they affect populations, causative agents, transmission patterns, latency and the development of resistance is incomplete (Sandmeier *et al*., 2009). *Mycoplasma agassizii* and *M. testudineum* are the most studied microparasites in this system and are considered the main causative agents of an upper respiratory tract disease. These pathogens are transmitted horizontally through direct contact between hosts but the potential for *Mycoplasma* spp. to survive temporarily outside the host in burrows has not been excluded (McLaughlin, 1997).

Infections with *Mycoplasma* spp. can cause recurring clinical disease interspersed by asymptomatic periods (Brown *et al*., 1994; Christopher *et al*., 2003; Sandmeier *et al*., 2009). It is unclear whether *Mycoplasma* spp. infection can be cleared from host tissues or if infected desert tortoises experience lifelong infection. Captive individuals inoculated with *M. agassizii* have been recorded shedding bacteria up to 1 year post inoculation (Brown *et al*., 1994). Prior infection does not appear to convey immunity or resistance based on studies with gopher tortoises *Gopherus polyphemus* – a host species similarly affected by *M. agassizii* infection (McLaughlin, 1997). Infected hosts appear to experience morbidity but low mortality, a condition that can still have meaningful impacts, particularly when interacting with other threats to a population (Smith, Acevedo-Whitehouse & Pedersen, 2009; Berish *et al*., 2010; Tompkins *et al*., 2011).

Current translocation guidelines recommend the movement of animals in good physical condition that do not exhibit moderate to severe clinical signs of disease (USFWS, 2013). This policy makes a number of assumptions: released animals with latent or mild infections will not progress to more virulent and transmissible infections, will integrate uniformly into the resident population, and will exhibit natural levels of contact and transmission. How infections in the translocated and resident population manifest and transmit through the population after release will largely depend on whether translocation alters disease parameters as discussed in above.

Current knowledge of desert tortoise translocations: implications for disease

Stress and virulence

Studies have found no statistical difference in potential stress indicators such as survival, egg production or corticosterone production (a common acute stress response

in reptiles) between translocated, resident and control tortoises (Drake *et al*., 2012; Nussear *et al*., 2012). Other stress-induced physiological changes may still weaken the immune response as observations suggest stressful environmental conditions may trigger severe upper respiratory tract disease symptoms (Sandmeier *et al*., 2009). No published studies have examined translocation effects on host susceptibility to Mycoplasma spp. infection or changes in intensity of existing infections and severity of clinical disease. Additionally, coinfections with parasites not considered in tortoise risk assessments may increase virulence of *Mycoplasma* spp. and the infectious period since naive tortoises inoculated with nasal discharge from infected individuals experienced more severe symptoms than hosts inoculated with *M. agassizii* cultures alone (Brown *et al*., 1994). If translocation affects immune response or increases virulent polymicrobial associations, rates of transmission-facilitating contact will likely increase (Anderson, 2009).

Population size and density

The number of tortoises relocated to each site during a translocation is often influenced by resident population density. Managers attempt to limit post-release densities to the average density of tortoise populations across the local recovery unit (a subdivision of the larger population) (USFWS, 2011) and encourage translocations to depopulated areas. While density limits are a positive step in preventing extreme changes in host numbers and avoiding density-dependent responses, population densities are difficult to estimate in this species (Nussear & Tracy, 2007; Inman, Nussear & Tracy, 2009). Underestimates of site resident density may result in more tortoises being released than should be, inducing a rapid change in population density. This contrasts with natural populations where increases in tortoise abundance are slow due to high juvenile mortality (Bjurlin & Bissonette, 2004) and slow recruitment (Woodbury & Hardy, 1948); as such, in undisturbed populations, host – parasite dynamics also are likely to change slowly over time.

Dispersal

Desert tortoise distributions exhibit substructuring within populations with small numbers of tortoises aggregated in discrete areas and empty habitat between (Duda, Krzysik & Meloche, 2002). While frequent interaction between adjacent groups is likely, longer distance movements greater than 3 km appear less common (Duda *et al*., 2002; Harless *et al*., 2009, 2010; Franks, Avery & Spotila, 2011). Limited movement between groups should protect tortoise populations from rapid spread of chronic upper respiratory tract disease, particularly if severe disease is triggered by low-resource conditions such as drought when tortoises responsively reduce movements across the landscape or if infection results in reduced surface activity (Brown *et al*., 1994; Duda, Krzysik & Freilich, 1999; Sandmeier *et al*., 2009). Desert tortoises frequently disperse after relocation often in the form of atypical straight-line paths, as illustrated in Fig. 2 (Field *et al*., 2007; Hinderle, 2011; Nussear *et al*., 2012). These dispersal paths can greatly exceed the maximum distances traveled by residents and are likely to connect several normally disconnected subgroups (Nussear *et al*., 2012).

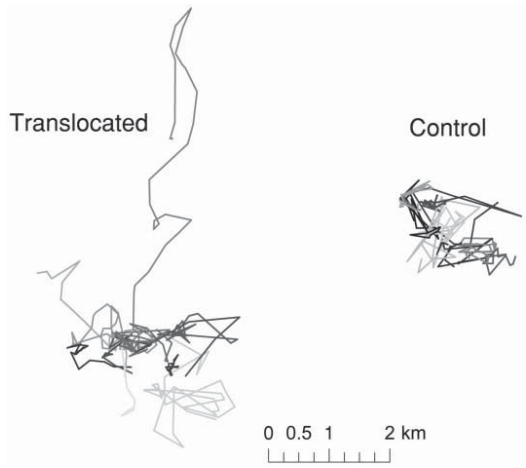

Figure 2 Movement paths of 10 control and 10 translocated tortoises during the first 2 months following release (3 April to 31 May 2008). Controls were resident tortoises at plots *c*. 2 km from the release site.

Pilot study: potential impacts to contact rates and connectivity in desert tortoises

The combined effect of increased population size and dispersal on population contact parameters can be explored with a dynamic network model. The dispersal of released tortoises creates temporal changes in spatial configuration that can influence how a pathogen travels through a population (Bansal *et al*., 2010). We used geographic locations for desert tortoises prior to and following a translocation at three translocation plots and three control plots to model dynamic contact networks and identify changes in contact rates and connectivity due to translocation. We estimated changes in disease risk by simulating infection through these networks and comparing resulting prevalence at translocation and control plots. Specifically, we asked: Did contact rate and percent animals connected in the network increase at translocation plots and not at controls immediately following translocation and, if so, how long did the perturbation last? If a network change occurred, is infection prevalence higher in residents at translocation plots than controls when we simulate infection transmission through the networks?

Materials and methods

Study site

Data for this study were collected during a translocation project that relocated animals displaced by the expansion of

Fort Irwin National Training Center, California, to nearby suitable habitat on public lands near Barstow, San Bernardino County, California, USA (Esque, Nussear & Medica, 2005). Multiple translocation sites were selected across a 1000 km^2 area based on habitat suitability, potential threats or disturbance, and existing tortoise densities (Esque *et al*., 2005; Heaton *et al*., 2008). Three of these sites were selected for this study because of the frequent tracking schedule of tortoises implemented at these sites and will be referred to as sites 1, 2 and 3. At each site, there are two 2.6 km^2 plots of similar habitat composition spaced at a distance of *c.* 2 km. Translocations occurred at one of the two plots at each site during 3–10 April 2008 while the other plot was left unaltered as a control.

Study animals and movement data

Surveys were conducted for samples of tortoises at all plots. Tortoises from the translocation plot are referred to as 'Residents' and tortoises at the control plots are referred to as 'Controls'. In spring of 2008, desert tortoises removed from the Fort Irwin expansion area were released within the translocation plots across the study site and following translocation, translocated (site 1 $n = 34$, site 2 $n = 33$, site 3 $n = 39$), resident (site 1 $n = 14$, site 2 $n = 20$, site 3 $n = 11$) and control (site 1 $n = 7$, site 2 $n = 14$, site 3 $n = 13$) animals were located approximately biweekly using radiotelemetry. For this study, all adult tortoises that were located within the boundary of the plot on at least one occasion in 2008 and relocated a minimum of 50 times during the post-translocation period of 3 April 2008 to 29 October 2008 were used in analysis so that animals missing for extended periods were not included.

Contact network construction and disease simulation

A contact network is typically represented with a graph of *nodes* connected by lines (referred to as *edges*) that signify a direct or inferred relationship. Each node in our graph represented a single tortoise and an edge between two tortoises signified an inferred contact. Inferred contacts were defined as a spatial proximity of 100 m or less (this distance is within the daily movement ranges observed for our tortoises) within 3 days time (all animals were typically tracked within 2–3 days time of one another). Spatial proximity or home-range overlap is often used to model networks in wild populations when interaction data are unavailable (Cross *et al*., 2004; Godfrey *et al*., 2010; Fenner, Godfrey & Bull, 2011). This dataset was originally collected to answer questions unrelated to social interaction and true observations of contact between tortoises were rare. We chose a fairly relaxed definition of contact to accommodate the potential error of handheld GPS (global positioning system) locations, short observation times during data collection, and temporal asynchrony of tracking events. These networks therefore reflect a nearest neighbor network and connected nodes represent tortoise pairs with a high potential for contact due to their proximity.

We constructed a dynamic contact network for each translocation and control plot that reflected the movement of animals through time. We created a pre-translocation graph for a 15-day period prior to translocation for tortoises at all plots (17 March to 31 March 2008). We then updated the graph with new edges based on changing tortoise locations at time steps of 10 days for a total of 21 time steps for the active season beginning with the first day of translocations (3 April 2008 to 29 October 2008). To simulate an infectious disease originating from the resident population, we selected a random resident to infect at time 0, and at each subsequent time step ($n = 21$) edges in the graph were updated to reflect tortoise movement, and any infected animals had a set probability of transmitting their infection to any animal they were connected to in the graph. We ran simulations with varied transmission probabilities of infected individuals to contacts from 0.1 to 1.0 in increments of 0.1 to explore the uncertainty in transmission on contact. No recovery or mortality due to infection was incorporated into simulations to reflect the characteristics of upper respiratory tract disease. Simulations were run 100 times for each transmission probability on each network. At the end of each simulation, the number of infected resident, translocated and control tortoises was recorded.

At each time step, we calculated the degree and betweenness centrality for each node (Christley *et al*., 2005). Degree is defined as the sum of all edges connected to that node, reflecting the total number of unique individuals that tortoise may have contacted, and the degree distribution reflects the variability of hypothetical contacts across a population. Betweenness centrality is the proportion of shortest paths connecting any two nodes in the network that pass through the node of interest, signifying an animal's role as a 'bridge' between other animals. Both measures have been associated with time to infection and infection risk in both simulation and field studies of pathogen transmission (Corner, Pfeiffer & Morris, 2003; Christley *et al*., 2005; Fenner *et al*., 2011). Additionally, we calculated the percentage of nodes connected in the largest component of each graph. A component is a group of nodes connected to each other but to no other nodes in the network. All networks and network measures were created using the package igraph in the program R (Csardi & Nepusz, 2006; R Development Core Team, 2012).

Results

We first compared the networks for the time period immediately prior to translocation and the 10-day period including the release of tortoises, as well as the days immediately following release. Degree distributions were similar at the control and translocation plots before release, with few potential contacts between tortoises (Fig. 3a). Once translocation occurred, high degrees were frequent at translocation plots (Fig. 3b), and translocated tortoises had the most opportunities for contact. We estimated the percentage of

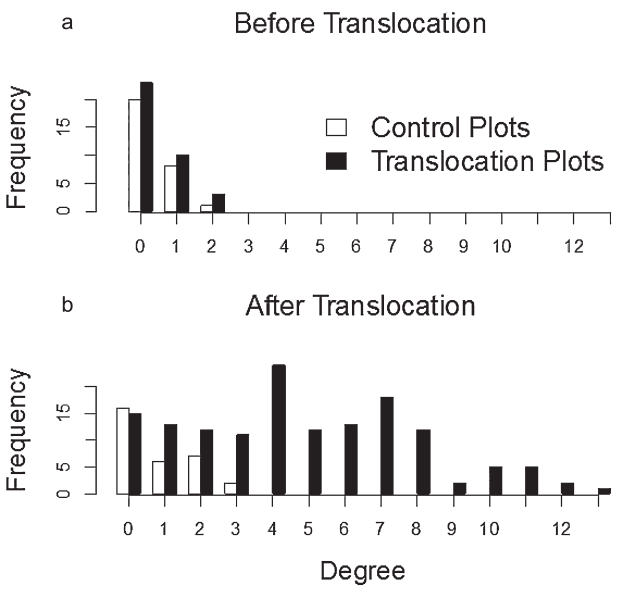

Figure 3 Degree distribution of tortoises at translocation and control plots at three sites for (a) a 15-day period prior to the release of translocated tortoises (17 March to 31 March 2008) and (b) a 10-day period when additional tortoises were released at each translocation plot over 2 days (3–12 April 2008). Degrees were based on inferred contact networks that considered two tortoises in contact based on spatial proximity < 100 m of locations made within 3 days time of each other. An individual's degree represents the total number of unique individuals a tortoise had the potential to contact.

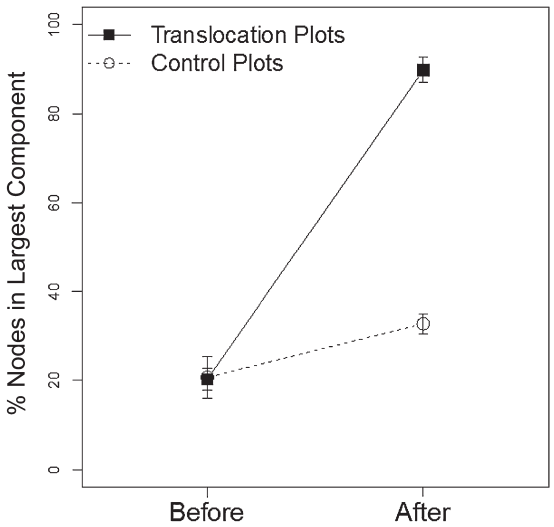

Figure 4 Average per cent of tortoises connected in the largest connected component of hypothetical contact networks for three sites prior to and following the release of animals at translocation plots. Control plots received no additional tortoises. Contact between tortoises was assumed based on spatial proximity < 100 m of locations made within 3 days time of each other. Network components are groups of tortoises connected to each other but to no other individuals in the network.

tortoises spatially 'connected' in the largest component or subgroup of each network (Fig. 4) and found that on average, 90% of tortoises at the translocation plots were potentially connected in one large subgroup compared to 33% at control plots at the time of releases.

The network changes at translocation sites were temporary, diminishing 10–20 days after translocation as released animals moved further from the site (Fig. 5). During this 20-day period following release, the translocated tortoises showed higher betweenness centrality scores compared to residents (Fig. 5). Even though local contact disruption was temporary, translocation sites often had a higher percentage of residents infected at the end of simulations than at control sites (Fig. 6).

Discussion

While wildlife managers recognize the potential for disease to affect translocation success, the potential for translocation to affect infection prevalence and the mechanisms through which this occurs needs more rigorous study. In this paper, we used the basic reproduction number R_0 and contact network methods to emphasize how changes in host susceptibility, population size, contact rates and connectivity between sub-populations can increase the likelihood of pathogen spread even if the pathogen did not originate from a translocated animal. Translocated animals, though often healthy at the time of selection, may be at high risk of acquiring infection from residents and facilitating spread. High mobility after release may increase contact opportunity, and stress associated with translocation may increase susceptibility or make even an avirulent infection more virulent. Indeed, a number of factors may increase R_0 after translocation and disrupt a potentially stable host – parasite relationship.

We describe the relevance of these translocation concerns in a heavily managed species, the desert tortoise, and show how pathogen spread may be affected by translocation using pilot data. A temporary change in spatial network characteristics occurred at translocation plots but not control plots. The change in degree distribution of our estimated translocation networks suggests higher contact rates are possible after translocation, especially for highly mobile translocated animals that were often observed within the vicinity of several unique neighbors following release. This post-release activity may also increase resident contact opportunities and facilitate increases in resident connectivity. We expect the movement of translocated tortoises away from the release site plays an important role in connecting distinct subgroups, as demonstrated by their high betweenness centrality in the first 10–20 days following release and the large percentage of tortoises incorporated in the largest connected component of networks after translocation. High degree and betweenness centrality is often associated with higher risk of acquiring and transmitting infection, especially if stress has compromised immune response (Corner *et al.*, 2003; Christley *et al.*, 2005; Fenner *et al.*, 2011; Plowright *et al.*, 2013).

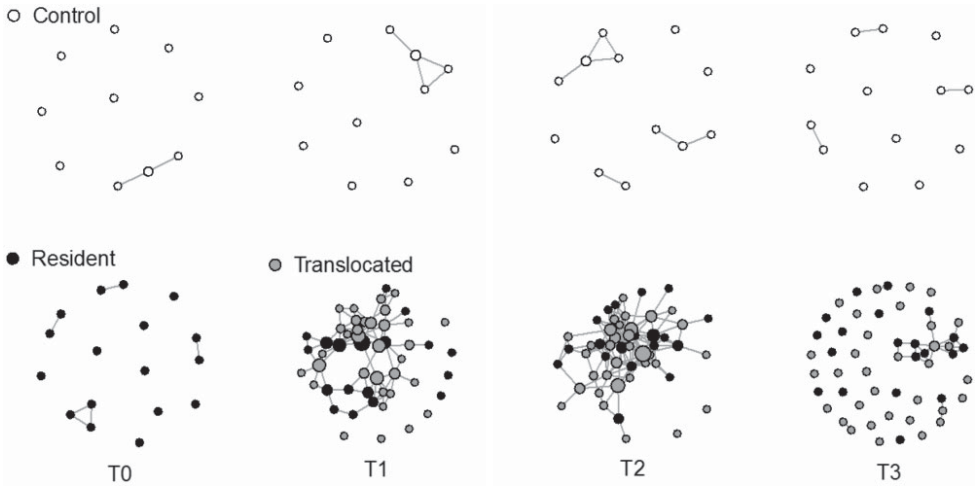

Figure 5 Inferred contact network of desert tortoises at site 2 control (top row) and translocation plot (bottom row) 15 days prior to translocation (T0; 17 March to 31 March 2008) and 10-day intervals during translocation (T1; 3–12 April 2008) and following translocation (T2 and T3; 13 April to 2 May 2008). Networks were drawn using the 'fruchterman reingold' algorithm to determine layout, therefore node (tortoise) location does not reflect geographic location (Fruchterman & Reingold, 1991). Lines connecting nodes represent an inferred contact between tortoises based on spatial proximity < 100 m of locations made within 3 days time of each other during that time step. Node size is scaled by its betweenness centrality score: a measure that reflects an individual's bridging role between other pairs or groups.

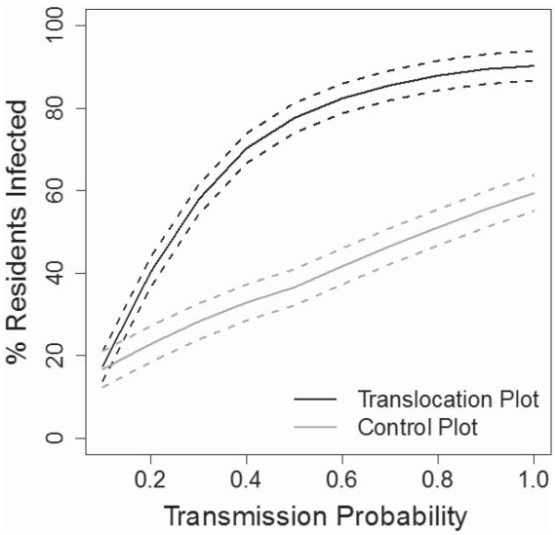

Figure 6 Infection simulation results for site 2 control and translocation plots represented by a loess smoothing curve with 95% confidence intervals. Simulations were run 100 times for each transmission probability where at each run, one random resident was infected at the start of a simulation and infection was allowed to spread based on the inferred contact networks derived from tortoise locations throughout the study period. Estimated contacts were updated at every time step to reflect tortoise movement during consecutive 10-day periods from 3 April 2008 to 29 October 2008.

Though the local connectivity changes appeared temporary, infection simulations that incorporated the movement and changing spatial configuration of these populations showed higher infection prevalence in residents at translocation networks, most notably at moderate transmission probabilities. This suggests increased disease risk at local scales, but larger impacts to population disease dynamics may also be possible. Released animals eventually moved away from the core resident group, likely returning the local connectivity to pre-translocation levels, but potentially creating connections between other groups at larger scales. Reduced clustering at the landscape level may increase outbreak risk for the wider population (Hess, 1994, 1996; Cross *et al.*, 2004; Griffin & Nunn, 2011). While this analysis suggests translocation affects contact network characteristics important to pathogen transmission, it relies on several assumptions discussed below and should be interpreted with care. This is only the first step for obtaining a more structured risk assessment for disease invasion after translocation and for developing translocation-focused research and modeling that would benefit from a risk analysis approach.

We constructed an approximation of tortoise contact networks based on radio telemetry data, but caution there are several reasons why these networks may not reflect the actual contact network. The magnitude of spatial and temporal proximity used to define a contact (100 m within 3 days), while appropriate for the coarse scale of the data available, may overestimate contacts in these populations. By estimating contact between individuals as a function of their physical proximity, we assume spatial distance between pairs is negatively correlated with contact probability, ignoring other variables that may also influence contact patterns. Translocated animals may avoid unfamiliar residents or seek out conspecifics or differences in gender between interacting animals may be important, regardless of their proximity (Serrano *et al.*, 2004; Pinter-Wollman, Isbell & Hart, 2009). We also have periods between observations when movement and contact potential is unknown.

Essentially, detailed data on true contact patterns in tortoise populations are needed to determine if such estimations are accurate.

Furthermore, even a well-documented contact network may not represent the actual *transmission* network. If long-exposure times or specific environmental conditions (e.g. high humidity in burrows) are needed to facilitate transmission between hosts, a general contact network may not be appropriate for disease transmission models. Indeed, the type, duration or location of contact can be necessary features for accurate transmission estimates as observed in both models of Sin Nombre virus prevalence in deer mice *Peromyscus* spp. and tuberculosis infections in meerkats *Suricata suricatta* (Clay *et al.*, 2009; Drewe, 2010).

There are several limitations to disease assessments in the desert tortoise system that will reflect uncertainties in the free-living, wild host – parasite systems. In addition to a lack of estimates for transmission likelihoods, the infectious period and the rate of parasite-induced host mortality, we also do not know if coinfections affect transmission, or whether infection alters host behavior such that activity and contact rates are reduced. We ran simulations using variable transmission probabilities and assumed infectiousness that lasted throughout the study period (7 months). Given the potential temporary nature of connectivity changes after translocation, a shorter or intermittent infectious period, or delayed infectiousness due to latency will influence whether short-term contact changes affect disease spread. The timing and duration of infectious period(s) will determine the time frame relevant to transmission networks; therefore, studies that provide estimates of its variation are invaluable to disease risk assessments.

Research agenda

The ultimate challenge is to collect data that we can apply to risk assessments that estimate the likelihood and consequences of an outbreak following translocation. Here, we outline a proposed research agenda on what data need to be obtained to provide a good understanding of the disease risks associated with translocation. We focus primarily on the issues relating to desert tortoise translocation, but this approach may help others in examining similar questions about host – parasite systems such as: Can we predict the transient dynamics of disease invasion with simple knowledge of infectiousness, infectious period and contact pattern? Can we predict contact networks from knowledge of population structure? Can we develop generic models that can be applied to a range of systems?

Step 1: identify the features of the parasite – host relationship at the individual level and obtain an understanding of variation between hosts

Undertake a series of transmission studies at the individual level, wherever possible, using a captive population. Estimate attributes of the infection and, in particular, understand the relationship between several features and transmission, including the likelihood of transmission with contact duration, frequency and intensity (i.e. contacts may be of similar duration but range in intensity from passive encounters, such as exploratory sniffing, to more dynamic interactions, such as combat). At the same time, seek to estimate shedding rates, the duration of infectiousness, and the influence of infection on host behavior and contact patterns.

Step 2: results from translocation and studies at the population level

Observe and quantify the effects of translocation on the contact network following experimental translocations of captive and wild animals at multiple scales. Seek to identify how the translocation influences contacts between and among translocated and resident individuals and how it disrupts previous contact patterns between residents. Proximity loggers can help record fine-resolution contact data in combination with radio telemetry data and larger scale survey data. This will help identify whether coarse scale data on distribution and abundance of residents may be used to estimate contact networks.

Step 3: application of findings to wildlife management

Combining results from steps 1 and 2 will allow managers to adjust contact networks with respect to likelihood of transmission events given levels of contact quality, predict how they may change with the proposed release, and simulate disease using parameters estimated from captive studies. If the process is successful, these models can advise managers whether a particular release site poses a high level of risk and should be avoided or whether particular release strategies may be preferred based on current population structure.

Given that epidemiology identifies the importance of heterogeneity of key host and pathogen characteristics to the transmission dynamics of populations, incorporating these features into risk assessments should be the next agenda in translocation research. Thorough knowledge of the host – pathogen relationship at an individual level will refine the parameter values applied to disease models, but equally necessary are data regarding population structure and contact rate. Most importantly, we must understand how translocation affects all of these features if our assessments are to reflect the dynamic nature of a population once hosts are added. Within the complexities of these relationships may exist dominant risk-causing characteristics that will inform managers of situations presenting the greatest impediment to translocation success.

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References

Altizer, A., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., Dobson, A.P., Ezenwa, V., Jones, K.E., Pedersen, A.B., Poss, M. & Pulliam, J.R.C. (2003). Social organization and parasite risk in mammals: integrating theory and empirical studies. *Ann. Rev. of Ecol. Evol. Syst.* **34**, 517–547.

Anderson, R.M. (2009). Epidemiology, In *Modern parasitology: a textbook of parasitology*, 2nd edn: 75–116. Cox, F.E.G. (Ed.). Oxford: Blackwell Publishing Ltd.

Armstrong, D., Jakob-Hoff, R. & Seal, U.S. (Eds) (2003). *Animal movements and disease risk: a workbook*: Apple Valley: IUCN/SSC Conservation Breeding Specialist Group.

Bansal, S., Grenfell, B.T. & Meyers, L.A. (2007). When individual behaviour matters: homogeneous and network models in epidemiology. *Royal Soc.* **4**, 879–891.

Bansal, S., Read, J., Pourbohloul, B. & Meyers, L.A. (2010). The dynamic nature of contact networks in infectious disease epidemiology. *J. Biol. Dynamics* **4**, 478–489.

Berish, J.E.D., Wendland, L.D., Kiltie, R.A., Garrison, E.P. & Cyndi, A.G. (2010). Effects of mycoplasmal upper respiratory tract disease on morbidity and mortality of gopher tortoises in northern and central Florida. *J. Wildlife Dis.* **46**, 695–705.

Bjurlin, C.D. & Bissonette, J.A. (2004). Survival during early life stages of the desert tortoise (*Gopherus agassizii*) in the south-central Mojave Desert. *J. Herpetol.* **38**, 527–535.

Brown, M.B., Schumacher, I.M., Klein, P.A., Harris, K., Correll, T. & Jacobson, E.R. (1994). *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infect. Immun.* **62**, 4580–4586.

Burns, C.E. (2005). Behavioral ecology of disturbed landscapes: the response of territorial animals to relocation. *Behav. Ecol.* **16**, 898–905.

Chamaillé-Jammes, S., Valeix, M. & Fritz, H. (2007). Managing heterogeneity in elephant distribution: interactions between elephant population density and surface-water availability. *J. Appl. Ecol.* **44**, 625–633.

Christley, R.M., Pinchbeck, G.L., Bowers, R.G., Clancy, D., French, N.P., Bennett, R. & Turner, J. (2005). Infection in social networks: using network analysis to identify high-risk individuals. *Am. J. Epidemiol.* **162**, 1024–1031.

Christopher, M.M., Berry, K.H., Henen, B.T. & Nagy, K.A. (2003). Clinical disease and laboratory abnormalities in free-ranging desert tortoises in California (1990–1995). *J. Wildlife Dis.* **39**, 35–56.

Clay, C.A., Lehmer, E.M., Previtali, A., St. Jeor, S. & Dearing, M.D. (2009). Contact heterogeneity in deer mice: implications for Sin Nombre virus transmission. *P. Roy. Soc. Lond. B. Bio.* **276**, 1305–1312.

Corner, L.A.L., Pfeiffer, D.U. & Morris, R.S. (2003). Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*). *Prev. Vet. Med.* **59**, 147–167.

Cross, P.C., Lloyd-Smith, J.O., Bowers, J.A., Hay, C.T., Hofmeyr, M. & Getz, W.M. (2004). Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park. *Ann. Zool. Fenn.* **41**, 879–892.

Csardi, G. & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal, Complex Systems* **1695**. [accessed 10 January 2013.] (http://igraph.sf.net).

Cunningham, A.A. (1996). Disease risks of wildlife translocations. *Conserv. Biol.* **10**, 349–353.

Deem, S.L., Karesh, W.B. & Weisman, W. (2001). Putting theory into practice: wildlife health in conservation. *Conserv. Biol.* **15**, 1224–1233.

Dickens, M.J., Delehanty, D.J. & Romero, L.M. (2010). Stress: an inevitable component of animal translocation. *Biol. Conserv.* **143**, 1329–1341.

Drake, K.K., Nussear, K.E., Esque, T.C., Barber, A.M., Vittum, K.M., Medica, P.A., Tracy, C.R. & Hunter, K.W. (2012). Does translocation influence physiological stress in the desert tortoise? *Anim. Conserv.* **15**, 560–570.

Drewe, J.A. (2010). Who infects whom? Social networks and tuberculosis transmission in wild meerkats. *P. Roy. Soc. Lond. B. Bio.* **277**, 633–642.

Duda, J.J., Krzysik, A.J. & Freilich, J.E. (1999). Effects of drought on desert tortoise movement and activity. *J. Wildlife Manage.* **63**, 1181–1192.

Duda, J.J., Krzysik, A.J. & Meloche, J.M. (2002). Spatial organization of desert tortoises and their burrows at a landscape scale. *Chelonian Conserv. Biol.* **4**, 387–397.

Eames, K.T. (2008). Modeling disease spread through random and regular contacts in clustered populations. *Theor. Popul. Biol.* **73**, 104–111.

Esque, T.C., Nussear, K.E. & Medica, P.A. (2005). *Desert tortoise translocation plan for Fort Irwin's land expansion program at the US Army National Training Center (NTC) at Fort Irwin*. Prepared for US Army National Training Center, Directorate of Public Works.

Ewen, J.G., Acevedo-Whitehouse, K., Alley, M.R., Carraro, C., Sainsbury, A.W., Swinnerton, K. & Woodroffe, R. (2012). Empirical considerations of parasites and health in reintroduction, In *Reintroduction biology: integrating science and management*: 290–335. Ewen, J.G., Armstrong, D.P., Parker, K.A. & Seddon, P.J. (Eds). Oxford: Wiley-Blackwell.

Faria, P.J., van Oosterhout, C. & Cable, J. (2010). Optimal release strategies for captive-bred animals in reintroduction programs: experimental infections using the guppy as a model organism. *Biol. Conserv.* **143**, 35–41.

Fenner, A.L., Godfrey, S.S. & Bull, C.M. (2011). Using social networks to deduce whether residents or dispersers spread parasites in a lizard population. *J. Anim. Ecol.* **80**, 835–843.

Field, K.J., Tracy, C.R., Medica, P.A., Marlow, R.W. & Corn, P.S. (2007). Return to the wild: translocation as a tool in conservation of the Desert Tortoise (*Gopherus agassizii*). *Biol. Conserv.* **136**, 232–245.

Fischer, J. & Lindenmayer, D.B. (2000). An assessment of the published results of animal relocations. *Biol. Conserv.* **96**, 1–11.

Franks, B.R., Avery, H.W. & Spotila, J.R. (2011). Home range and movement of desert tortoises *Gopherus agassizii* in the Mojave Desert of California, USA. *Endanger. Species Res.* **13**, 191–201.

Fruchterman, T.M.J. & Reingold, E.M. (1991). Graph drawing by force-directed placement. *Software Pract. Exper.* **21**, 1129–1164.

Gedeon, C.I., Boross, G., Németh, A. & Altbäcker, V. (2012). Release site manipulation to favour European ground squirrel *Spermophilus citellus* translocations: translocation and habitat manipulation. *Wildlife Biol.* **18**, 97–104.

Germano, J.M. & Bishop, P.J. (2008). Suitability of amphibians and reptiles for translocation. *Conserv. Biol.* **23**, 7–15.

Godfrey, S.S., Moore, J.A., Nelson, N.J. & Bull, C.M. (2010). Social network structure and parasite infection patterns in a territorial reptile, the tuatara (*Sphenodon punctatus*). *Int. J. Parasitol.* **40**, 1575–1585.

Grear, J.S. & Schmitz, O.J. (2005). Effects of grouping behavior and predators on the spatial distribution of a forest floor arthropod. *Ecology* **86**, 960–971.

Griffin, R.H. & Nunn, C.L. (2011). Community structure and the spread of infectious disease in primate social networks. *Evol. Ecol.* **26**, 779–800.

Griffith, B., Scott, J.M., Carpenter, J.W. & Reed, C. (1989). Translocation as a species conservation tool: status and strategy. *Science* **245**, 477–480.

Harless, M.L., Walde, A.D., Delaney, D.K., Pater, L.L. & Hayes, W.K. (2009). Home range, spatial overlap, and burrow use of the desert tortoise in the West Mojave desert. *Copeia* **2009**, 378–389.

Harless, M.L., Walde, A.D., Delaney, D.K., Pater, L.L. & Hayes, W.K. (2010). Sampling considerations for improving home range estimates of desert tortoises: effects of estimator, sampling regime, and sex. *Herpetol. Conserv. Biol.* **5**, 374–387.

Hartley, M. & Gill, E. (2010). Assessment and mitigation processes for disease risks associated with wildlife management and conservation interventions. *Vet. Rec.* **166**, 487–490.

Heaton, J.S., Nussear, K.E., Esque, T.C., Inman, R.D., Davenport, F.M., Leuteritz, T.E., Medica, P.A., Strout, N.W., Burgess, P.A. & Benvenuti, L. (2008). Spatially explicit decision support for selecting translocation areas for Mojave desert tortoises. *Biodivers. Conserv.* **17**, 575–590.

Heidinger, I.M.M., Poethke, H.J., Bonte, D. & Hein, S. (2009). The effect of translocation on movement behaviour – a test of the assumptions of behavioural studies. *Behav. Process.* **82**, 12–17.

Hess, G. (1996). Disease in metapopulation models: implications for conservation. *Ecology* **77**, 1617–1632.

Hess, G.R. (1994). Conservation corridors and contagious disease: a cautionary note. *Conserv. Biol.* **8**, 256–262.

Hinderle, D. (2011). *Desert tortoises (Gopherus agassizii) and translocation: homing, behavior, habitat and shell temperature experiments*. MSc Thesis, San Diego State University, San Diego, CA.

Hosseini, P.R., Dhondt, A.A. & Dobson, A. (2004). Seasonality and wildlife disease: how seasonal birth, aggregation and variation in immunity affect the dynamics of *Mycoplasma gallisepticum* in house finches. *P. Roy. Soc. Lond. B. Bio.* **271**, 2569–2577.

Hudson, P.J., Perkins, S.E., Cattadori, I.M., Ostfeld, R.S., Keesing, F. & Eviner, V.T. (2008). The emergence of wildlife disease and the application of ecology. In *Infectious disease ecology: the effects of ecosystems on disease and of disease on ecosystems*: 347–367. 11th Cary Conference, Millbrook, New York, USA, May 2005. Princeton, NJ: Princeton University Press.

Inman, R.D., Nussear, K.E. & Tracy, C. (2009). Detecting trends in desert tortoise population growth: elusive behavior inflates variance in estimates of population density. *Endangered Species Research* **10**, 295–304.

International Union for the Conservation of Nature (IUCN/SSC) (2013). *Guidelines for reintroductions and other conservation translocations, version 1.0*: Gland: IUCN Species Survival Commission, viiii + 57 pp.

Keeling, M.J. & Eames, K.T. (2005). Networks and epidemic models. *Royal Soc.* **2**, 295–307.

Keeling, M.J., Woolhouse, M.E.J., May, R.M., Davies, G. & Grenfell, B.T. (2002). Modelling vaccination strategies against foot-and-mouth disease. *Nature* **421**, 136–142.

Kesler, D.C., Cox, A.S., Albar, G., Gouni, A., Mejeur, J. & Plassé, C. (2012). Translocation of Tuamotu kingfishers, postrelease exploratory behavior, and harvest effects on the donor population. *Pac. Sci.* **66**, 467–480.

Kock, R.A., Woodford, M.H. & Rossiter, P.B. (2010). Disease risks associated with the translocation of wildlife. *Rev. Sci. Tech. OIE.* **29**, 329–350.

Lafferty, K.D. & Holt, R.D. (2003). How should environmental stress affect the population dynamics of disease? *Ecol. Lett.* **6**, 654–664.

Le Gouar, P., Mihoub, J. & Sarrazin, F. (2012). Dispersal and habitat selection: behavioral and spatial constraints for animal translocations, In *Reintroduction biology: integrating science and management*: 138–164. Ewen, J.G., Armstrong, D.P., Parker, K.A. & Seddon, P.J. (Eds). Oxford: Wiley-Blackwell.

Leighton, F.A. (2002). Health risk assessment of the translocation of wild animals. *Rev. Sci. Tech. OIE.* **21**, 187–195.

Letty, J., Marchandeau, S. & Aubineau, J. (2007). Problems encountered by individuals in animal translocations: lessons from field studies. *Ecoscience* **14**, 420–431.

Linklater, W.L. & Swaisgood, R.R. (2008). Reserve size, conspecific density, and translocation success for black rhinoceros. *J. Wildlife Manage.* **72**, 1059–1068.

Lloyd-Smith, J.O., Cross, P.C., Briggs, C.J., Daugherty, M., Getz, W.M., Latto, J., Sanchez, M.S., Smith, A.B. & Swei, A. (2005). Should we expect population thresholds for wildlife disease? *Trends Ecol. Evol.* **20**, 511–519.

Lopez, J.E., Gallinot, L.P. & Wade, M.J. (2005). Spread of parasites in metapopulations: an experimental study of the effects of host migration rate and local host population size. *Parasitology* **130**, 323–332.

Lovich, J.E. & Ennen, J.R. (2011). Wildlife conservation and solar energy development in the desert southwest, United States. *Bioscience* **61**, 982–992.

McLaughlin, G.S. (1997). *Upper respiratory tract disease in gopher tortoises, Gopherus polyphemus: pathology, immune responses, transmission, and implications for conservation and management*. PhD dissertation, University of Florida, Gainesville, FL, USA.

Miller, B., Ralls, K., Reading, R.P., Scott, J.M. & Estes, J. (1999). Biological and technical considerations of carnivore translocation: a review. *Anim. Conserv.* **2**, 59–68.

Miller, P.S. (2007). Tools and techniques for disease risk assessment in threatened wildlife conservation programmes. *Int. Zoo Yearbook* **41**, 38–51.

Nussear, K.E. & Tracy, C.R. (2007). Can modeling improve estimation of desert tortoise population densities? *Ecol. Appl.* **17**, 579–586.

Nussear, K.E., Tracy, C.R., Medica, P.A., Wilson, D.S., Marlow, R.W. & Corn, P.S. (2012). Translocation as a conservation tool for Agassiz's desert tortoises: survivorship, reproduction, and movements. *J. Wildlife Manage.* **76**, 1341–1353.

Parker, K.A., Dickens, M.J., Clarke, R.H. & Lovegrove, T.G. (2012). The theory and practice of catching, holding, moving and releasing animals, In *Reintroduction biology: integrating science and management*: 112–137. Ewen, J.G., Armstrong, D.P., Parker, K.A. & Seddon, P.J. (Eds). Oxford: Wiley-Blackwell.

Pinter-Wollman, N., Isbell, L.A. & Hart, L.A. (2009). The relationship between social behaviour and habitat familiarity in African elephants (*Loxodonta africana*). *P. Roy. Soc. Lond. B. Bio.* **276**, 1009–1014.

Plowright, R.K., Manlove, K., Cassirer, E.F., Cross, P.C., Besser, T.E. & Hudson, P.J. (2013). Use of exposure history to identify patterns of immunity to pneumonia in bighorn sheep (*Ovis canadensis*). *PLoS ONE* **8**, e61919.

Porphyre, T., Stevenson, M., Jackson, R. & McKenzie, J. (2008). Influence of contact heterogeneity on TB reproduction ratio R in a free-living brushtail possum *Trichosurus vulpecula* population. *Vet. Res.* **39**, 31.

R Development Core Team (2012). *R: a language and environment for statistical computing*: Vienna: R Foundation for Statistical Computing. [accessed 30 January 2013] (http://www.R-project.org).

Sainsbury, A.W. & Vaughan-Higgins, R.J. (2012). Analyzing disease risks associated with translocations. *Conserv. Biol.* **26**, 442–452.

Sandmeier, F.C., Tracy, C.R., DuPré, S. & Hunter, K. (2009). Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: a reevaluation. *Biol. Conserv.* **142**, 1255–1268.

Sasaki, A. (1997). Clumped distribution by neighbourhood competition. *J. Theor. Biol.* **186**, 415–430.

Serrano, D., Forero, M.G., Donázar, J.A. & Tella, J.L. (2004). Dispersal and social attraction affect colony selection and dynamics of lesser kestrels. *Ecology* **85**, 3438–3447.

Shier, D.M. (2006). Effect of family support on the success of translocated black-tailed prairie dogs. *Conserv. Biol.* **20**, 1780–1790.

Shier, D.M. & Swaisgood, R.R. (2012). Fitness costs of neighborhood disruption in translocations of a solitary mammal. *Conserv. Biol.* **26**, 116–123.

Smith, K.F., Acevedo-Whitehouse, K. & Pedersen, A.B. (2009). The role of infectious diseases in biological conservation. *Anim. Conserv.* **12**, 1–12.

Teixeira, C.P., De Azevedo, C.S., Mendl, M., Cipreste, C.F. & Young, R.J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Anim. Behav.* **73**, 1–13.

Thomas, C.D. (2011). Translocation of species, climate change, and the end of trying to recreate past ecological communities. *Trends Ecol. Evol.* **26**, 216–221.

Tompkins, D.M., Dunn, A.M., Smith, M.J. & Telfer, S. (2011). Wildlife diseases: from individuals to ecosystems. *J. Anim. Ecol.* **80**, 19–38.

Tsoar, A., Nathan, R., Bartan, Y., Vyssotski, A., Dell'Omo, G. & Ulanovsky, N. (2011). Large-scale navigational map in a mammal. *P. Natl. Acad. Sci. USA.* **108**, E718–E724.

United States Fish and Wildlife Service (USFWS) (1990). Endangered and threatened wildlife and plants; determination of threatened status for the Mojave population of the desert tortoise. *Fed. Regist.* **55**, 12178–12191.

United States Fish and Wildlife Service (USFWS) (1994). *Desert tortoise (Mojave population): recovery plan*: Portland: US Fish and Wildlife Service.

United States Fish and Wildlife Service (USFWS) (2011). *Revised recovery plan for the Mojave population of the desert tortoise* (*Gopherus agassizii*): Pacific Southwest Region: US Fish and Wildlife Service.

United States Fish and Wildlife Service (USFWS) (2013). *Health assessment procedures for the Mojave Desert tortoise (Gopherus agassizii): a handbook pertinent to translocation*: Reno: Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service.

Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A. & Hoffmann, A.A. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol. Appl.* **4**, 709–725.

Woodbury, A.M. & Hardy, R. (1948). Studies of the desert tortoise, *Gopherus agassizii*. *Ecol. Monogr.* **18**, 145–200.

HERPETOLOGICAL MONOGRAPHS

MULTIPLE FACTORS AFFECT A POPULATION OF AGASSIZ'S DESERT TORTOISE (*GOPHERUS AGASSIZII*) IN THE NORTHWESTERN MOJAVE DESERT

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MULTIPLE FACTORS AFFECT A POPULATION OF AGASSIZ'S DESERT TORTOISE (*GOPHERUS AGASSIZII*) IN THE NORTHWESTERN MOJAVE DESERT

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Abstract: Numerous factors have contributed to declines in populations of the federally threatened Agassiz's Desert Tortoise (*Gopherus agassizii*) and continue to limit recovery. In 2010, we surveyed a low-density population on a military test facility in the northwestern Mojave Desert of California, USA, to evaluate population status and identify potential factors contributing to distribution and low densities. Estimated densities of live tortoises ranged spatially from 1.2/km^2 to 15.1/km^2. Although only one death of a breeding-age tortoise was recorded for the 4-yr period prior to the survey, remains of 16 juvenile and immature tortoises were found, and most showed signs of predation by Common Ravens (*Corvus corax*) and mammals. Predation may have limited recruitment of young tortoises into the adult size classes. To evaluate the relative importance of different types of impacts to tortoises, we developed predictive models for spatially explicit densities of tortoise sign and live tortoises using topography (i.e., slope), predators (Common Raven, signs of mammalian predators), and anthropogenic impacts (distances from paved road and denuded areas, density of ordnance fragments) as covariates. Models suggest that densities of tortoise sign increased with slope and signs of mammalian predators and decreased with Common Ravens, while also varying based on interaction effects involving these predictors as well as distances from paved roads, denuded areas, and ordnance. Similarly, densities of live tortoises varied by interaction effects among distances to denuded areas and paved roads, density of ordnance fragments, and slope. Thus multiple factors predict the densities and distribution of this population.

Key words: Denuded areas; Ordnance; Predators; Roads

Agassiz's Desert Tortoise (*Gopherus agassizii*, hereafter Desert Tortoise; Murphy et al., 2011), a species of the southwestern United States, was federally listed as threatened in 1990 because populations were declining and habitats were deteriorating or lost from multiple sources (US Fish and Wildlife Service [USFWS], 1990). One challenge to recovery of the tortoise is identification of critical drivers of population declines, both locally and regionally, and prioritization of recovery actions (USFWS, 2011; Averill-Murray et al., 2012; Darst et al., 2013). These drivers may be common on a landscape scale throughout the geographic range (e.g., roads, utility corridors) or specific to a particular land managing agency. Military installations, for example, contain 3.76% of designated critical habitat and 13.5% of habitat available within the geographic range (USFWS, 1994a, 2010; Berry, 1997). They can contribute to population and habitat losses from development of facilities, force-on-force military training with vehicles, testing of missiles and explosives, and other ground-disturbing activities (Tazik and Martin, 2002; USFWS, 2010). They also can be refuges where threatened and endangered species thrive in relatively undisturbed environments (Stein et al., 2008). Some recovery units and critical habitats for the Desert Tortoise are affected more than others by military use, e.g., military installations occupy 28% of habitat in the Western Mojave Recovery Unit (Fig. 1; USFWS, 2010).

The effects of military activities on the Desert Tortoise have been studied in the Western Mojave Recovery Unit at the National Training Center (NTC), Fort Irwin, California, USA (e.g., Krzysik, 1997; Berry et al., 2006), where troops are trained for ground maneuvers using tanks and other

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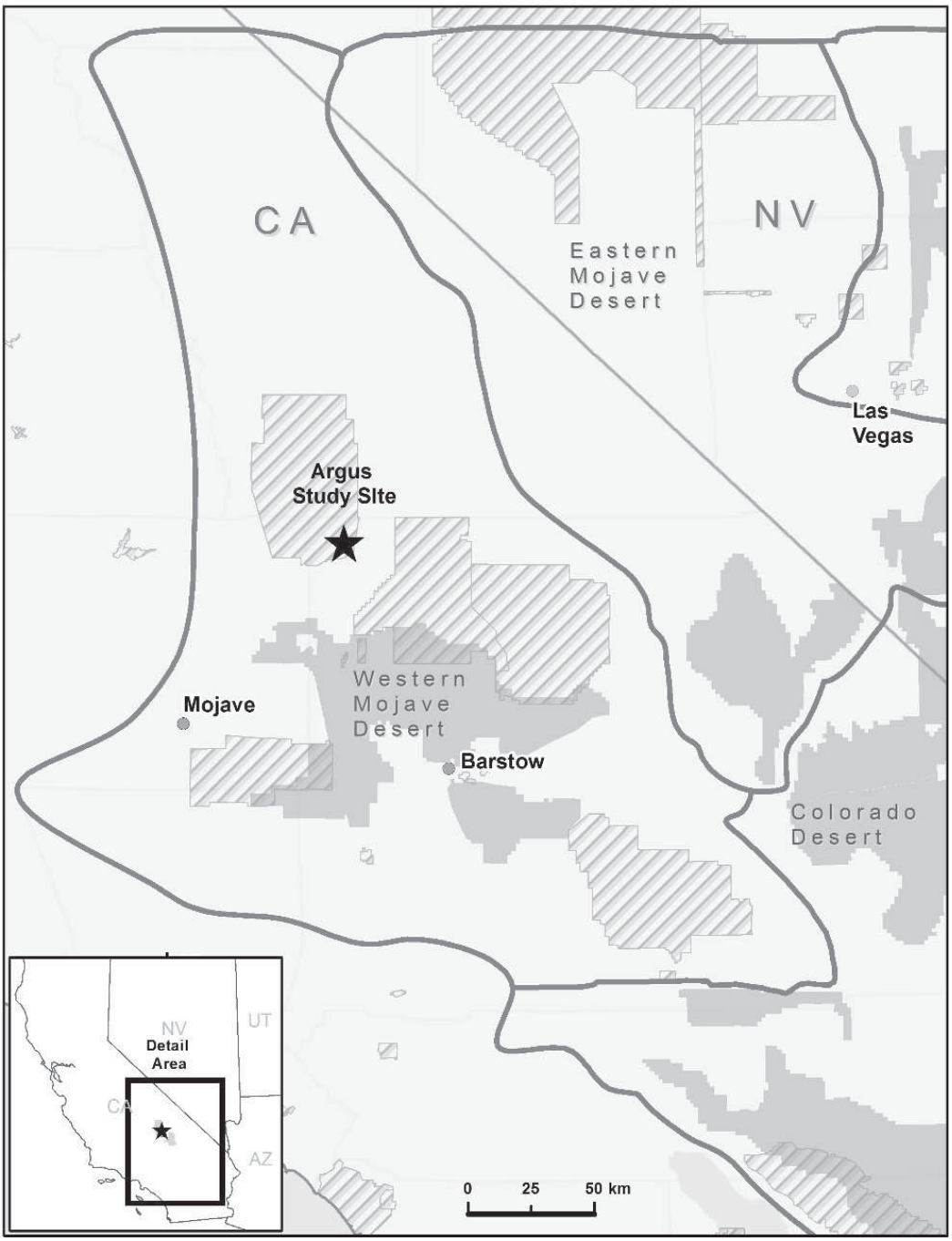

Fig. 1.—Location of the Argus study area for Desert Tortoises (star) with respect to the Western Mojave Desert Recovery Unit, boundaries of other Recovery Units, military installations (hatched), and the nearest Desert Tortoise critical habitats (solid grey polygons).

armored vehicles. Mortality of tortoises from vehicles was high (Berry et al., 2006). A recent expansion of NTC operations into critical habitat required off-site translocation of several hundred tortoises (Esque et al., 2005). At another military installation in Arizona, USA, Grandmaison et al. (2010) examined the effects of multiple factors—live artillery fire and firing boxes, a network of gravel roads, livestock grazing, and recreation—on microhabitat use by the closely related Morafka's Tortoise (*Gopherus morafkai*). They reported

that a greater proportion of tortoise locations were found outside of firing box boundaries where shelter sites and vegetation showed little human impact than inside the firing boxes. They also noted that tortoises selected areas with a higher percentage of canopy cover of plants and where evidence of cattle activity was absent.

We analyzed the effects of multiple factors (munitions research, roads, predators, and topography) on an isolated population of the Desert Tortoise on the China Lake Naval Air Weapons Station (NAWS) in the northwestern part of the geographic range in California. The NAWS is a research-oriented facility with minimal ground-disturbing activities and with substantial areas that have been inaccessible to the public since 1942. Our objectives were to (1) evaluate the status of the Desert Tortoise population at a site inaccessible to the public; (2) model the effects of predators, topography, munition test areas, ordnance, and a paved road on distribution and density of Desert Tortoises; and (3) identify the variables with positive and negative relationships to the Desert Tortoise.

Study Area

The 5.42-km^2 study area (35°41′33″N, 117°28′20″W; datum = WGS84) is in the southeastern portion of the China Lake NAWS (Fig. 2) in the foothills of the southern Argus Range and at the edge of Salt Wells Valley, San Bernardino County, California. Composed of low hills with scattered rock outcrops and small valleys at elevations of 597–732 m, the site is part of an estimated 130-km^2 fragment of habitat that is isolated from other tortoise populations by the steep topography of the Argus Range and anthropogenic activities. The 130 km^2 habitat fragment was in close proximity to high-density human populations 6.5 km to the east at Trona and Westend (1757 people; United States Census Bureau (USCB), 2010) and 15 km to the west, at NAWS, China Lake Acres, Ridgecrest, and Inyokern (30,591 people; USCB, 2010). To the south, Highway 178 parallels and is 3.2 km from the southern study area boundary, effectively isolating the fragment on NAWS from Desert Tortoise populations to the south. Populations to the south also have been depleted from intensive recreational vehicle use in the US Department of the Interior, Bureau of Land Management's (BLM) Spangler Hills Off-Highway Vehicle Area (USBLM, 1973, 1980, 2006). The study area is in part of the NAWS with no public access since the mid-1940s. Between the late 1940s and 1973, the site received occasional use from NAWS scientists and engineers. In 1973, testing of ordnance began, generating unexploded ordnance (UXO), areas devoid of perennial vegetation, and vehicle traffic on roads.

The dominant perennial vegetation association was white bur-sage and creosote bush (*Ambrosia dumosa-Larrea tridentata;* California Department of Fish and Game (CADFG), 2010a). The long-term annual mean for precipitation was 104.4 mm for the hydrologic year (1 October–30 September) and 80.5 mm for winter rainfall (1 October–31 March; Trona weather station, 35°46′N, 117°23′W, 516.6 m; National Oceanic and Atmospheric Administration, 2009–2010). Prior to the survey, an estimated 116.84 mm of rain fell between 1 October 2009 and 31 March 2010, resulting in an abundance of winter annual plants, which were forage for tortoises during spring 2010.

Materials And Methods

Field Sampling

We collected data on live and dead tortoises, tortoise sign (shelter sites, scats, tracks, egg shells, courtship rings, drinking sites), observations and signs of predators, and anthropogenic impacts. We recognize that detection of live and dead tortoises, other signs of tortoises and predators, and anthropogenic impacts is imperfect so we designed the field survey to maximize detection of live tortoises, tortoise sign, predator sign, and anthropogenic impacts. Since tortoises spend much of their lives underground, we selected spring, the time of greatest aboveground activity for all sizes of tortoises (Nagy and Medica, 1986). In addition, we chose a spring following a winter with above average precipitation when tortoise forage was abundant (Henen et al., 1998; Duda et al., 1999; Jennings, 2002). Based on previous research showing strong relationships between live

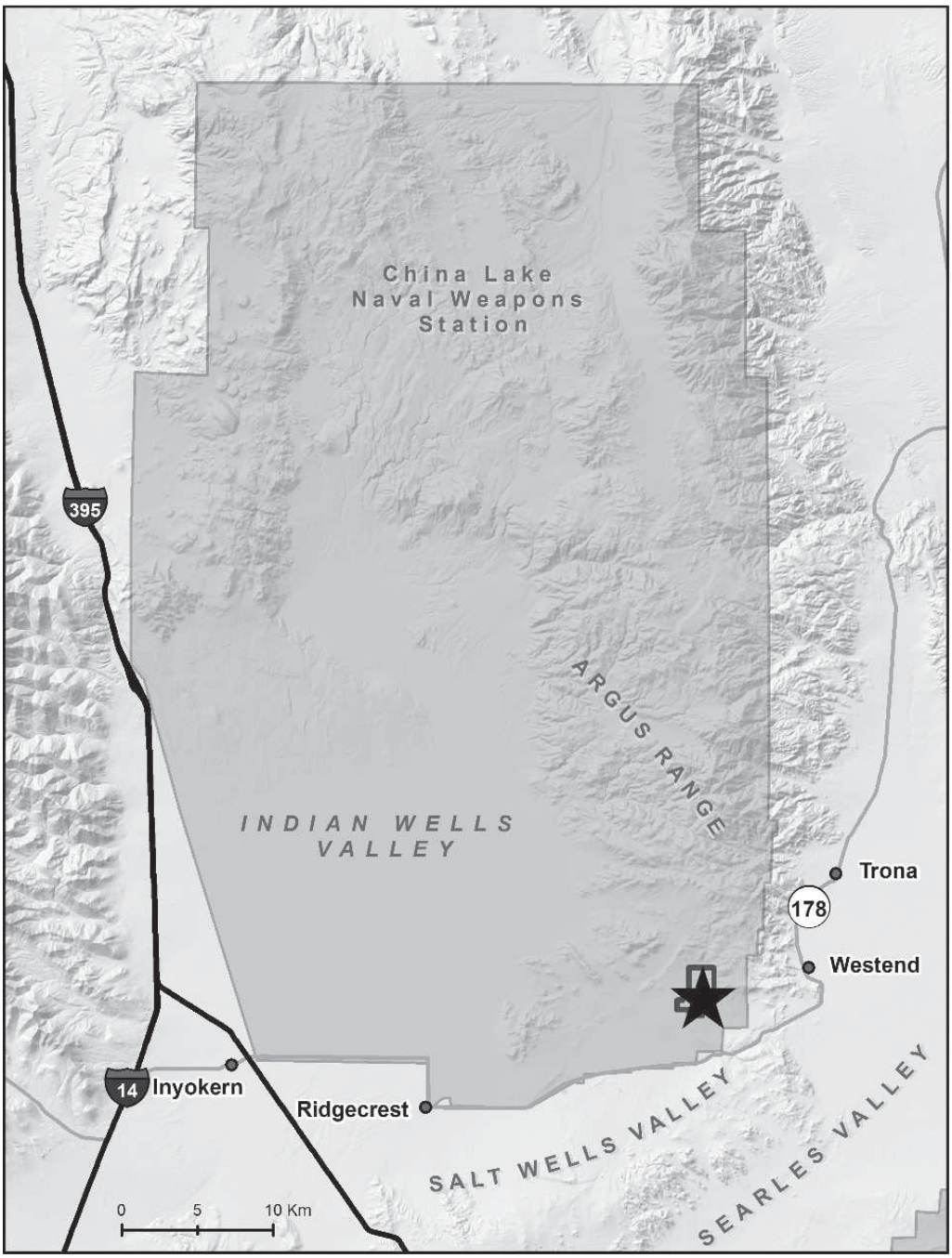

Fig. 2.—Location of the Argus study area for Desert Tortoises (star) within the Naval Air Weapons Station China Lake, San Bernardino County, California, and the proximity to local towns and highways.

tortoises and tortoise sign (e.g., burrows, scats, tracks, courtship rings), we assumed that tortoise sign at a location represented use for shelter, travel, feeding, and social interactions, and that we observed representative samples of each variable (Krzysik, 2002). We made similar assumptions for sign of mammalian predators and anthropogenic impacts. The low cover of perennial shrubs, sandy soils, and areas denuded of vegetation by anthropogenic activities facilitated observations. We had an additional constraint and safety requirement: the field crew was to be in view of an NAWS UXO expert at all times.

A team of eight surveyed the area for a total of 583 h between 12 and 22 May searching for live tortoises, tortoise shell–skeletal remains, and other tortoise sign. The field team walked 10 m apart and covered the study area completely once, thus ensuring detection of most sign. In fall, the same field workers searched for tortoises for 86.5 h by revisiting shelters that were recently used or active during the previous May. The field team followed standard protocols to handle, mark, and evaluate health of and trauma on the tortoises (Berry and Christopher, 2001). They took digital images of carapace, plastron, eyes, beak, nares, and any lesions. They weighed the tortoises, measured carapace length at the midline (MCL), assigned a sex if ≥180 mm MCL, and noted locations in Universal Transverse Mercator coordinates. Field workers recorded clinical signs of health and disease, e.g., signs of lethargy, starvation and dehydration, trauma, or abnormalities (Berry and Christopher, 2001; Berry et al., 2002). Clinical signs of upper respiratory tract disease (Jacobson et al., 1991, 1995; Brown et al., 1994; Jacobson and Berry 2012), herpesvirus (Jacobson et al., 2012), shell disease (Jacobson et al., 1994; Homer et al., 1998), and trauma were rated as none, mild, moderate, severe, or unknown for each variable (e.g., palpebrae, periocular area, nares). Signs of severe trauma to the shell and limbs, such as vehicle hits or chewing by dogs and other predators were also noted (e.g., Boyer and Boyer, 2006). No blood samples were taken for laboratory tests for infectious diseases.

Shell-skeletal remains.—All shell-skeletal remains found within plot boundaries were collected and catalogued. When remains or parts of remains were discovered, the team examined the vicinity for signs of potential cause (or causes) of death. The team took digital images of remains in situ and recorded details of location, size and sex of the tortoise, condition, signs of predators and scavengers (e.g., predator scats), and human impacts (e.g., vehicle tracks) associated with the remains.

Tortoise sign.—We recorded all tortoise sign. We defined cover or shelter sites as burrows, pallets, rock shelters, and caves (after Burge, 1978).

Potential predators of tortoises.—We collected data on avian and mammalian predators to determine predator pressure and to assess potential sources of mortality. We noted date, time, location, behavior, and species for all avian predators observed or heard (e.g., Common Raven, *Corvus corax*; Golden Eagle, *Aquila chrysaetos*; Greater Roadrunner, *Geococcyx californianus*; Red-tailed Hawk, *Buteo jamaicensis*; and Northern Shrike, *Lanius ludovicianus*; Boarman, 1993; K.H. Berry, personal observation). We examined areas with concentrations of predator sign (e.g., perches, nests, dens, marking sites, feeding posts, roosts) for evidence of tortoise remains. The field team broke apart scats of mammalian predators (coyote, *Canis latrans*; kit fox, *Vulpes macrotis*; badger, *Taxidea taxus;* bobcat, *Lynx rufus*) to look for remains of tortoises.

Anthropogenic impacts.—We used four methods to measure different aspects of anthropogenic impacts. First, to calculate surface disturbances from roads and areas partially or completely denuded of vegetation, we used aerial imagery from the National Agriculture Imagery Program with a summer natural color (ArcGIS Image Service from the CADFG, 2010b). We digitized disturbed areas (polygons) and road features (lines) on the screen and saved them as feature classes in a geo-database. To calculate disturbed surface areas for roads, we measured widths of the paved road with berm, several bulldozed dirt roads with berms, and old graded and ungraded dirt roads. For the second method, we used a geographic information system to generate 50 random points with a minimum distance of 200 m between points to sample anthropogenic impacts (ordnance from explosive testing, trash, vehicle tracks, mining excavations, utility lines, fences, and other miscellaneous types of human disturbances). Each of the 50 points was outside of denuded areas and was the starting point for 10 m × 100 m transects. We defined ordnance as metal shell casings, pieces and particles of explosives, wires, circuit boards, nuts, bolts, and rocket fins ranging in size from 1 cm^3 in volume to 0.5 m in length. Pieces of ordnance averaged 3 cm in diameter. For the third method, we established 40 additional 10 m × 100 m transects specifically for ordnance. These transects extended from the center of areas denuded by explosive

testing to 100–200 m beyond the boundaries of the denuded zone. For the fourth method, we counted all ordnance, trash, balloons, vehicle tracks off road, power poles and lines, fences, and burned areas (total counts only, no locational data recorded) while surveying the entire study area in May.

Data Analysis

Live tortoises.—We assigned live and dead tortoises to one of six size-age classes according to MCL: juvenile 1 = <60 mm, juvenile 2 = 60–99 mm; immature 1 = 100–139 mm; immature 2 = 140–179; subadult or small adult = 180–207 mm; adult = ≥208 mm. We used the exact binomial proportion test and 90% confidence intervals (CIs) to determine if the sex ratio of subadult and adult tortoises was significantly different than the expected 1:1 ratio at $P \leq 0.10$ (SAS Institute Inc., 2010). Tortoises with moderate to severe clinical signs of one or more diseases and trauma (e.g., upper respiratory tract disease, cutaneous dyskeratosis) were noted.

Shell–skeletal remains.—We evaluated shell-skeletal remains to determine size-age class, sex, whether previously marked or captive (e.g., painted numbers on shell), approximate time of death, and cause of death. We determined carapace length by one of three methods depending on the condition of remains in the following order of priority: direct measure of MCL; estimation of the MCL using scutes or impressions of scutes in the bony shell and previously derived regression equations based on measurements of selected scutes from the carapace and plastron (Berry and Woodman, 1984); or estimation of MCL by matching the fragments of scutes and bones to similarly sized whole tortoise shells from the US Geological Survey's (USGS's) collection of shell-skeletal remains. To estimate time since death (specifically, time elapsed between death and collection of remains), we used keys in Berry and Woodman (1984), and placed each tortoise in one of two classes: dead ≤4 yr or >4 yr.

The general appearance, location, and forensic evidence associated with the remains provided information on cause of death, e.g., firearms or Common Ravens (Berry, 1986; Boarman, 1993). Tortoises hit by a vehicle have cracked and/or crushing injuries to the shell. Tortoises killed by a mammalian predator are likely to have chew or gnaw marks, puncture wounds, and twisting of scute and bone. The twisting and deformation of scutes and bones occur when scutes and bones are pliable and the tortoise is alive or dying. In contrast, when remains are scavenged, scutes and bones are dry and tend to break and crack rather than showing signs of twisting and deformation. Some causes of death are ambiguous or unknown, e.g., a tortoise dying of disease and then scavenged by a predator or if only fragments of shell remain. Subadult and adult tortoises were used for a 4-yr retrospective calculation of crude death rate.

Spatial data layers.—In a preliminary evaluation of the data on anthropogenic impacts, we noted that the major surface disturbances were areas denuded of vegetation from testing, paved roads, and density of ordnance. We hypothesized that distance from denuded area, distance from the paved road, and density of ordnance would correlate with densities of live tortoises and tortoise sign. For the purposes of statistical modeling, we divided the study area into 31 sampling units based on a 500-m × 500-m grid snapped to major grid units using the Create Fishnet tool in ArcGIS Version 9.3.1 (ESRI, Inc., 2009a). Each grid was 0.25 km^2, with the exception of grids truncated by the study area boundary. Those grids ranged in size from 0.024 km^2 to 0.216 km^2.

We identified two response variables: density of live tortoises and density of tortoise sign. Density of live tortoises was measured as the count of live tortoises relative to the area surveyed within each grid square. For all statistical analyses the first known location of an individual tortoise was used. Tortoise sign density, the second response variable, was measured as the count of all tortoise sign relative to the area surveyed in each grid.

We evaluated three types of predictor variables: topography (slope), densities of predator sign, and densities of and distances to anthropogenic impacts. Degree of slope (hereafter, "Slope") was calculated from the 1/3-Arc Second (approximately 10 m) National Elevation Dataset (USGS, 2012) using the Slope tool in ArcGIS Version 9.3.1 (ESRI, Inc., 2009b). Predictive surfaces of predator

densities, Common Ravens and mammalian predator sign per km^2 (hereafter "Raven" and "Mammal," respectively), were created using the Kernel Density tool in ArcGIS Version 9.3.1 (ESRI, Inc., 2009c). Slope and predator density surfaces were averaged across each grid square. Two predictor variables for anthropogenic impacts were distances from the center point of each grid square to the paved road and to nearest boundary of a denuded area (hereafter "Paved" and "Denuded," respectively). Distances were determined using the Near tool (ESRI, Inc., 2009d). Density of ordnance was calculated for each of the 90 transects by dividing the number of ordnance pieces by the area of the transect ($100\ m \times 10\ m = 1000\ m^2$). Transformed ordnance densities (using a fourth-root transformation and then back-transformed) were interpolated with ordinary kriging using a spherical model based on the nearest 20 neighbors with the Kriging tool in ArcGIS Version 9.3.1 (ESRI, Inc., 2009e). Based on kriging estimates, the density of ordnance at the center point of each grid square was identified (hereafter "Ordnance").

Models of live tortoises and tortoise sign vs. slope and anthropogenic and predator variables.—We used generalized linear models (GLMs) to analyze the densities of live tortoises and densities of tortoise sign in relationship to Slope, Mammals, Ravens, Paved, Denuded, and Ordnance (Venables and Ripley, 2002; R Development Core Team, 2012). We modeled live tortoise counts and tortoise sign counts using GLMs based on the Poisson distribution and a log link function, which equates to density models when including an offset based on the log-transformed search area within each grid (McCullagh and Nelder, 1989).

Our use of GLMs carried implicit assumptions about the data, which we relaxed whenever possible or evaluated using second-order corrected Akaike's information criteria (AIC_c) based on 31 sampling units (Mazerolle, 2012). We assumed that counts followed a Poisson distribution, which holds many features that are compatible to the behavior of count data in natural systems. Poisson variables are integer-valued, nonnegative, and have a variance equal to its mean. The latter feature is appropriate for data that exhibit increasing variance with increasing mean; however, it can also be overly restrictive when data are overdispersed, i.e., having variances that exceed the mean. We relaxed this constraint by incorporating an overdispersion factor in our models so that the variance was allowed to differ from the mean. Another conventional assumption of GLM is that the response data (i.e., tortoises) are independent among sampling units (i.e., grids). We relaxed this assumption by incorporating random effects into the model based on clusters of grids to account for spatial correlations between adjacent grids.

Prior to modeling the predictor effects, we assessed the data for overdispersion and spatial correlation based on grids grouped into two-by-two clusters (Dormann et al., 2007). We first accounted for any potential covariate effects by fitting a GLM to each response variable saturated with all covariates and their two-way interaction effects. We then extended the GLM to a generalized linear mixed model (GLMM; Bates et al., 2012) by including a random effect for grid cluster to model spatial correlation, a random effect for each unique grid identifier to model overdispersion, or both, and compared with a GLM based on ordinary dispersion and independence. We compared AIC_c values among the GLM and the three GLMM (Mazerolle, 2012; AICcmodavg package). We identified the best model based on smallest AIC_c to determine whether to include overdispersion or spatial correlation when analyzing predictors (Zuur et al., 2009).

Because of the small number of grids, we analyzed models with less than three predictors and one interaction. Prior to analysis we believed any of these predictor combinations could describe the variation in tortoise response. Therefore we examined all combinations equally to avoid biasing the results toward preselected combinations, except we excluded nearly all interaction terms involving Ordnance due to high correlations ($r > 0.70$) between Ordnance terms, for a total of 96 predictor combinations. Models with the smallest AIC_c best represented the data with the smallest loss of information, and additional models within two AIC_c units were worthy of consideration (Burnham and Anderson, 2002).

We regarded the null model with no covariates as an uninformative model, and presented models only when they surpassed the null model by an AIC_c difference > 2.

Because the large number of models increases the potential for model selection uncertainty, we calculated the main effect of each covariate by model-averaging its coefficient (β) across all models containing that covariate, excluding models in which that covariate has an interaction effect (Mazerolle, 2012). We standardized all covariates ($[x - \bar{x}]/s$) so that model coefficients represent the effect on tortoises for every one standard deviation (s) increase in the covariate. The coefficient β represents positive or negative effects on tortoise sign and live tortoise densities, on the log scale, per s increase for each covariate. We expressed these effects (± 1 SE) as percentage changes by using the transformation $(\exp[\beta] - 1) \times 100\%$. We similarly calculated each interaction effect between two covariates by model-averaging the interaction coefficient across all models containing the interaction. Interaction coefficients cannot be interpreted independently from other coefficients; therefore we summarized the cumulative effects by calculating spatially explicit predictions of tortoise response by model-averaging predicted responses across all 96 models (Burnham and Anderson, 2002). We created predictive surfaces for tortoise sign and live tortoises to visualize the outputs of these model-averaged estimates using the Inverse Distance Weighted Spatial Analyst tool in ArcGIS Version 9.3.1 (ESRI, Inc., 2009f).

Our predictive surfaces for tortoise sign and live tortoise densities are based on the weight of evidence from multiple models, each of which assumes a log-linear relationship between tortoises and predictor variables. This assumption ensures that each model predicts positive densities; it can also produce misleading predictions if the predictor–response relationships are not actually log-linear. Our sample was too sparse to formally evaluate this assumption; however, our use of a multi-model framework accounts for some uncertainty in specifying a single correct model. We calculated standard errors to quantify the uncertainty around mean predictions. We calculated correlations between predicted estimates and actual data, paired by grid, to describe the quality of the fit. Squared correlations are analogous to r^2 values in regression analyses.

The unique conditions of the Argus study area precluded the availability of independent data from other sites that could be used to assess the robustness of our models. Instead, we used live tortoise sightings to corroborate our results based on the tortoise sign model. We tested the Pearson correlation between counts of live tortoises and model-predicted tortoise sign, paired by grid (R Development Core Team, 2012). We note that this correlation is positively biased because live tortoises were among the tortoise sign used to develop the predictive model; however, since the majority of tortoise sign were not live tortoises, we believe the correlation is only mildly biased. We similarly tested the correlations between counts of live tortoises and tortoise remains to determine similarities in the distributions of live and dead tortoises. We analyzed the correlation using remains aged <4 yr, as well as all remains.

Results

Population Attributes of Live Tortoises

We found 28 live tortoises, of which 27 were captured in spring, and one, an immature 2, that was located for the first time in fall (Table 1). In the brief fall visit, 10 of the 27 were recaptured. The sample was almost equally composed of adults (46.5%) and immature and juvenile tortoises (53.6%). The immature 2 class was poorly represented and no subadults were observed. The sex ratio of subadult and adult tortoises was 10 females to 3 males, which differed from the expected 1:1 sex ratio (Exact binomial test, $P = 0.0923$, 90% CI = 0.51–0.93). The crude density of adult tortoises was 2.4 tortoises/km^2 (13 adults /5.42 km^2). Densities of tortoises of all sizes, based on the predicted live tortoise density models, ranged from 1.2/km^2 to 15.1/km^2 depending on location (Fig. 3). The distribution of live tortoises differed throughout the study area, with lower predicted densities associated with the end of the paved road and near denuded areas where testing occurs and higher predicted densities to the northwest

TABLE 1.—Size–age class distributions of live Desert Tortoises and shell–skeletal remains, with estimated age of shell–skeletal remains collected in 2010 within the Argus study area at the Naval Air Weapons Station China Lake, San Bernardino County, California.

Size–age class structure (carapace length at the midline)	Live tortoise count	Dead tortoise count	
		≤4 yr since death	>4 yr since death
Juvenile 1 (<60 mm)	3	1	—
Juvenile 2 (60–99 mm)	5	7	1
Immature 1 (100–139 mm)	5	6	1
Immature 2 (140–179 mm)	2	2	1
Subadult (180–207 mm)	—	1	1
Adult (≥208 mm)	13	—	11
Totals	28	17	15

and northeast of the denuded areas (Fig. 3; see also models below).

The 28 tortoises appeared to be robust, active, and behaving normally. Juveniles had wide bands of new growth at the seams between the scutes, representing the ample forage available during the spring. No tortoises had wet nares or a purulent discharge, typical clinical signs of mycoplasmosis caused by the pathogens *Mycoplasma agassizii* (Jacobson et al., 1991; Brown et al., 1994; Homer et al., 1998) and *M. testudineum* (Brown et al., 2004; Jacobson and Berry, 2012). Some clinical signs suggestive of mycoplasmosis were evident, however. Eleven tortoises had mild and moderate occlusions of one or both naris. The occlusions appeared to be dirt or soil associated in most cases with layers of soil and plant sap from foraging, not mucus characteristic of mycoplasmosis. Twelve tortoises had moderate to severe edema of the palpebrae and/or periocular area, seven had dried or wet mucus crusts on the palpebrae or in the fornix, and the globes bulged in six tortoises. Such ocular signs are often observed in years of abundant forage and may be the result of sap and detritus from food plants or from disease (e.g., mycoplasmosis or cutaneous dyskeratosis). Twenty-four of the 28 tortoises had signs of active cutaneous dyskeratosis on and between the scales of the fore- and hind limbs covering 40% or less of the limbs. For all but one tortoise, severity of the lesions was rated as mild. Ten tortoises also had mild, active signs of cutaneous dyskeratosis on either plastron or carapace or both.

Signs of trauma were evident on 16 tortoises, all of which were ≥163 mm MCL. The injuries were healing or had healed. One adult had severe healed injuries to the plastron and gular horn, which had been chewed away (injury typical of a domestic dog; A. Carlson and K. Berry, personal observation; Boyer and Boyer, 2006). Twelve juvenile and small immature tortoises (54–128 mm MCL) had no signs of past or recent injuries. Between the spring survey and early October of 2010, two tortoises were injured by predators: one was a 123-mm-MCL immature with bites and tears to the gular horn, plastron, and carapace and the other tortoise was an adult with injuries to the foreleg.

Death Rates and Causes of Death

We collected shell-skeletal remains of 32 tortoises, 17 of which represented tortoises estimated to have died between 2006 and 2010 (Table 1; Fig. 3). Sixteen juvenile and immature tortoises and one subadult female tortoise (MCL = 180 mm) died during that interval. No tortoises marked in spring were found dead during the fall visit; however, one juvenile hatched in summer of 2010 was found dead during the fall visit, killed by a raven. Most remains showed signs of traumatic deaths: broken scutes and bones, and signs of crushing, gnaws, or punctures. For these 17 dead tortoises, potential or probable causes of death included predation by mammalian carnivores (seven) and Common Ravens (two or three), trauma (which could include predation, two), and unknown (five). Remains of three tortoises (one juvenile in a coyote scat) were found at predator sign concentration areas: two sites were scat marking areas and the third was at a coyote rock shelter. Fifteen of the 32 tortoises died >4 yr previously; 12 of the 15 tortoises were subadult or adults. Several had signs of traumatic deaths or predation, but most could not be assigned a cause of death because of the deteriorated condition. The crude annual death rate based on 14 adults and subadult tortoises found to survive or die during 2006–2010 was 1.8% (1 dead out of 14, divided by 4 yr; Table 1).

Tortoise Sign

We located 285 tortoise sign: 140 shelter sites, 138 scats, 3 courtship rings, 2 sets of egg

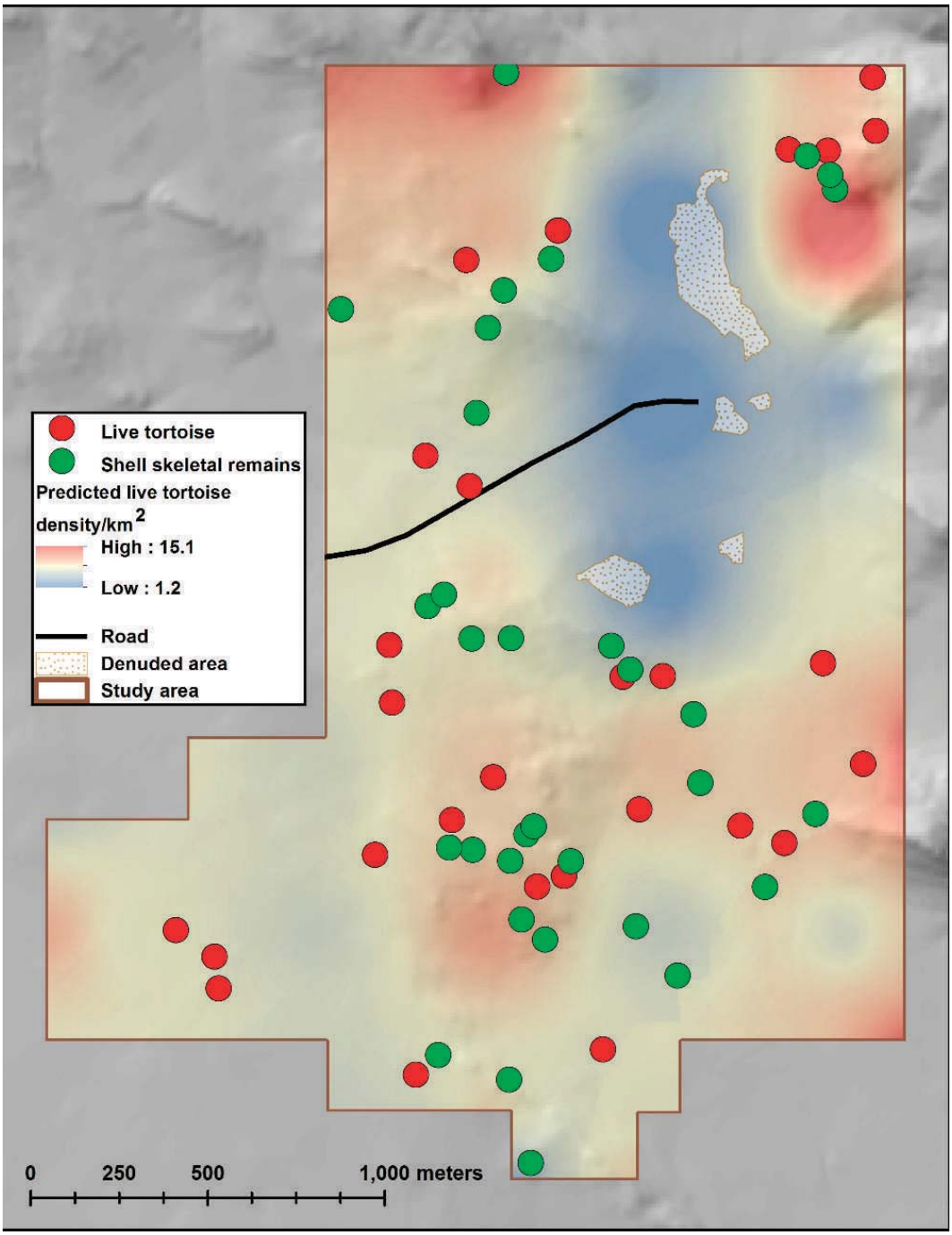

FIG. 3.—The locations of live and dead Desert Tortoises and the modeled predicted densities and distribution of live Desert Tortoises at the Argus study area, Naval Air Weapons Station China Lake, San Bernardino County, California, in 2010. The blue and red colors reflect low and high densities of Desert Tortoises, respectively.

shell fragments, and 2 sets of foot tracks (Fig. 4). Most shelters were burrows (132, 94.3%); only four rock shelters, two caves in calcic soils, and two pallets were observed. Most shelter sites (110, 78.6%) were of sizes used by adults; 26 and 4 burrows were of sizes used by immature and juvenile tortoises, respectively. Tortoise sign counts (including

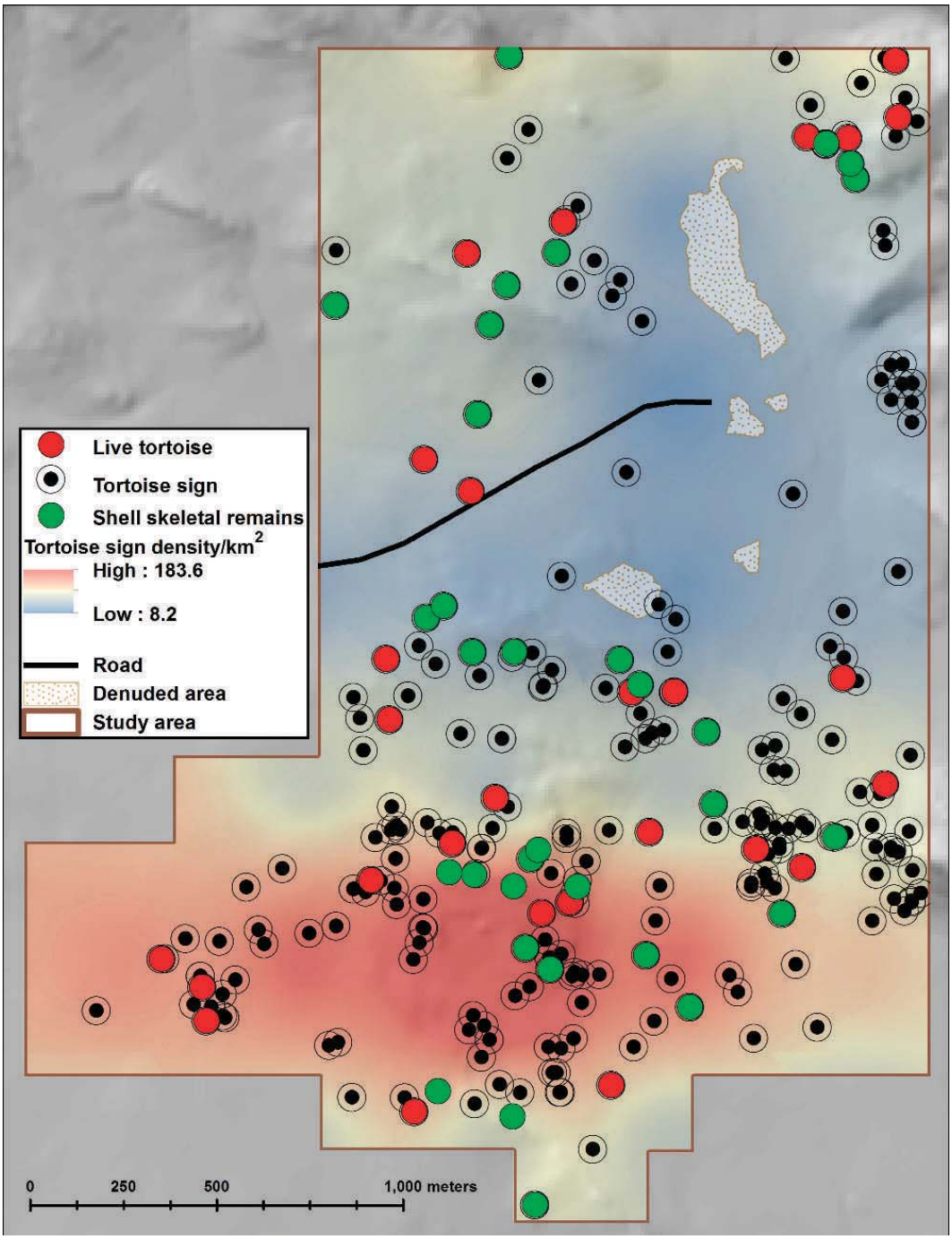

FIG. 4.—The locations of Desert Tortoise sign and the modeled-predicted distribution and density of tortoise sign at the Argus study area, Naval Air Weapons Station China Lake, San Bernardino County, California, in 2010. The blue and red colors reflect low and high densities of tortoise sign, respectively.

live tortoises and shell–skeletal remains) were lower in the northern two-thirds of the study area than in the south (Fig. 4). Predicted densities for sign counts ranged from 8.2/km^2 to 183.6/km^2 depending on location.

Predators

We recorded from one to three Common Ravens flying or perching on power poles for a total of 16 sightings in spring/583 person-h

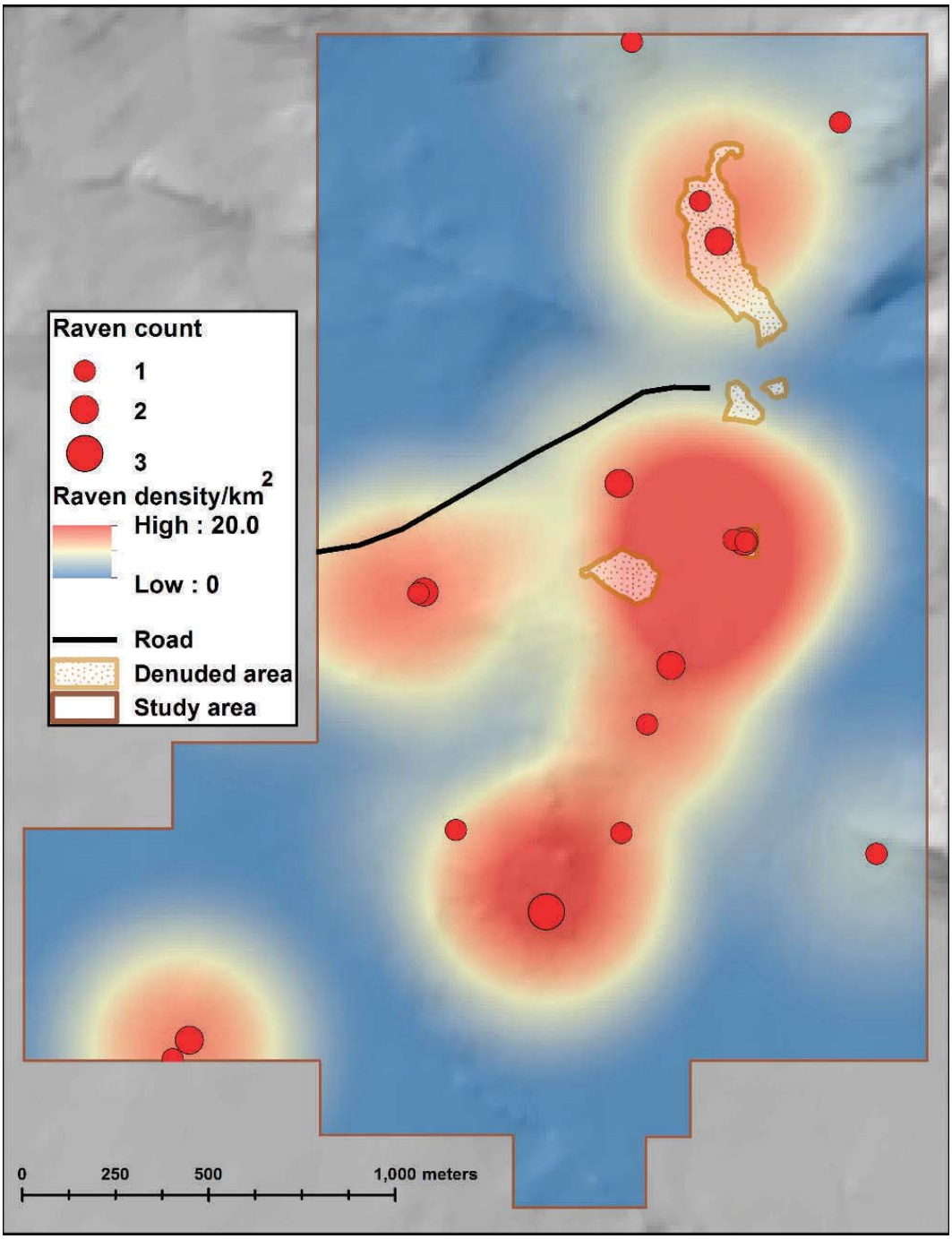

Fig. 5.—Observations of the Common Raven in May 2010 with predicted densities using a 500 m search radius at the Argus study area, Naval Air Weapons Station China Lake, San Bernardino County, California.

and 3 in fall/144 person-h with modeled estimates ranging from 0/km^2 to 20.0 Common Ravens/km^2 (Fig. 5). We also saw one each of three other avian predator species: Prairie Falcon (*Falco mexicanus*), Red-tailed Hawk, and Northern Shrike. We identified 63 sign concentration areas (scat marking sites, dens, and other shelters) created by mammalian predators (Fig. 6) and of these, 46 were scat marking sites and 17 were dens, tunnels, or

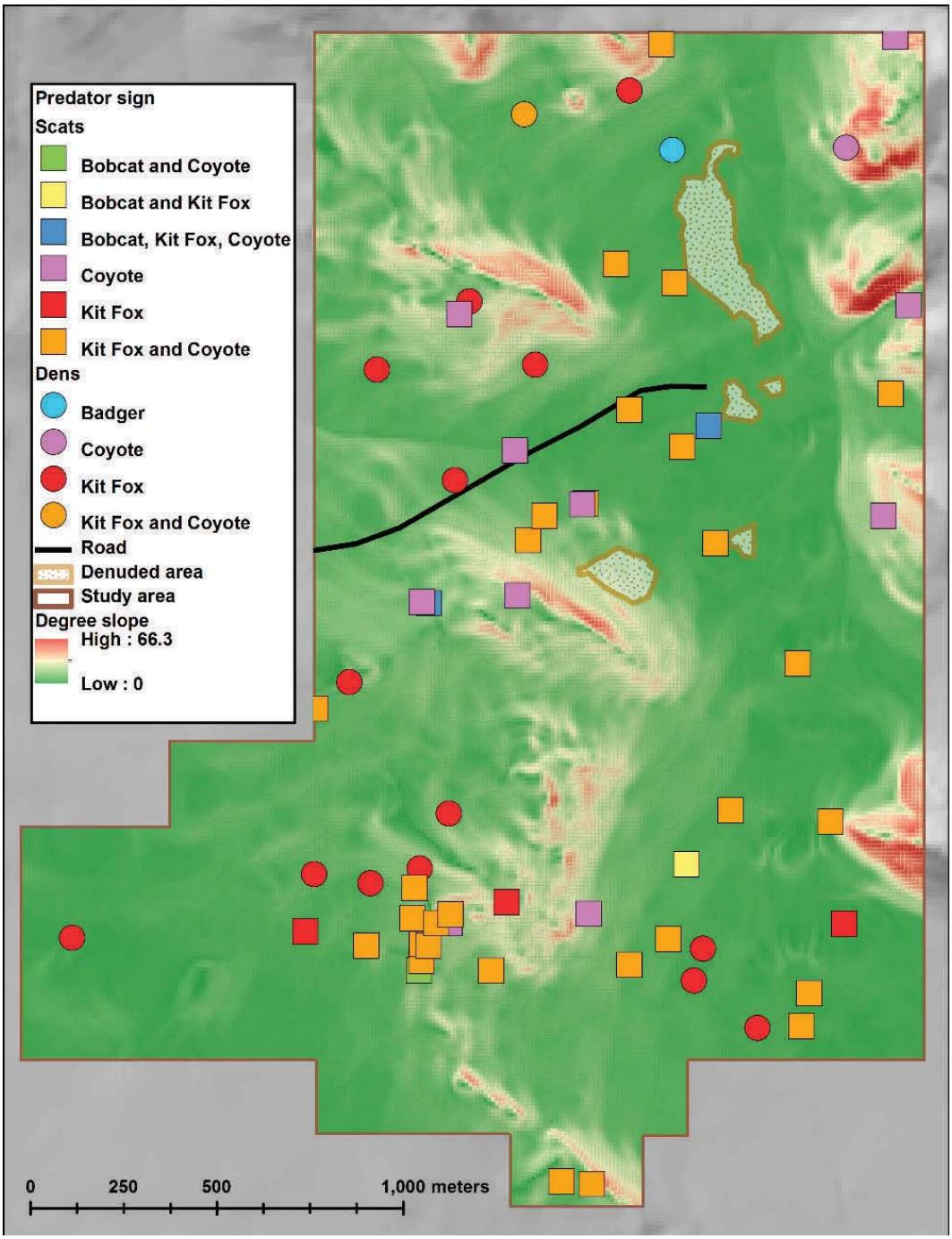

FIG. 6.—Slope and locations of mammalian predator sign at the Argus study area, Naval Air Weapons Station China Lake, San Bernardino County, California.

overhanging rocks used as shelters. These concentration areas were used by combinations of coyotes, kit foxes, and bobcats. Densities of mammalian predator signs ranged from a low of 0/km^2 to a high of 70.3 signs/km^2 (Fig. 7).

Anthropogenic Disturbances

We estimated that areas denuded or partially denuded of perennial vegetation covered 34.52 ha or 6.38% of the area: 4.89 ha were totally denuded of vegetation in the test areas;

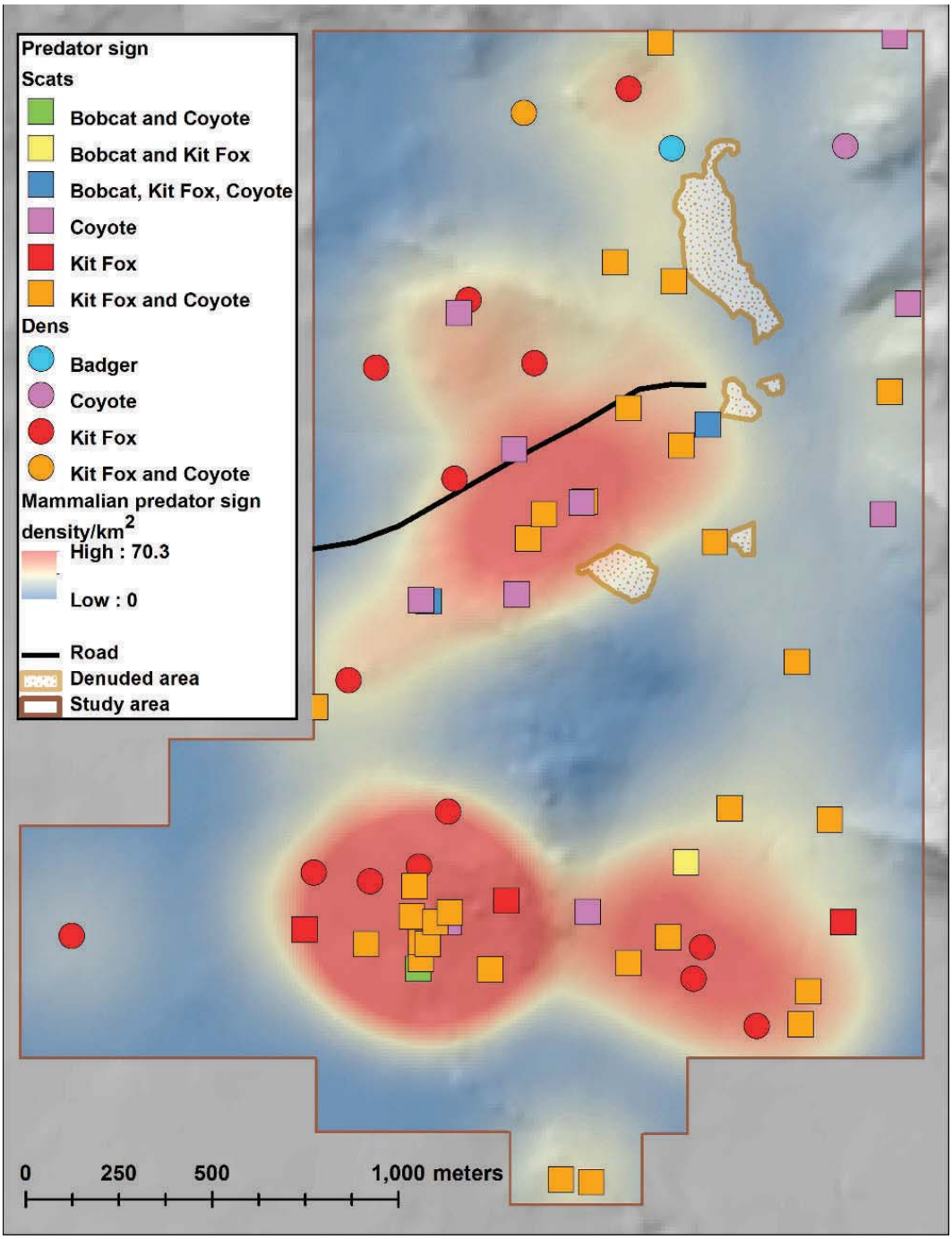

FIG. 7.—Modeled surfaces of mammalian predator sign density using a search radius of 400 m at the Argus study area, Naval Air Weapons Station China Lake, San Bernardino County, California, in 2010.

15.49 ha were partially denuded of vegetation in test areas and boundary trenches; 2.47 ha were composed of pavement, road shoulders, and adjacent denuded areas; and 11.67 ha were dirt roads. The field team counted 10,292 instances of ordnance, trash, balloons, burned areas, utility lines, fences, and vehicle tracks. Ordnance accounted for 93.1%, followed in descending order by trash (5.4%), burned areas and balloons (each 0.5%), and vehicle tracks (0.4%). Counts of ordnance made on 50 randomly placed transects ranged from 0 to

TABLE 2—Models ranked according to corrected Akaike's Information Criterion (AIC_c) using a generalized linear mixed effects model for three Desert Tortoise response variables at the Argus study area in San Bernardino County, California: density of tortoise sign and density of live tortoises. Models are based on one or more of the following covariates, except for the null model: distance from denuded areas (Denuded), distance from paved roads (Paved), density of ordnance (Ordnance), degree of slope (Slope), density of Common Ravens (Raven), and density of mammalian sign (Mammal). The symbol "+" denotes an additive effect and "×" denotes interaction effects. All models are ranked by second-order corrected AIC_c and listed from best to worst with the number of model parameters (K), log likelihood (LL), difference in corrected AIC_c relative to the best model (ΔAIC_c), Akaike weight (ωAIC_c), and cumulative weight (Cum wt). Only the models with Akaike weight >0.05 and AIC_c at least 2 units better than the null model AIC_c are shown.

Model	K	LL	AICc	ΔAICc	ωAICc	Cum wt
Density of tortoise sign						
Denuded × Paved + Mammal	6	−44.05	103.59	0	0.72	0.72
Paved × Raven + Slope	6	−45.30	106.10	2.51	0.20	0.92
Density of live tortoises						
Ordnance × Slope	4	−30.90	71.33	0	0.20	0.20
Ordnance × Slope + Mammal	5	−30.56	73.51	2.18	0.07	0.26

1536 and averaged 40.8; whereas counts of ordnance made on 40 transects associated with the denuded areas ranged from 0 to 327 and averaged 87.3.

Models of live tortoise density and tortoise sign density with predictor variables and interactions.—The AICc statistics indicated no support for overdispersion, therefore our prediction models are based on conventional Poisson distributions. We found spatial correlations in the tortoise sign data and retained this spatial correlation structure in the remainder of those analyses. We did not find spatial correlations in the live tortoise data, however. Densities of tortoise sign were best predicted by distances from denuded areas and paved roads and densities of mammalian predator signs in a model supported by 72% of the total Akaike weight of evidence of all models combined (Table 2). A second model with 20% Akaike weight suggested that Common Ravens and slopes, which ranged from 0 to 66.3% (Fig. 6), were also important predictors. The two best models predicting live tortoise density included ordnance and slope and had a cumulative weight of 26% (Table 2). Certain individual covariates or interaction effects appeared repeatedly in supported models, indicating that specific covariates could be more important than specific models.

Tortoise sign density was positively related to degree of slope, increasing 51 ± 18% per 2.26° increase in slope (Table 3). Tortoise sign density was also positively associated with mammalian predator signs, increasing 40 ± 11% per increase of 7.5 mammal signs/km^2. However, tortoise sign density was negatively related to Common Ravens, decreasing 31 ± 10% for every 3.6 additional sightings/km^2. Model-averaged main effects based on anthropogenic predictors were not significant, based on CIs overlapping with zero; however, model-averaged interaction effects involving anthropogenic predictors were consistently significant whenever they appeared (Table 3).

No clear relationship emerged for live tortoises, which were observed in considerably lower numbers than tortoise sign (Tables 2 and 3). Only two models appeared to be better than the null model, and none of the main effects estimated in those models was significant. Although two of the model-averaged interaction terms were significant, the model-specific percentage changes associated with those interactions were not significant. Although all covariates seemed to play a role in predicting tortoise density, significant interactions suggest that the effect of covariates on prediction generally varied depending on other variables. We spatially mapped the cumulative effects of the covariates (Figs. 3 and 4). The quality of the regression models is modest but significant. Correlations between model-averaged predictions and observed data were 0.67 for tortoise sign and 0.70 for live tortoises ($P < 0.0001$), analogous to $r^2 = 0.45$ and $r^2 = 0.49$, respectively. While these models support general spatial patterns, the predicted values are subject to considerable error (Appendix I).

TABLE 3—Model-averaged effects and standard errors (SE) on estimated Desert Tortoise response (tortoise sign density, live tortoise density) in relationship to distances from denuded areas (Denuded) and paved roads (Paved), densities of ordnance (Ordnance), mammalian predator (Mammal) and Common Raven (Raven) densities, slope (Slope), and their interaction effects (denoted by ×). The model-averaged effects are for the Argus study area, Naval Air Weapons Station China Lake, San Bernardino County, California.

	Tortoise sign density		Live tortoise density	
	Estimate ± SE	90% CI	Estimate ± SE	90% CI
% change[a]				
Denuded	3 ± 22	−28, 46	22 ± 44	−40, 146
Paved	18 ± 18	−9, 52	−4 ± 24	−42, 58
Ordnance	−9 ± 21	−38, 33	−32 ± 25	−67, 39
Mammal	40 ± 11	22, 60[c]	−16 ± 17	−43, 24
Raven	−31 ± 10	−46, −12[c]	5 ± 26	−35, 69
Slope	51 ± 18	24, 84[c]	54 ± 36	−3, 144
Interaction coefficient[b]				
Denuded × Paved	−0.60 ± 0.11	−0.78, −0.41[c]	−0.80 ± 0.35	−1.49, −0.10[c]
Denuded × Mammal	0.42 ± 0.11	0.24, 0.59[c]	0.62 ± 0.40	−0.16, 1.41
Denuded × Raven	0.48 ± 0.10	0.32, 0.65[c]	0.43 ± 0.27	−0.09, 0.96
Denuded × Slope	0.61 ± 0.15	0.36, 0.86[c]	−0.27 ± 0.26	−0.79, 0.25
Paved × Mammal	0.44 ± 0.11	0.26, 0.63[c]	0.17 ± 0.30	−0.43, 0.76
Paved × Raven	0.54 ± 0.11	0.37, 0.71[c]	0.54 ± 0.32	−0.09, 1.17
Paved × Slope	0.54 ± 0.14	0.31, 0.77[c]	0.24 ± 0.27	−0.29, 0.77
Ordnance × Slope	−0.37 ± 0.13	−0.58, −0.15[c]	1.87 ± 1.17	0.37, 3.37[c]
Mammal × Raven	0.07 ± 0.11	−0.11, 0.25	−0.11 ± 0.28	−0.66, 0.44
Mammal × Slope	−0.01 ± 0.08	−0.14, 0.13	0.32 ± 0.25	−0.17, 0.82
Raven × Slope	−0.42 ± 0.21	−0.77, −0.07[c]	0.12 ± 0.33	−0.53, 0.77

[a] Main effects (a) are expressed as percent changes, $(\exp[\beta]-1) \times 100\%$, where β is the coefficient for tortoise response variables on the log scale, and
[b] Interaction effects (b) are expressed as β.
[c] Effects with 90% confidence interval (CI) not overlapping zero were significant at $P < 0.10$.

Live tortoise sightings corresponded significantly with model-predicted tortoise sign ($r = 0.37$, $P = 0.042$, 90% CI = 0.07–0.60). Live tortoises also correlated positively with all tortoise remains ($r = 0.39$, $P = 0.028$, 90% CI = 0.11–0.62), but not significantly for tortoise remains from within the previous 4 yr ($r = 0.28$, $P = 0.12$, 90% CI = −0.02 to 0.54).

DISCUSSION

Desert Tortoise populations were more widespread and higher in density in the western, central, and southern Mojave Desert regions during the 1970s and early 1980s (Berry et al., 1986a, 1986b; USFWS, 1994b; Berry and Medica, 1995) than they are today. The existing Argus population fragment (~130 km^2) was part of this larger, higher-density, and interconnected population that extended from the Argus Range and Searles and Indian Wells valleys south to the southwest border of the geographic range (now the Western Mojave Recovery Unit; Figs. 1 and 2). By 2010, populations were fragmented, tortoise densities were much lower than previously described, and recovery efforts were challenged by numerous anthropogenic impacts (USFWS, 2010). Although detection of tortoises is imperfect, our 2010 surveys were conducted under conditions that increase visibility, e.g., peak spring activity with abundant forage following above-average precipitation, and few concealment opportunities due to sandy soils and low cover of perennial shrubs. Despite favorable conditions for detection, our estimated densities at Argus for all sizes of tortoises were low; our estimates for densities of adult tortoises (2.4/km^2) were similar to those reported by the USFWS for the nearest critical habitat 32 km to the south (Fremont-Kramer critical habitat, 2.5/km^2 in 2010; USFWS, 2012).

Low-density population fragments are potentially more vulnerable to loss and extirpation than are large, high-density, and robust populations (Simberloff and Abele, 1982; USFWS, 1994b). The Argus population

fragment has three positive demographic attributes favoring persistence and recovery: (1) the sex ratio favors female adults, (2) juvenile and small immature tortoises composed 53.6% of the sample, and (3) individual tortoises appeared robust and healthy. Other demographic attributes suggest vulnerability to stochastic events such as drought (Intergovernmental Panel on Climate Change, 2007; Seager et al., 2007) and hyperpredation (Kristan and Boarman, 2003; Esque et al., 2010). These attributes limit the potential for population growth and include (1) low population density; (2) small size of the fragment; (3) low survival of immature tortoises and low recruitment into the young, small-adult size class; and (4) frequent predator attacks on all sizes of tortoises, judging from signs of trauma on live tortoises, shell–skeletal remains, and tortoise parts in coyote scat.

The Argus area has several features in common with tortoise critical habitats managed by non-Defense Department agencies: subsidized predators, paved and dirt roads, areas with partially and completely denuded vegetation, patchy distribution of disturbances in the habitats, and close proximity to urban and exurban lands (USFWS, 1994b, 2011). Substantial differences exist too: only scientists and engineers use the site for research-related testing; the general public has had no access since 1942 or earlier; and shooting, hunting, mining, and livestock grazing are not permitted. Surface disturbances have been confined to specific areas, some of which have been in place for decades.

Although our sample was too small to model all covariates simultaneously, we found varying effects on tortoise sign density and live tortoise density in relationship to each of the anthropogenic, topographic, and predator variables across a variety of models. A large degree of evidence, based on Akaike weights, identified the best models for tortoise sign densities, while a lesser weight of evidence identified predictors for live tortoise densities. The significant effects on tortoise sign densities were positive with slope and mammal signs, negative for Common Ravens, and included interaction effects among these predictors as well as ordnance and distances to denuded areas and paved roads; for live tortoise densities there were significant interaction effects between distances to denuded and paved areas, and between ordnance and slope. Our multi-model inferences allow the cumulative effects of these relationships to be mapped by averaging, weighted by Akaike evidence, all prediction surfaces for tortoise sign density and live tortoise density (Figs. 3 and 4).

Predators and Their Role in Models

Predators were prominent predictors in models of tortoise sign density, the population attribute with the most evidence. Although densities of tortoises were low at the study area in 2010, female tortoises were producing eggs as evidenced by egg shells, 14 live juvenile and immature tortoises, and remains of 16 dead juvenile and immature tortoises. However, no live subadult or young, small adult tortoises (180–220 mm MCL) were located during the study. Thus recruitment of immature tortoises into the adult population has been limited for several years. The probable cause of low survivorship was predation: the remains of most recently dead juvenile and immature tortoises showed signs of traumatic deaths (broken scutes/bones) typical of avian or canid predators. Further, pressure from subsidized mammalian predators was evident in new attacks on two tortoises between May and October field visits in 2010 and a fresh raven kill of a juvenile in early fall.

Common Ravens and coyotes present obstacles to recovery of low-density, fragmented Desert Tortoise populations through increased mortality. Common Ravens, for example, engage in hyperpredation of juveniles (Campbell, 1983; Boarman, 1993) and can also attack and kill adult tortoises (A.P. Woodman, A. Walde, W. Boarman, personal observations). Common Raven populations have grown substantially in the Mojave Desert, subsidized by resources available in cities, towns, and rural areas, including sewage ponds and landfills, road-killed animals, and perching opportunities from power poles (Boarman and Berry, 1995; Knight et al., 1995; Boarman et al., 2006). Common Ravens have the potential of driving local tortoise populations to extinction (Kristan and Boarman, 2003). Coyotes also are subsidized predators, and subsidies in the form of food and

water are readily available in the urban and exurban developments in Indian Wells and Searles valleys. They can move back and forth between sources of subsidies and adjacent wild lands, where tortoises are more common (Fedriani et al., 2001). During periods of drought, subsidized predators such as coyotes may turn to tortoises as food sources when other prey, such as rodents and rabbits, are in low numbers (Woodbury and Hardy, 1948; Esque et al., 2010). Domestic dogs, ranging from housing areas in the Indian Wells and Searles valleys, also may be responsible for attacks on tortoises, e.g., the tortoise with the severely damaged shell (Boyer and Boyer, 2006).

Tortoise sign density increased with increasing slope on the low hills and with mammalian predator sign (Table 3), a distribution pattern evident in Figs. 4 and 7. Based on sign, both tortoises and their mammalian predators appeared to spend more time in the hills and away from the low areas, where the denuded test areas and the paved road occurred. In contrast, observations of ravens were more concentrated at lower elevations, at the terminus of the road, and in denuded areas, sites with more human use but with fewer tortoise sign (Fig. 4). Thus tortoises are exposed to predator pressure whether in the hills or on flat terrain.

Paved Roads and Denuded Areas

Paved roads and denuded areas were important predictors of tortoise densities, as evidenced by the most successful model of tortoise sign. The locations and densities of tortoise sign and live tortoises were low near and including the terminus of the paved road and the denuded areas where testing occurs (Figs. 3 and 4). The areas with low tortoise densities extended for distances of 100–300 m beyond the denuded surfaces, thus encompassing about 15% to 25% of the study area. Spatial differences in detectability are unlikely to have contributed to lower tortoise counts in these locations, which tend to be more open than other parts of Argus and have fewer perennial shrubs where tortoises can hide. The effects of paved roads, especially roads with high traffic volume, on depleting adjacent tortoise populations are well known (e.g., von Seckendorff Hoff and Marlow, 2002; Boarman and Sazaki, 2006). At the Argus site, habitat has been lost from the road, and regardless of the low traffic volume, the roadway itself serves as an attractant to the Common Raven (Knight and Kawashima, 1993; Knight et al., 1995). Areas partially or completely denuded of vegetation—whatever the source of damage—are unsuitable for tortoises: canopy cover of shrubs used for protection from extremes of temperature and predators is minimal or absent, compacted and denuded soils lack forage of annual wildflowers, burrows excavated in denuded areas are vulnerable to human disturbance, and deaths are likely to be higher than in intact habitats (e.g., Bury and Luckenbach, 2002; Berry et al., 2006; Grandmaison et al., 2010). Throughout the geographic range of the Desert Tortoise, roads and denuded areas are frequently coupled: roads terminate in disturbed areas e.g., campsites, off-highway vehicle recreation sites, livestock grazing piospheres, and mining operations. Where roads terminate in anthropogenic uses, we might expect to see more loss of habitat for tortoises than from the denuded surfaces alone, regardless of whether the surface is used for testing explosives and other ordnance or as a livestock watering area, mine, or recreational vehicle encampment.

Denuded areas may have served as a proxy for ordnance in some models. Although ordnance was not a major factor in the models, it may affect health and survival of individuals. Ordnance fragments, common in the denuded and partially denuded test areas, may be eaten by tortoises; chelonians in general are known to consume trash and foreign objects (Boyer and Boyer, 2006). Consumption of potentially toxic materials may contribute to limb and shell lesions, i.e., cutaneous dyskeratosis (Jacobson et al., 1994; Homer et al., 1998), which were common in the Argus tortoises. Static testing of explosives and other ordnance may have negative impacts on tortoises from noise and ground vibrations but we did not measure these impacts.

Management Implications

Many factors affect the well-being, distribution, survival, and ultimately the recovery

potential of Desert Tortoise populations. Suites of predictor variables and their interactions are likely to be complex and to vary over time and space. Separating the most from the least important variables can be challenging. The multiple impacts affecting the Argus tortoises are typical of anthropogenic uses occurring on lands managed by the Department of Defense and other government agencies throughout the geographic range of the species. Of the predictor variables we evaluated, only ordnance is primarily restricted to Department of Defense holdings.

Low-density population fragments with few adults and low recruitment of adults are especially susceptible to stochastic events. Networks of roads and denuded areas contribute to habitat loss, reduce the integrity of the fragments, and increase the likelihood of injury and death to the tortoises. Subsidized predators can also increase vulnerability of the population fragment by limiting recruitment of immature tortoises into the adult population and periodically reducing densities of adult tortoises. The close proximity of expanding towns and cities (Hunter et al., 2003) adds to potential predator subsidies. Unless impacts to Desert Tortoises from anthropogenic impacts are limited or mitigated effectively and unless predation from subsidized predators is reduced, the long-term persistence of this and other similar population fragments will be in doubt.

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Literature Cited

Averill-Murray, R.C., C.R. Darst, K.J. Field, and L.J. Allison. 2012. A new approach to conservation of the Mojave desert tortoise. BioScience 62:893–899.

Bates, D., M. Maechler, and B. Bolker. 2012. Package 'lme4': Linear mixed-effects models using S4 classes. Available at http://cran.r-project.org/web/packages/lme4/lme4.pdf. Archived by WebCite at http://www.webcitation.org/6G9cwvmOB on 25 April 2013.

Berry, K.H. 1986. Incidence of gunshot deaths in desert tortoises in California. Wildlife Society Bulletin 14:127–132.

Berry, K.H. 1997. The Desert Tortoise Recovery Plan: An ambitious effort to conserve biodiversity in the Mojave and Colorado deserts of the United States. Pp. 430–440 in J. Van Abbema (Ed.), Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles: An International Conference. New York Turtle and Tortoise Society and Wildlife Conservation Society Turtle Recovery Program, USA.

Berry, K.H., and M.M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Diseases 37:427–450.

Berry, K.H., and P. Medica. 1995. Desert tortoises in the Mojave and Colorado deserts. Pp. 135–137 in E.T. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac (Eds.), Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. United States Department of the Interior, National Biological Service, USA.

Berry, K.H., and A.P. Woodman. 1984. Methods used in analyzing mortality data for most tortoise populations in California, Nevada, Arizona, and Utah. Appendix 7 in K.H. Berry (Ed.), The Status of the Desert Tortoise (*Gopherus agassizii*) in the United States. Desert Tortoise Council Report to United States Fish and Wildlife Service, Sacramento, California, USA. Order No. 11310–0083–81.

Berry, K.H., L.L. Nicholson, S. Juarez, and A.P. Woodman. 1986a. Changes in desert tortoise populations at four study sites in California. Proceedings of the Desert Tortoise Council Symposium 1986:60–80.

Berry, K.H., T. Shields, A.P. Woodman, T. Campbell, J. Roberson, K. Bohuski, and A. Karl. 1986b. Changes in desert tortoise populations at the Desert Tortoise Research Natural Area between 1979 and 1985. Proceedings of the Desert Tortoise Council Symposium 1986: 100–123.

Berry, K.H., E.K. Spangenberg, B.L. Homer, and E.R. Jacobson. 2002. Deaths of desert tortoises following periods of drought and research manipulation. Chelonian Conservation and Biology 4:436–448.

Berry, K.H., T.Y. Bailey, and K.M. Anderson. 2006. Attributes of desert tortoise populations at the National Training Center, Central Mojave Desert, California, USA. Journal of Arid Environments 67S:165–191.

Boarman, W.I. 1993. When a native predator becomes a pest: A case study. Pp. 191–206 in S.K. Majumdar, E.W. Miller, D.E. Baker, E.K. Brown, J.R. Pratt, and R.F. Schmalz (Eds.), Conservation and Resource Management. Pennsylvania Academy of Sciences, USA.

Boarman, W.I., and K.H. Berry. 1995. Common ravens in the southwestern United States, 1968–92. Pp. 73–75 in E.T. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac (Eds.), Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. United States Department of the Interior, National Biological Service, USA.

Boarman, W.I., and M. Sazaki. 2006. A highway's road-effect zone for desert tortoises (*Gopherus agassizii*). Journal of Arid Environments 65S:94–101.

Boarman, W.I., M.A. Patten, R.J. Camp, and S.J. Collis. 2006. Ecology of a population of subsidized predators: Common ravens in the central Mojave Desert, California. Journal of Arid Environments 67S:248–261.

Boyer, T.H., and D.M. Boyer. 2006. Turtles, tortoises, and terrapins. Pp. 78–99 in D.R. Mader (Ed.), Reptile Medicine and Surgery, 2nd ed. Saunders Elsevier Inc., USA.

Brown, D.R., J.L. Merritt, E.R. Jacobson, P.A. Klein, J.G. Tully, and M.B. Brown. 2004. *Mycoplasma testudineum* sp. nov. from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. International Journal of Systematic and Evolutionary Microbiology 43:1527–1529.

Brown, M.B., I.M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E.R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62:4580–4586.

Burge, B.L. 1978. Physical characteristics and patterns of utilization of cover sites used by *Gopherus agassizi* in southern Nevada. Proceedings of the Desert Tortoise Council Symposium 1978: 80–111.

Burnham, K.P., and D.R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd ed. Springer, USA.

Bury, R.B., and R.A. Luckenbach. 2002. Comparison of desert tortoise (*Gopherus agassizii*) populations in an unused and off-road vehicle area in the Mojave Desert. Chelonian Conservation and Biology 4:457–463.

[CADFG] California Department of Fish and Game. 2010a. Hierarchical list of natural communities with Holland types, Sept. 2010. Available at: http://www.dfg.ca.gov/biogeodata/vegcamp/natural_comm_list.asp. Archived by WebCite at http://webcitation.org/6GAdpJ8wq on 26 April 2013.

[CADFG] California Department of Fish and Game. 2010b. Available at: http://imaps.dfg.ca.gov/ArcGIS/services (when in ArcGIS). Archived by WebCite at http://webcitation.org/6GAoCjasE on 26 April 2013.

Campbell, T. 1983. Some natural history observations of desert tortoises and other species on and near the Desert Tortoise Natural Area, Kern County, California. Proceedings of the Desert Tortoise Council Symposium 1983:80–88.

Darst, C.R., P.J. Murphy, N.W. Stout, S.P. Campbell, K.J. Field, L. Allison, and R.C. Averill-Murray. 2013. A strategy for prioritizing threats and recovery actions for at-risk species. Environmental Management 51:786–800.

Dormann, C.F., J.M. McPherson, M.B. Araújo, R. Bivand, J. Bolliger, G. Carl, R.G. Davies, A. Hirzel, W. Jetz, W.D. Kissling, I. Kühn, R. Ohlemüller, P.R. Peres-Neto, B. Reineking, B. Schröder, F.M. Schurr, and R. Wilson. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography 30:609–628.

Duda, J.J., A.J. Krzysik, and J.E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. Journal of Wildlife Management 63:1181–1192.

Esque, T.C., K.E. Nussear, and P.A. Medica. 2005. Desert tortoise translocation plan for Fort Irwin's land expansion program at the U.S. Army National Training Center (NTC) at Fort Irwin. Prepared for United States Army National Training Center, Directorate of Public Works. Available at www.fortirwinlandexpansion.com/Documents.htm. Archived by WebCite at http://webcitation.org/6GAdvLceL on 26 April 2013.

Esque, T.C., K.E. Nussear, K.K. Drake, A.D. Walde, K.H. Berry, R.C. Averill-Murray, A.P. Woodman, W.I. Boarman, P.A. Medica, J. Mack, and J.S. Heaton. 2010. Effects of subsidized predators, resource variability, and human population density on desert tortoise populations in the Mojave Desert, USA. Endangered Species Research 12:167–177.

ESRI, Inc. 2009a. ArcGIS 9.3.1 Desktop help: Create Fishnet (data management). Available at http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?id=1782&pid=1777&topicname=Create_Fishnet_(Data_Management). Archived by WebCite at http://webcitation.org/6GAe6Uzes on 26 April 2013.

ESRI, Inc. 2009b. ArcGIS 9.3.1 Desktop help: Slope (Spatial Analyst). Available at http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?TopicName=slope. Archived by WebCite at http://webcitation.org/6GAeErROP on 26 April 2013.

ESRI, Inc. 2009c. ArcGIS 9.3.1 Desktop help: Kernel Density. Available at http://webhelp.esri.com/arcgisdesktop/9.3/body.cfm?id=6190&pid=6188&topicname=Kernel%20Density&tocVisable=0. Archived by WebCite at http://webcitation.org/6GAeLH5en on 26 April 2013.

ESRI, Inc. 2009d. ArcGIS 9.3.1 Desktop help: An overview on calculating the distance from each feature to the nearest feature, Release 9.3.1. Available at http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?id=1273&pid=1268&topicname=Near (Analysis). Archived by WebCite at http://webcitation.org/6GAePqJm8 on 26 April 2013.

ESRI, Inc. 2009e. ArcGIS 9.3.1 Desktop help: Using kriging. Available at http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?TopicName=Using%20Kriging. Archived by WebCite at http://webcitation.org/6GAeX3PxG on 26 April 2013.

ESRI, Inc. 2009f. ArcGIS 9.3.1 Desktop help: IDW. Available at http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?id=5579&pid=5577&topicname=IDW. Archived by WebCite at http://webcitation.org/6GAeeYfsv on 26 April 2013.

Fedriani, J.M., T.K. Fuller, and R.M. Sauvajot. 2001. Does availability of anthropogenic food enhance densities of omnivorous mammals? An example with coyotes in southern California. Ecography 24:325–331.

Grandmaison, D.D., M.F. Ingraldi, and F.R. Peck. 2010. Desert tortoise microhabitat selection on the Florence Military Reservation, south-central Arizona. Journal of Herpetology 44:581–590.

Henen, B.T., C.C. Peterson, I.R. Wallis, K.H. Berry, and K.A. Nagy. 1998. Effects of climatic variation on field metabolism and water relations of desert tortoises. Oecologia 177:365–373.

Homer, B.L., K.H. Berry, M.B. Brown, G. Ellis, and E.R. Jacobson. 1998. Pathology of diseases in wild desert tortoises from California. Journal of Wildlife Diseases 34:508–523.

Hunter, L.M., G. Gonzalez, M. Stevenson, K.S. Karish, R. Toth, T.C. Edwards, R.J. Lilieholm, and M. Cablk. 2003.

Population and land use change in the California Mojave: natural habitat implications of alternative futures. Population Research and Policy Review 22:373–397.

Intergovernmental Panel on Climate Change. 2007. Climate change 2007: The physical science basis. Summary for policy makers. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Available at http://www.ipcc.ch. Archived by WebCite at http://webcitation.org/6GAepoecA on 26 April 2013.

Jacobson, E.R., and K.H. Berry. 2012. *Mycoplasma testudineum* in free-ranging desert tortoises, *Gopherus agassizii*. Journal of Wildlife Diseases 48:1063–1068.

Jacobson, E.R., J.M. Gaskin, M.B. Brown, R.K. Harris, C.H. Gardiner, J.L. LaPointe, H.P. Adams, and C. Reggiardo. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). Journal of Wildlife Diseases 27:296–316.

Jacobson, E.R., T.J. Wronski, J. Schumacher, C. Reggiardo, and K.H. Berry. 1994. Cutaneous dyskeratosis in free-ranging desert tortoises, *Gopherus agassizii*, in the Colorado Desert of southern California. Journal of Zoo and Wildlife Medicine 25:68–81.

Jacobson, E.R., M.B. Brown, I.M. Schumacher, B.R. Collins, R.K. Harris, and P.A. Klein. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. Chelonian Conservation and Biology 1:279–284.

Jacobson, E.R., K.H. Berry, J.F.X. Wellehan, Jr., F. Origgi, A.L. Childress, J. Braun, M. Schrenzel, J. Yee, and B. Rideout. 2012. Serologic and molecular evidence for Testudinid herpesvirus 2 infection in wild Agassiz's desert tortoises, *Gopherus agassizii*. Journal of Wildlife Diseases 48:747–757.

Jennings, W.B. 2002. Diet selection by the desert tortoise in relation to the flowering phenology of ephemeral plants. Chelonian Conservation and Biology 4:353–358.

Knight, R.L., and J.Y. Kawashima. 1993. Responses of raven and red-tailed hawk populations to linear right-of-ways. Journal of Wildlife Management 57:266–271.

Knight, R.L., H.A.L. Knight, and R.J. Camp. 1995. Common ravens and number and type of linear rights-of-way. Biological Conservation 74:65–67.

Kristan, W.B. III, and W.I. Boarman. 2003. Spatial pattern of risk of common raven predation on desert tortoises. Ecology 84:2432–2443.

Krzysik, A.J. 1997. Desert tortoise populations in the Mojave Desert and a half-century of military training activities. Pp. 61–73 in J. Van Abbema (Ed.), Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles: An International Conference. New York Turtle and Tortoise Society and Wildlife Conservation Society Turtle Recovery Program, USA.

Krzysik, A.J. 2002. A landscape sampling protocol for estimating distribution and density patterns of desert tortoises at multiple spatial scales. Chelonian Conservation and Biology 4:366–379.

Mazerolle, M.J. 2012. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.25. Available at http://CRAN.R-project.org/package=AICcmodavg. Archived by WebCite at http://webcitation.org/6GAey9PRk on 26 April 2013.

McCullagh, P., and J.A. Nelder. 1989. Generalized Linear Models, 2nd ed. Chapman and Hall/CRC, USA.

Murphy, R.W., K.H. Berry, T. Edwards, A.E. Leviton, A. Lathrop, and J.D. Riedle. 2011. The dazed and confused identity of Agassiz's land tortoise, *Gopherus agassizii* (Testudines, Testudinidae) with the description of a new species, and its consequences for conservation. ZooKeys 113:39–71.

Nagy, K.A., and P.A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica 42:73–92.

National Oceanic and Atmospheric Administration. 2009–2010. Climatological Data for California. National Climatic Data Center, Asheville, North Carolina. Available at http://www.ncdc.noaa.gov/oa/climate/normals.html, Archived by WebCite at http://webcitation.org/6GAf6v80z on 26 April 2013.

R Development Core Team. 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Austria. Available at http://www.R-project.org. Archived by WebCite at http://webcitation.org/6GAfEGifb on 26 April 2013.

SAS Institute Inc. 2010. SAS OnlineDoc® 9.2. SAS Institute Inc, USA.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.-P. Huang, N. Harnik, A. Leetmaa, N.-C. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science, New Series 316: 1181–1184.

Simberloff, D., and L.G. Abele. 1982. Refuge design and island biogeographic theory: effects of fragmentation. America Naturalist 120:41–50.

Stein, B.A., C. Scott, and N. Benton. 2008. Federal lands and endangered species: The role of military and other federal lands in sustaining biodiversity. BioScience 58:339–347.

Tazik, D.J., and C.O. Martin. 2002. Threatened and endangered species on U.S. Department of Defense lands in the arid West, USA. Arid Land Research and Management 16:259–276.

[USBLM] US Department of the Interior, Bureau of Land Management. 1973. Interim critical management plan for recreational vehicle use in the California Desert. United States Department of the Interior, Bureau of Land Management, California Desert Program, USA.

[USBLM] US Department of the Interior, Bureau of Land Management. 1980. The California Desert Conservation Area Plan, 1980. United States Department of the Interior, Bureau of Land Management, USA.

[USBLM] US Department of the Interior, Bureau of Land Management. 2006. Record of decision, West Mojave Plan, amendment to the California Desert Conservation Area Plan. March 2006. United States Department of the Interior, Bureau of Land Management, USA.

[USCB] US Census Bureau. 2010. Population finder. Available at http://www.census.gov/. Archived by WebCite at http://webcitation.org/6GAoHm36O on 26 April 2013.

[USFWS] US Fish and Wildlife Service. 1990. Endangered and threatened wildlife and plants; determination of threatened status for the Mojave population of the desert tortoise. Federal Register 55:12178–12191.

[USFWS] US Fish and Wildlife Service. 1994a. Endangered and threatened wildlife and plants; determination of critical habitat for the Mojave population of the desert tortoise. Federal Register 59:5820–5866.

[USFWS] US Fish and Wildlife Service. 1994b. Desert Tortoise (Mojave Population) Recovery Plan. United States Fish and Wildlife Service, USA.

[USFWS] US Fish and Wildlife Service. 2010. Mojave population of the desert tortoise (*Gopherus agassizii*). 5-Year review: Summary and evaluation. September 30, 2010. United States Fish and Wildlife Service, USA.

[USFWS] US Fish and Wildlife Service. 2011. Revised recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*). United States Fish and Wildlife Service, USA.

[USFWS] United States Fish and Wildlife Service. 2012. Range-wide monitoring of the Mojave desert tortoise (*Gopherus agassizii*): 2010 Annual Report. United States Fish and Wildlife Service, Desert Tortoise Recovery Office, USA. Available at http://www.fws.gov/nevada/desert_tortoise/dtro_monitor.html. Archived by WebCite at http://www.webcitation.org/6GGvNNIwY on 30 April 2013.

[USGS] US Geological Survey. 2012. National elevation dataset. Available at http://ned.usgs.gov/. Archived by WebCite at http://www.webcitation.org/6GB2nyOlb on 26 April 2013.

Venables, W.N., and B.D. Ripley. 2002. Modern Applied Statistics with S, 4th ed. Springer, USA.

von Seckendorff Hoff, K., and R.W. Marlow. 2002. Impacts of vehicle road traffic on desert tortoise populations with consideration of conservation of tortoise habitat in southern Nevada. Chelonian Conservation and Biology 4:449–456.

Woodbury, A.M., and R. Hardy. 1948. Studies of the desert tortoise, *Gopherus agassizi*. Ecological Monographs 18:145–200.

Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev, and G.M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer Science+Business Media, LLC, USA.

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Appendix I

Predicted densities (counts/km^2 ± 1 SE) and predicted counts (per grid ± 1 SE) of tortoise signs and live tortoises at 31 grids (500 m × 500 m or smaller) comprising the Argus core study area, San Bernardino County, California. Predictions are averaged across all models, weighted by AIC_c weight, and shown with observed counts. Coefficient of variations (CV = SE/prediction) represent accuracy of predictions.

Gridno	Grid area (km^2)	Predicted tortoise sign density	Predicted live tortoise density	Observed tortoise sign	Predicted tortoise sign	CV	Observed live tortoises	Predicted live tortoises	CV
1	0.04	49 ± 20	8.0 ± 4.4	0	2.1 ± 0.8	0.41	0	0.34 ± 0.18	0.54
2	0.10	63 ± 31	9.4 ± 5.1	1	6.3 ± 3.1	0.48	0	0.94 ± 0.51	0.54
3	0.10	55 ± 20	3.2 ± 3.0	0	5.5 ± 2.0	0.36	0	0.32 ± 0.30	0.93
4	0.09	61 ± 28	7.2 ± 3.8	15	5.3 ± 2.5	0.46	2	0.63 ± 0.34	0.53
5	0.11	46 ± 15	6.2 ± 1.9	2	5.1 ± 1.7	0.33	0	0.69 ± 0.20	0.30
6	0.25	43 ± 27	6.4 ± 1.8	11	10.8 ± 6.7	0.62	3	1.61 ± 0.45	0.28
7	0.25	14 ± 6	1.2 ± 1.8	3	3.4 ± 1.5	0.44	0	0.30 ± 0.44	1.44
8	0.22	37 ± 30	10.0 ± 6.6	13	7.9 ± 6.6	0.83	2	2.15 ± 1.42	0.66
9	0.11	44 ± 18	5.3 ± 1.6	0	4.9 ± 2.0	0.40	0	0.59 ± 0.17	0.30
10	0.25	42 ± 28	5.3 ± 1.7	5	10.4 ± 7.1	0.68	2	1.33 ± 0.44	0.33
11	0.25	8 ± 5	1.5 ± 1.8	2	2.0 ± 1.3	0.64	0	0.37 ± 0.45	1.21
12	0.22	27 ± 28	3.1 ± 2.4	15	5.8 ± 6.1	1.06	0	0.68 ± 0.53	0.78
13	0.11	20 ± 11	5.3 ± 2.0	1	2.2 ± 1.3	0.57	1	0.58 ± 0.22	0.38
14	0.25	17 ± 7	5.8 ± 2.7	13	4.1 ± 1.8	0.42	0	1.46 ± 0.66	0.45
15	0.25	10 ± 5	2.0 ± 2.1	4	2.6 ± 1.3	0.52	0	0.51 ± 0.52	1.02
16	0.22	24 ± 11	5.4 ± 1.7	9	5.3 ± 2.4	0.45	1	1.18 ± 0.36	0.31
17	0.06	75 ± 41	5.6 ± 4.0	0	4.4 ± 2.4	0.54	0	0.33 ± 0.24	0.71
18	0.19	45 ± 17	4.9 ± 1.7	11	8.7 ± 3.3	0.38	1	0.95 ± 0.33	0.35
19	0.25	35 ± 12	6.1 ± 1.6	35	8.8 ± 2.9	0.33	2	1.53 ± 0.39	0.26
20	0.25	38 ± 14	6.2 ± 2.0	27	9.4 ± 3.4	0.36	4	1.56 ± 0.50	0.32
21	0.22	52 ± 17	6.2 ± 1.8	32	11.3 ± 3.8	0.33	2	1.35 ± 0.38	0.28
22	0.25	89 ± 61	5.3 ± 2.9	20	22.3 ± 15.2	0.68	3	1.33 ± 0.73	0.55
23	0.25	105 ± 62	4.7 ± 2.1	24	26.2 ± 15.4	0.59	1	1.17 ± 0.51	0.44
24	0.25	184 ± 112	7.0 ± 3.6	35	45.9 ± 27.9	0.61	2	1.74 ± 0.89	0.51
25	0.25	103 ± 57	4.6 ± 1.9	16	25.8 ± 14.3	0.55	0	1.15 ± 0.48	0.42
26	0.22	70 ± 37	4.9 ± 1.7	31	15.1 ± 8.1	0.53	0	1.07 ± 0.38	0.35
27	0.03	62 ± 41	5.5 ± 5.1	0	1.8 ± 1.2	0.67	0	0.16 ± 0.15	0.93
28	0.07	46 ± 25	4.7 ± 2.6	1	3.3 ± 1.8	0.54	0	0.33 ± 0.18	0.55
29	0.17	47 ± 22	5.7 ± 2.4	11	7.7 ± 3.7	0.47	1	0.95 ± 0.39	0.41
30	0.12	40 ± 24	5.1 ± 2.3	2	4.9 ± 2.9	0.59	1	0.63 ± 0.28	0.44
31	0.02	29 ± 23	4.1 ± 2.6	0	0.7 ± 0.6	0.80	0	0.10 ± 0.06	0.64
Correlation between observed and predicted					0.67			0.70	

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PROTECTION BENEFITS DESERT TORTOISE (*GOPHERUS AGASSIZII*) ABUNDANCE: THE INFLUENCE OF THREE MANAGEMENT STRATEGIES ON A THREATENED SPECIES

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Abstract: We surveyed an area of ~260 km^2 in the western Mojave Desert to evaluate relationships between condition of Agassiz's Desert Tortoise populations (*Gopherus agassizii*) and habitat on lands that have experienced three different levels of management and protection. We established 240 1-ha plots using random sampling, with 80 plots on each of the three types of managed lands. We conducted surveys in spring 2011 and collected data on live tortoises, shell-skeletal remains, other signs of tortoises, perennial vegetation, predators, and evidence of human use. Throughout the study area and regardless of management area, tortoise abundance was positively associated with one of the more diverse associations of perennial vegetation. The management area with the longest history of protection, a fence, and legal exclusion of livestock and vehicles had significantly more live tortoises and lower death rates than the other two areas. Tortoise presence and abundance in this protected area had no significant positive or negative associations with predators or human-related impacts. In contrast, the management area with a more recent exclusion of livestock, limited vehicular traffic, and with a recent, partial fence had lower tortoise densities and high death rates. Tortoise abundance here was negatively associated with vehicle tracks and positively associated with mammalian predators and debris from firearms. The management area with the least protection—unfenced, with uncontrolled vehicle use, sheep grazing, and high trash counts—also had low tortoise densities and high death rates. Tortoise abundance was negatively associated with sheep grazing and positively associated with trash and mammalian predator scat.

Key words: Desert Tortoise Research Natural Area; Fence; Land use legacy; Mojave Desert; Protected areas; Sheep grazing; Vehicles

Agassiz's Desert Tortoise, *Gopherus agassizii* (hereafter called Desert Tortoise or tortoise), is both a federally and state-listed threatened species with designated critical habitat and recovery plans (US Fish and Wildlife Service [USFWS], 1990, 1994a, 1994b, 2011; California Department of Fish and Wildlife, 2013). Despite recovery efforts, populations have continued to decline and available habitat has been reduced largely because of human-related uses (USFWS, 2010). In 2011, *G. agassizii* was split into two species, *G. agassizii* and *G. morafkai*, and the geographic range of *G. agassizii* was reduced by ~66% (Murphy et al., 2011).

Few populations and places within the geographic range of *G. agassizii* are more threatened than the western Mojave Desert (USFWS, 1994a). Since the arrival of explorers and settlers in the mid-1800s, the desert ecosystem has experienced many uses and changes that have affected the tortoise. The historical patterns of land use have left a legacy that is important for understanding not only the decline of the tortoise and its habitat but also the potential effects of this legacy on current management strategies to recover the species (Foster et al., 2003; Leu et al., 2008). Briefly, in the mid- to late 1800s, early explorers and settlers engaged in dry-land farming and agriculture (Norris, 1982), livestock grazing and ranching (Wentworth, 1948; Powers, 1988, 2000), and mining (Vredenburg et al., 1981). The 1900s were characterized by growth and expansion of human populations, military bases and facilities, energy and transportation corridors, energy developments and facilities, and off-highway vehicle-oriented recreation (US Bureau of Land Management [USBLM], 1973, 1980, 2006; Hunter et al., 2003). With the exception of dry-land farming, these uses have continued and the

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total amount of land with surface disturbances has grown.

Livestock grazing, ranching, and other surface-disturbing activities have caused profound changes to the distribution and composition of annual and perennial vegetation once typical of the Mojave Desert (Minnich, 2008). The invasion and establishment of alien annual grasses, such as the fire-prone bromes (*Bromus madritensis* subsp. *rubens, B. tectorum*) and Arab grasses (*Schismus barbatus, S. arabicus*), have severely altered the biomass and composition of the annual flora in the region (Brooks and Berry, 2006; Brooks et al., 2006) and led to a fire-prone regime in some areas (Brooks and Matchett, 2006). Introduced forbs, such as *Erodium cicutarium* and members of the mustard family, also are highly successful and form a substantial part of the biomass in many areas (Brooks and Berry, 2006; Minnich, 2008). The alien annuals have altered the foods available to the tortoise; the alien grasses, in particular, are not preferred foods in this region (Jennings, 2002) and may be detrimental in diets of juvenile and adult tortoises (Nagy et al., 1998; Hazard et al., 2009, 2010).

Paved and dirt roads and recreational use of off-highway vehicles not only have created surface disturbances but also have provided access to previously remote wild lands (Brooks and Lair, 2009). Roads, whether paved or dirt, vehicle trails and routes, and tracks contribute to invasion and establishment of alien annual plants (Brooks and Berry, 2006). They can alter surface flow of water and nutrients, fragment the ecosystem, and reduce the surface available for food and shelter for the tortoises and other animals (Brooks and Lair, 2009). The increased access and recreational play areas also have created numerous areas denuded or partially denuded of vegetation (Busack and Bury, 1974; Bury and Luckenbach, 2002).

Another legacy associated with growth and expansion of human populations in the Mojave Desert is the concomitant growth of subsidized predator populations. Subsidized predators are predators with population sizes supported by anthropogenic sources of food, water, shelter, and perch sites. Subsidized predators have the potential to increase in numbers far beyond levels provided by the natural desert prey base by using food and water sources in nearby desert towns and settlements. They include the Common Ravens (*Corvus corax*), coyotes (*Canis latrans*), and domestic dogs (*Canis lupus familiaris*). These predators have used the subsidies to expand their populations and have engaged in hyperpredation of tortoises in some areas (Boarman, 1993; Boarman and Berry, 1995; Fedriani et al., 2001; Boyer and Boyer, 2006; Esque et al., 2010).

We designed this research project to evaluate how contiguous areas with three different land-use histories and types of management have affected Desert Tortoise populations and the habitats where they live. For comparisons, we selected the fenced Desert Tortoise Research Natural Area (Tortoise Natural Area) as most protected. The Tortoise Natural Area is internationally recognized as a protected area with an irreplaceability rank for threatened species in the top 6% of protected areas worldwide (Le Saout et al., 2013). We chose critical habitat for the tortoise in the Western Rand Area of Critical Environmental Concern as moderately protected. Critical habitat in this region is contained within three designated management areas: the Western Rand Area of Critical Environmental Concern, western parts of the Rand Mountains Management Area, and Fremont–Kramer Desert Wildlife Management Area and Area of Critical Environmental Concern (USBLM, 1980, 2006; USFWS, 1994b). The Fremont–Kramer Area of Critical Environmental Concern is also on the list of globally protected areas and has an irreplaceability rank for threatened species in the top 2% of protected areas worldwide (Le Saout et al., 2013). Private lands are least protected. Henceforth, we use the terms Tortoise Natural Area, critical habitat, and private lands to refer to the three management areas. These areas are interconnected and experienced similar land-use histories through the 1950s and 1960s, when nearby California City was established and off-highway vehicle-oriented recreation became a change agent in the region (USBLM, 1973). Throughout the interconnected areas, densities of tortoise populations were high in the late 1970s and early 1980s, ranging from 110 to 147

Table 1.—Historic density estimates of Desert Tortoise populations from long-term plots in the three management areas between 1979 and 1981, eastern Kern County, California. The density estimates are for all sizes of tortoises and are based on mark–recapture techniques and stratification of the tortoises into size classes for analysis.

Name of plot, size	Location in management area	Year of survey	Tortoise density/km^2 (95% interval)	Reference
Desert Tortoise Natural Area (interior), 1.1 km^2	Tortoise Natural Area	1979	147 (113–192)	Berry et al. (1986a), Berry and Medica (1995)
Desert Tortoise Natural Area Interpretive Center (inside fence), 4.53 km^2	Tortoise Natural Area	1979	131 (111–155)	Berry et al. (1986a)
Fremont Valley, 2.59 km^2	Critical habitat	1981	110 (83–144)	Turner and Berry (1984)
Desert Tortoise Natural Area Interpretive Center (outside fence), 3.24 km^2	Private lands	1979	114 (90–146)	Berry et al. (1986a)

tortoises/km^2, depending on location (Table 1; Turner and Berry, 1984; Berry et al., 1986a; Berry and Medica, 1995). For the three management areas, our objectives were to (1) describe the historical legacy and identify recent differences in management, (2) compare tortoise abundance and other population attributes in 2011, (3) identify natural and anthropogenic factors positively or negatively associated with tortoise abundance in 2011, (4) evaluate differences in mammalian and avian predators in 2011, and (5) discuss factors relevant to future recovery efforts for tortoises and their habitats.

Study Area

General Description

The northern and northeast boundaries of the study area are the Red Rock–Randsburg, Garlock, and Goler paved roads; from these roads, the ~260-km^2 study area extends south through the Fremont Valley and Rand Mountains to the southern boundary of the Tortoise Natural Area (Figs. 1, 2). The only paved road within the study area is a ~8-km stretch of the Red Rock–Randsburg Road, which traverses the northern part of critical habitat. The nearest paved roads to study area boundaries are 1.6 km distant in the west (Neuralia Road), 3.2 km distant in the south (within California City), and 3.2 to 16.0 km in the east (US Highway 395). The settlements of Cantil and Goler Heights are within 4 km in the northwest and 0.8 km in the north, respectively; the towns of Randsburg and Johannesburg (populations 69 and 172, respectively) and the settlement of Red Mountain are within 2.9 km in the northeast; and urbanized California City (population of 14,327; US Census Bureau estimate for 2011) is 3.2 km to the south. More important, four high-use recreation areas are within 5 to 14 km of study area boundaries (Fig. 1): Red Rock Canyon State Park and three recreation areas with unrestricted access for off-highway vehicles (Jawbone Canyon, Dove Springs, and Spangler Hills).

Within the study area, elevations range from 590 m near the edge of Koehn Dry Lake to 1240 m at the crest of the Rand Mountains. Perennial vegetation is predominantly composed of white bur-sage (*Ambrosia dumosa*) and creosote bush (*Larrea tridentata*) alliances (California Department of Fish and Game, 2010), which change with elevation and surface disturbance. At the edge of Koehn Dry Lake in the north, allscale saltbush (*Atriplex polycarpa*) and other *Atriplex* species are common. On the floor of the Fremont Valley and the toeslope of the Rand Mountains, perennial vegetation grades into creosote bush scrub with white bur-sage and many different species of shrubs: cheesebush (*Ambrosia salsola*), goldenhead (*Acamptopappus sphaerocephalus*), spiny senna (*Senna armata*), silver cholla (*Cylindropuntia echinocarpa*), Nevada ephedra (*Ephedra nevadensis*), winter fat (*Krascheninnikovia lanata*), hop-sage (*Grayia spinosa*), Mojave indigo bush (*Psorothamnus arborescens*), Acton encelia (*Encelia actoni*), Mojave Desert California buckwheat (*Eriogonum fasciculatum*), Anderson box-thorn (*Lycium andersonii*), Cooper's box-thorn (*L. cooperi*), and wishbone bush (*Mirabilis laevis* var. *retrorsa*).

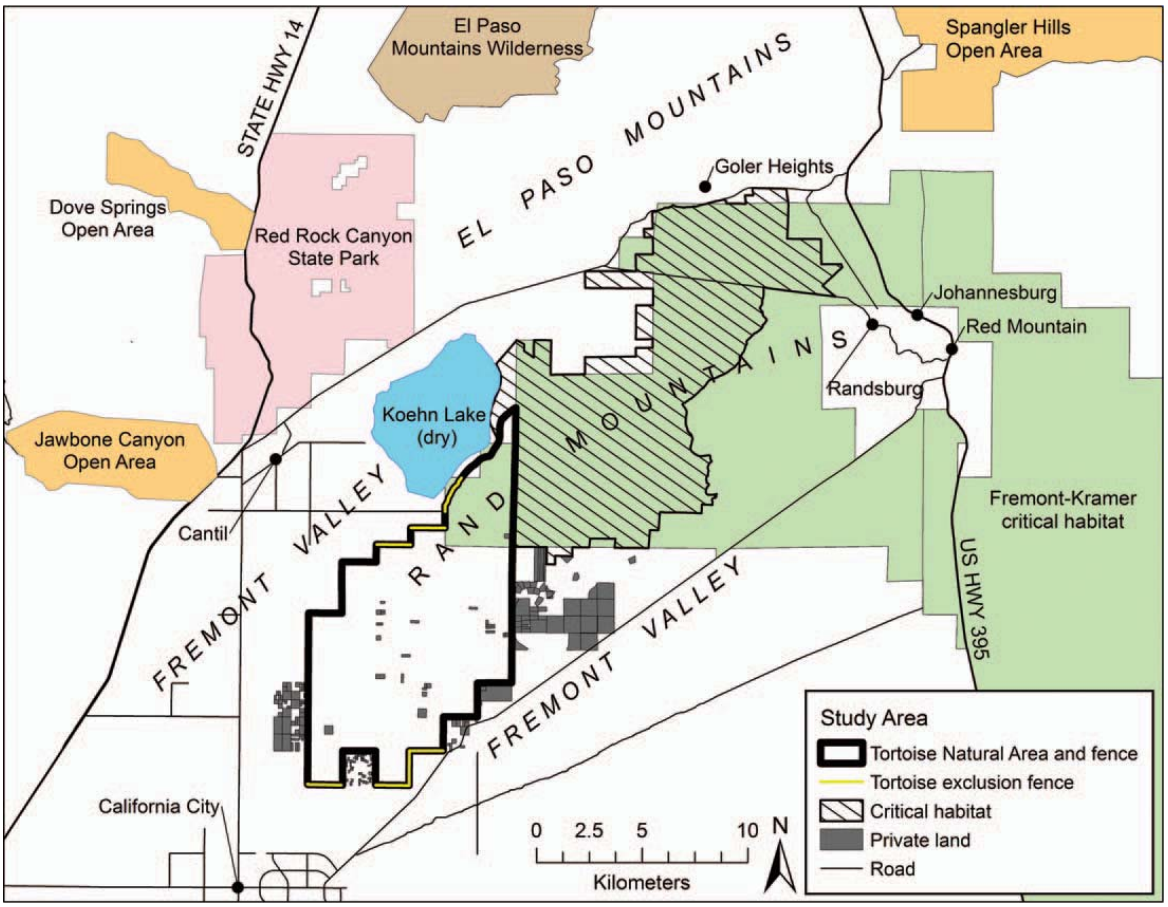

FIG. 1.—General location of the study area and the three interconnected management areas in the western Mojave Desert, eastern Kern County, California. In proximity to the study area are three areas designated for unrestricted recreational vehicle use (orange), a state park (pink), and wilderness (brown). Critical habitat in the western Mojave Desert is shown as green.

Joshua trees (*Yucca brevifolia*) are occasional on the slopes and crest of the Rand Mountains. Plant nomenclature follows Baldwin et al. (2012).

The climate is typical of the western Mojave Desert. The nearby Tehachapi Mountains to the west act as a rain shadow, influencing the amount of precipitation, frequency and velocity of winds, and temperatures. One long-term weather station (Randsburg station) has relevant precipitation data: average annual rainfall is estimated to be 174.24 mm (National Oceanic and Atmospheric Administration [NOAA], 2010–2011). More than 80% of precipitation occurs in fall and winter, between October and March. Overall, the study area is situated sufficiently close to the Tehachapi Mountains to receive more rain than many desert areas at similar elevations farther inland.

Management Areas, Land Uses, and Protections

The three management areas have had different histories of human uses for agriculture, mining, grazing, human settlements, and recreation during the last 40 yr, based in part on land ownership (Table 2; Fig. 2). In the late 1960s and early 1970s, most land within the study area was public and managed by the Bureau of Land Management; the rest was private. The Bureau of Land Management administers public lands for sheep grazing, mining, rights-of-way for transportation and utility corridors, leases, and sales; sheep grazing and mining were predominant uses

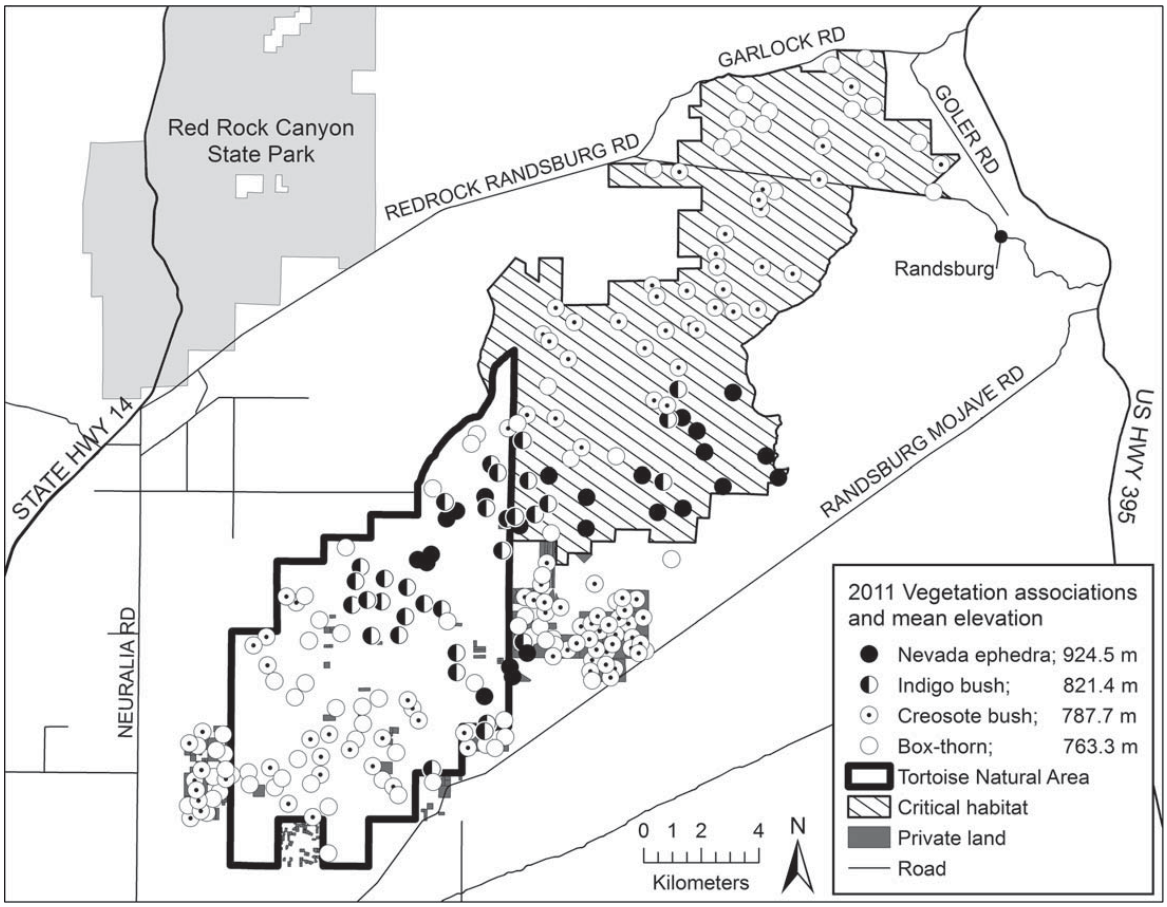

FIG. 2.—The locations of the 240 sampling points and the distribution of the four perennial vegetation associations determined by *k* clustering of means within the study area in the western Mojave Desert, eastern Kern County, California.

of public lands in the area. In the mid-1960s, off-highway vehicle-oriented recreation became a driving force and by the early 1970s, it was a major use in the region (USBLM, 1973). Private lands, mostly unfenced, undeveloped, and with absentee owners, were often in 5- to 10-acre parcels. Sheep also grazed on these properties under the open range provisions of the Kern County Estray Ordinance (established in 1942). Mining exploration occurred, as did uncontrolled, unauthorized off-road vehicle use.

In 1973, the Bureau of Land Management designated specific management areas on public lands as open or closed to recreational vehicle use under a California desert-wide recreation plan for off-highway vehicles (USBLM, 1973). The land that was to officially become the Tortoise Natural Area was closed to recreational vehicles and signs were erected along the boundaries. The land that was later to become critical habitat was designated as open to recreation vehicle use throughout, with no requirement to stay on trails or designated routes (Table 2).

Seven years later, in 1980, two of the three management areas received another, significant change toward protection, with publication of the Bureau of Land Management's *California Desert Conservation Area Plan, 1980* (USBLM, 1980; Table 2). The Tortoise Natural Area was designated as an Area of Critical Environmental Concern and Research Natural Area, and the US Congress formally withdrew these federal lands from the mining laws and livestock grazing. In the same year the Bureau of Land Management finished fencing the boundaries with hog-wire fencing (raised ~25 cm off the ground to permit movements of wild animals) and

signed the area as closed to livestock grazing and recreational vehicles. Thus, the first Research Natural Area was created in the California deserts. Also as part of the same management plan, the land later to become critical habitat received two separate designations: the West Rand Area of Critical Environmental Concern and the Rand Mountain–Fremont Valley Recreation Area (USBLM, 1980). Under the 1980 plan, vehicles in both the West Rand Area of Critical Environmental Concern and Rand Mountains–Fremont Valley Recreation Area were restricted to existing routes.

Between 1980 and 2011, additional major changes occurred at the Tortoise Natural Area and on private lands. The Bureau of Land Management and California Department of Fish and Game, in conjunction with the Desert Tortoise Preserve Committee, Inc., a nonprofit corporation, developed a management plan for the Tortoise Natural Area (Bureau of Land Management and California Department of Fish and Game, 1988; Table 2). All three entities purchased small and large private inholdings within the Tortoise Natural Area boundaries for conservation purposes and for providing a wider connecting corridor to the adjacent critical habitat. From the 1990s to 2011, both the Desert Tortoise Preserve Committee and California Department of Fish and Game also began acquisition of private lands adjacent and to the west and east of the Tortoise Natural Area with the purpose of expanding the Tortoise Natural Area. At the time of our study in 2011, these private lands, recently acquired for conservation purposes, were in small and large blocks, had not been fenced, and were unprotected from sheep grazing, recreational vehicle use, shooting, and dumping of trash.

Between 1980 and 2011, the West Rand Area of Critical Concern and Rand Mountains–Fremont Valley Recreation Area experienced three significant protective actions (Table 2). When the Desert Tortoise was federally listed as threatened in 1990 (USFWS, 1990), the Bureau of Land Management closed the lands to livestock grazing to better protect the tortoise. In 1994, critical habitat was formally designated for the Desert Tortoise, and the West Rand Area of Critical Environmental Concern and Rand Mountains–Fremont Valley Recreation Area became part of critical habitat (USFWS, 1994b). However, during these three decades, unauthorized vehicle travel off existing routes was a significant and continuing issue (e.g., Goodlett and Goodlett, 1992; USBLM, 2002) and became the subject of legal action (US District Court, 2009, 2011). To protect the habitat, the Bureau of Land Management fenced parts of the West Rand Area of Critical Environmental Concern and closed the fenced portion to off-highway vehicle use from 2002 to 2008, and again in 2009 because of unauthorized use (details in Table 2).

Methods

Collection of Data on Vegetation, Desert Tortoises, Predators, and Human Impacts

We confined surveys within the study area to public land and to private lands that we had permission to access (lands held by the Desert Tortoise Preserve Committee, Inc.). We assumed, on the basis of 40 yr of experience within the region, that these private lands were representative of much larger areas to the east and west of the Tortoise Natural Area both within and outside our study area boundaries (Fig. 1). To randomly select the hectare plots for the study, we acquired geographic information system layers of land ownership from the Bureau of Land Management (USBLM, 2012) and Kern County (Kern County Engineering, Surveying and Permit Services Department, 2011). We established 80 1-ha plots using random sampling in each of the three types of managed lands for a total of 240 plots across the study area (Fig. 2). The 240 plots constituted a 0.92% sample of the study area. The 80 plots in the West Rand Area of Critical Environmental Concern were within or on the edge of critical habitat.

Field teams surveyed the plots in spring of 2011 (3 April–25 May) using methods similar to those described in Keith et al. (2008). Because detection of live and dead tortoises and other sign is imperfect, we designed field surveys to maximize detection of live tortoises, tortoise sign, predator sign, and anthropogenic impacts. We scheduled surveys to coincide with high aboveground activity levels for all sizes of tortoises, as well as for counting sign

TABLE 2.—Time line for management actions for the three management areas in the Fremont Valley and Rand Mountains, eastern Kern County, California.

Management area	Years	Land use and management action	References
All management areas	1950s–1970	Mix of public land managed by the Bureau of Land Management and private lands with absentee landowners; checkerboard of public and private land in southern part of study area.	Bureau of Land Management files, land-ownership maps; master title plats; Vredenburg et al. (1981),
		Sheep grazing: Cantil Common allotment with 15 operators and ~20,000–30,000 sheep. Mining exploration (bulldozed roads, exploration pits).	Starry (1974); USBLM (1993)
	1960–1970	Growth of off-road vehicle recreation.	
Tortoise Natural Area	1973	Early boundaries of what was to become the Desert Tortoise Research Natural Area were established in a management plan; area posted with signs as closed to recreational vehicle use.	USBLM (1973)
	1974	Desert Tortoise Preserve Committee, Inc., formed to help establish a Tortoise Natural Area.	
	1979–1980	Desert Tortoise Preserve Committee begins land acquisition.	
	1980	US Congress withdraws Tortoise Natural Area from mining; Bureau of Land Management formally designated the Tortoise Natural Area as both a Research Natural Area and Area of Critical Environmental Concern; sheep grazing terminated; fencing of the Tortoise Natural Area completed, excluding recreational vehicles and sheep; interpretive kiosk constructed.	USBLM (1980)
	1988	Management plan for the Tortoise Natural Area published.	USBLM and California Department of Fish and Game (1988)
	1990s–2011	Ongoing land acquisition of inholdings by Bureau of Land Management, Desert Tortoise Preserve Committee, and California Department of Fish and Game, first inside Tortoise Natural Area boundaries then on adjacent private lands.	
	2006	Bureau of Land Management publishes West Mojave Plan; affirms expansion of Tortoise Natural Area through acquisition of private lands to west and east.	USBLM (2006)
	2010	Fence extended to ground in several areas (11.8 linear km) to prevent tortoises from leaving the Tortoise Natural Area and being hit by vehicles.	
Critical habitat	1973	Public lands in the Fremont Valley and Rand Mountains, not including Tortoise Natural Area, open to unrestricted recreational vehicle use (off-road vehicle racing, mass events). Between 1973 and 1987, from 3 to 34 competitive events occurred annually with numbers of participants (not including observers) ranging from 994 to 10,845. Other land uses continue (e.g., sheep grazing, mining).	USBLM (1973, 1993)
	1980	Bureau of Land Management modified the 1973 decision and designated the West Rand Area of Critical Environmental Concern (72.3 km^2) and the Rand Mountain–Fremont Valley Recreation Area. Recreational vehicle use limited to existing roads and routes but not enforced. Other land uses continued (e.g., sheep grazing, mining).	USBLM (1980)
	1980–2011	Unauthorized vehicle activities off existing routes continue.	USBLM (2002, 2006)
	1990	USFWS formally lists the Desert Tortoise as a threatened species; Bureau of Land Management closes potential critical habitat in the West Mojave to sheep grazing.	USFWS (1990)

TABLE 2.—Continued.

Management area	Years	Land use and management action	References
	1993	Bureau of Land Management publishes Rand Mountains–Fremont Valley Plan, closing some off-highway vehicle routes, thereby reducing route density from 5.1 km/km^2 to 0.86 km/km^2 in the West Rand Area of Critical Environmental Concern and on lands scheduled to be designated as critical habitat in 1994. The boundaries of the West Rand Area of Critical Environmental Concern are proposed for expansion to the north and northeast by 53.1 km^2.	USBLM (1993)
	1994	Parts of the Fremont Valley and Rand Mountains were designated as critical habitat. Most of the West Rand Area of Critical Environmental Concern is in critical habitat.	USFWS (1994b)
	1995, 2002, 2005	Southern boundary of West Rand Area of Critical Environmental Concern fenced in 1995 to curtail unauthorized off-route use by recreational vehicles; northern and eastern boundaries fenced in 2002; two internal routes fenced in 2005 to protect tortoise habitat from off-highway vehicle use	
	2006	West Rand Area of Critical Environmental Concern is expanded north and northeast to include 53.1 km^2.	USBLM (2006)
	2002–2008, 2009–2011	Original West Rand Area of Critical Environmental Concern boundaries closed to off-highway vehicle travel to protect the tortoise and manage noncompliance of off-highway vehicle use; reopened temporarily to off-highway vehicle use between 2008 and 2009, then closed again.	USBLM (2002)
	2009	Center for Biological Diversity and other plaintiffs file suit in US District Court seeking protection from unauthorized off-highway vehicle use and reduction in route density.	US District Court (2009, 2011)
Private lands	1970–2011	Private land in a few large and many small parcels, not posted; absentee landowners, often unaware of location of parcels.	
		Private lands are open to sheep grazing under Estray Ordinance, unless fenced. Grazing often intensive, unregulated.	County of Kern, California (1942)
		Off-highway recreation intensive, unauthorized, without regard to land ownership.	
	1995–2011	Desert Tortoise Preserve Committee and California Department of Fish and Game acquire lands to east and west of the Tortoise Natural Area to add to the Tortoise Natural Area. In 2011 these private lands remain unfenced.	
	2011	Desert Tortoise Preserve Committee begins installation of protective signage on recently acquired private lands abutting the eastern boundary of the Tortoise Natural Area.	

(Zimmerman et al., 1994; Lance and Rostal, 2002). We selected a field team with demonstrated expertise in finding all sizes of live tortoises, shell-skeletal remains (including fragments), and other tortoise sign. Field team members also were experienced in finding predator sign and counting anthropogenic impacts. Each plot was surveyed twice and on the same day, with rare exceptions, by walking 10-m-wide transects: once in a N–S direction and then in an E–W direction to collect data on perennial vegetation, live tortoises, tortoise sign, shell-skeletal remains, predators, and human-related impacts. If sign was difficult to see with rough terrain or dense vegetation, the transect width was narrowed.

Vegetation.—Because tortoise distribution and abundance may differ among vegetation associations, we prepared a list of all perennial plant species likely to occur within the study area (shrubs, bunch grasses, cacti). For each plot, field-workers recorded data on these species by relative abundance: (0) absent from the plot, (1) one or two individuals, (2) rare, (3) sparse, (4) common, or (5) dominant or ubiquitous (for definitions, see Glossary in Baldwin et al., 2012). The surveyor finalized ratings for each plant species after covering the plot twice.

Live tortoises.—We processed live tortoises encountered on and off plots using protocols described by Berry and Christopher (2001). We recorded tortoise location, activity, and sex, and took measurements on carapace length at the midline, plastron length from notch to notch, and weight. We collected data on clinical signs of infectious disease, shell disease, and trauma. Observations for potential infectious diseases included general condition and behavior (e.g., active, listless, unresponsive); appearance of the nares (e.g., presence or absence of a nasal discharge; amount, color, and opacity of discharge; occlusion of nares); presence of a discharge from the chin glands during the nonbreeding seasons in adult males and at any time of year in adult females and juveniles; appearance of eyes (e.g., sunken, wet, or crusted); presence of caked dirt in, on, or near the beak or on the forelegs; and ulcers, plaques, or other lesions in the oral cavity. Of particular interest were signs of infectious disease, e.g., upper respiratory tract disease caused by mycoplasmosis (Jacobson et al., 1991; Brown et al., 1994, 2004; Johnson et al., 2006), lesions in the mouth typical of herpesvirus (Johnson et al., 2005; Jacobson, 2007), a shell disease described as cutaneous dyskeratosis (Jacobson et al., 1994; Homer et al., 1998), and trauma from dog attacks (Boyer and Boyer, 2006). We ranked the distribution, severity, and chronicity of clinical signs as none, mild, moderate, or severe on forms similar to those published in Berry and Christopher (2001).

We took digital photographs of the carapace, plastron, posterior left costal, right and left eyes and periocular areas, and a frontal view of the nares and beak. If the tortoise had ectoparasites, or unusual anomalies or injuries (e.g., missing limb), we took additional images.

Shell-skeletal remains.—Field-workers collected shell-skeletal remains encountered on and off plots. Before collecting the remains, they noted condition of the remains and signs of human activities or predators that may have caused or contributed to the death (e.g., trail or tracks of vehicles, human footprints, or predator scat). They took photographs of the remains in situ to document the setting where death may have occurred. In addition, they took at least three images of each shell-skeletal remains before handling: (1) a general picture showing the remains within the context of soils, vegetation, and land uses (if any); (2) a close-up image of the remains; and (3) a close-up image of the oldest and most deteriorated portion of the scutes and bones. Then they placed remains in a heavy-duty ziplock plastic bag for transfer to the US Geological Survey Field Station for further analysis.

Tortoise sign.—We primarily collected data on two types of tortoise sign: cover sites and scat. We measured and assigned shelters or cover sites (defined as burrows, caves, pallets, and rock shelters used by tortoises after Burge, 1978) to one of five classes on the basis of recency of use and condition (Berry et al., 2008; Keith et al., 2008). Similarly, we measured, aged, and recorded the number and sizes of tortoise scats using three age classes of recency and states of deterioration (Berry et al., 2008; Keith et al., 2008). Additionally, we identified and estimated recency of tortoise tracks by looking for

impressions of scales and marks from toenails and tails. For courtship rings, we looked for depressions with freshly churned soil, tortoise tracks, drag marks from plastrons, and small areas of dried, clotted soil. At drinking sites, we looked for impressions of toenails in dried mud and scats.

Predators.—To evaluate geospatial relationships between predators and live and dead tortoises and other tortoise sign, the field team searched for perches, nests, roosts, burrows, dens, and scats of avian and mammalian predators. They recorded numbers and locations of these sites and also examined them for fragments of tortoise skin, scutes, and bones.

Human impacts.—To document historical and ongoing land uses, field-workers collected data on types and amounts of human disturbance.

Data Analysis

Vegetation.—We categorized plots by vegetation association by performing *k*-means clustering analysis on perennial plant data (Version 6.0; StatSoft, Inc., 2001) using the six ordinal categories of abundance. We specified $k = 4$ clusters for the analysis, and then verified that the four associations of perennial species were of biological significance by evaluating composition, relative abundance, and diversity within each of the vegetation associations and comparing each cluster to the Hierarchical List of Natural Communities with Holland Types (California Department of Fish and Game, 2010) to assign a vegetation community name. We used ANOVA to compare elevations among different vegetation associations and Tukey honestly significant difference (Tukey HSD) tests to conduct pair-wise comparisons. We considered those species with mean relative abundance values greater than the midpoint between the minimum and maximum relative abundance values (2.35) as "abundant species." We also used exact binomial tests to perform pair-wise comparisons among the numbers of plots associated with each vegetation association to identify differences both within and among management areas (binom.test function; R Development Core Team, 2013).

Underlying all statistical tests was the assumption that our plots were independently random in their representation of each management area. This assumption was met by the random process in which the plots were selected; however, we systematically excluded certain private lands not held by the Desert Tortoise Preserve Committee because of privacy considerations. We have no reason to expect that our sample plots differed substantially from the excluded private lands. The ANOVA tests further assume that data are normally distributed and homoscedastic. We did not need to check these assumptions, because our models are highly robust to nonnormal distributions due to the well-established asymptotic properties of the model test statistics (Arnold, 1981). For example, Jacqmin-Gadda et al. (2007) used sample sizes of 50 and 200 simulated nonnormal data to demonstrate that general linear mixed models, as a generalization of ANOVA models, had accuracy rates similar to those when performed on correctly specified normal data. We considered $P < 0.05$ as statistically significant for the ANOVA, Tukey HSD, and binomial exact tests.

Live and dead tortoises.—We assigned a size–age class to live tortoises by carapace length at the midline: juvenile $\leq$ 99 mm, immature = 100–179 mm, small adult/subadult = 180–207 mm, and adult $\geq$ 208 mm. To assess whether sex ratios of subadult and adult tortoises differed significantly from the expected 1:1 ratio, we used exact binomial tests to compare numbers of females and males. We used a 1000-simulation bootstrap to estimate densities (tortoises/km^2) and 95% confidence intervals of live subadult and adult tortoises and all sizes of tortoises for each of the three management areas, as well as for the entire study area (Barreto and Howland, 2006). To avoid cumbersome reporting, we presented the estimated densities/km^2 followed by the 95% interval in parentheses. We also estimated relative age of adults using age classes developed by Berry and Woodman (1984). For shell-skeletal remains, we measured or estimated carapace length, estimated time since death (dead $\leq$ 4 yr, $>$ 4 yr), and assigned causes of death (see details of methods in Berry et al., 2013). We calculated crude annual death rates for each management area, specifically for adult tortoises

found dead on plots and with an estimated time since death of ≤ 4 yr; we used the equation $d = D/N$, where D is the number of dead adults/4 yr, and where N is the number of live and dead adult tortoises on plots.

Comparisons of predator activity and anthropogenic impacts among management areas.—We compared counts of Common Ravens (Ravens) and mammalian predator scat (Mammals) found on plots. We similarly compared surrogate variables for anthropogenic impacts, measured specifically as counts of sheep scat (Sheep); vehicle tracks (Vehicles); trash (Trash); shooting debris, including casings, shells, and targets (Firearms); and mines (Mines). We calculated amount of surface disturbance (m^2) for partially and completely denuded areas, dirt roads, and vehicle routes, trails, and tracks. For variables with abundant counts (> 10 total per management area), we used generalized linear models (McCullagh and Nelder, 1989) with a log link function to analyze differences among management areas (glm function; R Development Core Team, 2013). We used quasi-Poisson distributions in our models when overdispersion was evident (i.e., variance > mean μ). Thus, we corrected the SEs by modeling variance as $\mu \times \phi$ (mean × overdispersion parameter; Zuur et al., 2009). For variables with low counts, we conducted exact binomial tests between pairs of areas to determine significant differences (binom.test function; R Development Core Team, 2013). We considered variables with $P < 0.10$ as statistically significant for the models and used 90% confidence intervals.

Models of tortoise presence across the study area.—We used generalized linear models with a logit link for binary outcomes of tortoise presence to examine the relationship between tortoise presence and the covariates for the three management areas combined and for each management area separately (R Development Core Team, 2013). Tortoise presence was indicated by the occurrence of live tortoises, shell-skeletal remains, and tortoise sign on each plot. Tortoise sign can be used as a surrogate for live tortoises, because a positive correlation has been demonstrated between tortoise sign and tortoise densities (Krzysik, 2002). Hereafter live tortoises, shell-skeletal remains, and tortoise sign collectively are called tortoise sign. We treated tortoise sign as a binary response variable where 0 = no live or dead tortoises or sign detected on the plot, and 1 = live, dead, or sign detected on the plot.

We evaluated all variables for indications of outliers that had >10% influence on correlations with tortoise sign using the Pearson r influence feature in Systat (Systat Software, Inc., 2007). We removed one plot on the Tortoise Natural Area that had large trash counts, apparently as a result of an old habitation or mining operation. All explanatory variables except Mines were log-transformed ($\log_{10}$) to normalize their distributions and reduce the influence of unusually large values. We conducted correlation analyses and calculated generalized variance inflation factors to assess multicollinearity (vif function; Fox and Weisberg, 2011). We omitted surface disturbance from models because of high correlations with other predictors ($r > 0.8$). Because multicollinearity can obscure the effects of predictors when used in combination, we used two complementing strategies to model Desert Tortoise presence. Our first approach was to model the relationship between Desert Tortoise presence with each individual predictor alone in a separate model, but including management area and vegetation association effects in every model to control for differences in plot types. Our second approach was to select a set of variables that best predict Desert Tortoise occurrence; we performed backward removal model selection by starting with a full model with all predictors, including interaction effects with management area, and sequentially dropping variables that were not significant according to chi-square tests at the 0.1 significance level (drop1 function; R Development Core Team, 2013). We arrived at a final model when no further variables could be dropped, and estimated the probability and odds of Desert Tortoise presence with respect to significant effects (lsmeans function; Lenth, 2013). For continuous predictors with model coefficients β, we estimated the percent change in the odds of Desert Tortoise presence as $(\exp[\beta] - 1) \times 100\%$.

Models of tortoise abundance for each management area.—Using a separate gener-

TABLE 3.—Vegetation associations found in the three management areas, eastern Kern County, California, in descending order by total plots assigned. Number of species includes perennial species (shrubs, herbaceous perennials, and bunch grasses). Bold values for creosote bush indicate that significantly more plots were assigned to this association than to other vegetation associations in the respective management areas. Elevation values sharing a superscript capital letter were not statistically different at $P < 0.05$.

Vegetation association name	Total plots assigned				No. of species	No. and % abundant species	Elevation range (m)	Elevation mean (m) ± SE
	Tortoise Natural Area	Critical habitat	Private lands	Total plots assigned				
Creosote bush	20	**37**	**49**	106	26	2 (7.7%)	590–960	788 ± 10.6^{BC}
Box-thorn	31	21	26	78	36	5 (13.9%)	613–1027	763 ± 10.6^{C}
Indigo bush	22	8	2	32	31	9 (29.0%)	682–1002	821 ± 13.3^{B}
Nevada ephedra	7	14	3	24	30	11 (36.7%)	753–1210	924 ± 26.2^{A}

alized linear model for each of the three management areas, based on a Poisson (or quasi-Poisson, if overdispersion was evident) distribution and log link function, we modeled tortoise sign counts to examine tortoise abundance in relationship to vegetation associations and anthropogenic and predator variables. As with our presence models, we performed backward removal starting with full models that included the same variables: Vegetation, Sheep (private lands only), Vehicles, Trash, Firearms, Mines (Tortoise Natural Area and critical habitat only), Ravens, and Mammals. Only one sheep scat was found in the Tortoise Natural Area and no sheep scat was found in critical habitat. No mines were found in the private lands. We dropped variables using F and chi-square tests, for quasi-Poisson and Poisson models respectively, until only variables significant at the 0.1 level remained. For continuous predictors with model coefficients β, we used β to calculate the percent change in mean tortoise sign.

RESULTS

Precipitation

Precipitation during the hydrologic year (1 October 2010–30 September 2011) was >218 mm for the fall–winter of 2010–2011 (1 October–30 March), and was above normal. The 2011 records from the National Climate Data Center were incomplete with missing values, and therefore precipitation could have been higher. Rainfall was recorded in every month between 1 October and 30 March. Wildflowers and therefore tortoise forage plants were abundant.

Perennial Vegetation

We identified 39 species of perennial shrubs and four species of perennial grasses on the plots. Creosote bushes and white bur-sage were common to dominant within all four associations. We assigned each plot to one of four vegetation associations (Table 3; Fig. 2): (1) creosote bush/white bur-sage (hereafter creosote bush) had only two abundant species among the 26 species found on these plots; (2) creosote bush/white bur-sage/Anderson box-thorn (hereafter box-thorn) had a total of five abundant species, including cheesebush and goldenhead; (3) creosote bush/white bur-sage/Mojave indigo bush (hereafter indigo bush) had nine dominant species (Anderson box-thorn, cheesebush, goldenhead, Nevada ephedra, Mojave California buckwheat, and desert trumpet, *Eriogonum inflatum*); and (4) creosote-bush/white bur-sage/Nevada ephedra (hereafter Nevada ephedra) was the most diverse, with 11 dominant species (goldenhead, Mojave indigo bush, Mojave California buckwheat, Anderson box-thorn, winter fat, hop-sage, and Mojave aster [*Xylorhiza tortifolia*]). The vegetation associations differed significantly in elevations ($F_{3,236} = 16.158$, $P = 0.001$), with Nevada ephedra occurring at higher elevations than the other three vegetation associations, and indigo bush occurring at a higher elevation than box-thorn (Tukey HSD, $P = 0.034$; Table 3). More plots in critical habitat and on private lands were assigned to the creosote bush association, the least diverse association with only two abundant species, than to the more diverse vegetation associations with more abundant species (exact binomial $P < 0.05$; Table 3).

TABLE 4.—Size–age classes of live Desert Tortoises and shell-skeletal remains occurring on and off plots on the Desert Tortoise Research Natural Area, critical habitat, and private lands in the study area in the western Mojave Desert, eastern Kern County, California.

Plot location	Tortoise Natural Area, on plot (off plot)			Critical habitat, on plot (off plot)			Private lands, on plot (off plot)		
		Dead			Dead			Dead	
Size–age class	Live	≤4 yr	>4 yr	Live	≤4 yr	>4 yr	Live	≤4 yr	>4 yr
Juvenile	2	2 (1)		(3)	1				
Immature	2 (1)	2		(1)	2 (1)			(1)	
Subadult	1		8 (1)	1 (1)	1	3 (2)			
Adult	7 (10)	1 (1)	9 (11)	1 (7)	8 (3)	8 (5)	3 (4)	1	1 (1)
Totals	12 (11)	5 (2)	17 (12)	2 (12)	12 (4)	11 (7)	3 (4)	1 (1)	1 (1)

Live Tortoises

The field team located 17 live tortoises on the 240 plots: 12 in the Tortoise Natural Area, 2 in critical habitat, and 3 on private lands. They observed an additional 27 tortoises off plots, when walking from one plot to another or to and from vehicles (Tortoise Natural Area, 11; critical habitat, 12; private lands, 4). In critical habitat, they observed a tortoise on a vehicle route, a juvenile on a motorcycle trail, and a third tortoise on a road.

The density and confidence intervals for adult tortoises on all 240 plots combined for the entire study area were 5.5 (5.4–5.6)/km^2. When we analyzed densities of adult tortoises separately for the three types of managed lands, the results differed significantly from one another by management area: for the Tortoise Natural Area, 10.2 (9.9–10.4) tortoises/km^2; for critical habitat, 2.4 (2.3–2.6) tortoises/km^2; and for private lands, 3.7 (3.6–3.8) tortoises/km^2. When we estimated densities and confidence intervals for all sizes of tortoises separately for the three types of managed lands, the results were 14.8 (14.6–15.1)/km^2 for the Tortoise Natural Area, 2.4 (2.3–2.6)/km^2 for critical habitat, and 3.7 (3.6–3.8)/km^2 for private lands.

Adults composed the majority of the samples for both on- and off-plot tortoises. For the 17 on-plot tortoises, the composition of the tortoises by size–age class was 11 adults ≥208 mm carapace length, 2 small adults, 2 large immature tortoises, and 2 juveniles (Table 4). The sex ratio of female-to-male subadult and adult tortoises was 9:4 and not statistically different from 1:1 ($P = 0.27$). Juvenile and immature tortoises were observed only on the Tortoise Natural Area plots. For the 27 off-plot tortoises, the size–age class composition was similar, with 21 adults, 1 small adult, 2 immature tortoises, and 3 juveniles, and the female-to-male sex ratio was 10:12, which was not statistically different from 1:1 ($P = 0.83$). Overall, for both on- and off-plot tortoises, the female-to-male sex ratio was 19:16 and also not significantly different from 1:1 ($P = 0.74$). Of the 34 adult tortoises that could be assigned an age class, 38% were young and growing, 24% were of middle age, and 38% were in the old-age classes.

Of the 44 tortoises observed during the surveys, 34 received comprehensive health evaluations. We could not handle the remaining tortoises because of federal permit constraints, but instead recorded some data for each individual on the basis of field observations. Two adult male tortoises, one on private lands and one in the Tortoise Natural Area management area, had moderate clinical signs of upper respiratory tract disease characteristic of mycoplasmosis (damp or wet beak from exudate or bubbles from the nares). Both tortoises also had other clinical signs, such as ocular discharge, crusts on the palpebrae and periocular area, mucus on the globe, and exposed conjunctiva. The nares of 19 other tortoises were partially or completely occluded, potentially from dried exudate or from plant sap or dirt and mud from drinking during rainstorms.

Without exception, signs of predator attacks were evident on all 25 adult tortoises that we handled. Twelve of the 25 had moderate to severe damage to the gular horn; for nine tortoises the gular horn was severely reduced or chewed away completely. Some signs of trauma (extensive chewing) appeared typical

of domestic dog attacks. One adult tortoise had a healed injury from crushing, potentially by a vehicle. Two juvenile tortoises had ant heads attached to soft parts of the integument.

Shell-Skeletal Remains and Death Rates

We found shell-skeletal remains of 47 tortoises on plots, and observed 27 off plots (Table 4). We collected more remains from the Tortoise Natural Area and critical habitat plots than from private land plots. Eighteen of the 47 (38.3%) on-plot remains were from tortoises dead ≤ 4 yr: 11 adults, 4 immatures, and 3 juveniles. We assigned the 11 on-plot adult tortoises to two general categories: (1) unknown and found in desert woodrat (*Neotoma lepida*) middens (n = 5), and (2) traumatic deaths (n = 6). We separated traumatic deaths (fractured scutes, bones) by cause, where possible: three showed signs of mammalian predation (one, domestic dog), one had both pellet holes (gunshot death) and punctures, one was probably a vehicle kill, and one was killed by a predator at a site with a Common Raven perch and mammalian predator scats and prey remains. Three of the seven recent juvenile and immature remains showed signs of having been killed by Common Ravens or small mammals; we could not determine the causes of death for the other four. Causes of death for off-plot tortoises were similar and included predation by canids and Common Ravens, gunshot and vehicle kills, and unknown. Remains with evidence of gunshots occurred both within the Tortoise Natural Area and critical habitat; remains of tortoises likely to have been killed by vehicles were in the Tortoise Natural Area and on private lands.

Crude annual death rates for adults for the 4 yr preceding the survey differed by management area. The lowest rate was in the Tortoise Natural Area, with 2.8%/yr, followed in ascending order by private lands with 6.3%/yr and critical habitat with 20.4%/yr.

Tortoise Sign on Plots

We observed sign on 55.0% (44/80) of the Tortoise Natural Area plots, 47.5% (38/80) of critical habitat plots, and 12.5% (10/80) of private land plots (Fig. 3). Sign counts were highest (n = 190) on the Tortoise Natural Area plots, followed in descending order by critical habitat plots (n = 90) and private land plots (n = 20). Scats (n = 159) were the most common type of sign observed, followed by burrows and pallets (n = 77) and shell-skeletal remains (n = 47). The Tortoise Natural Area had ~2 × the total sign count of critical habitat and ~9 × the count of private lands. Shell-skeletal remains accounted for 25.6% of on-plot sign in critical habitat, compared with 11.6% at the Tortoise Natural Area and 10.0% on private lands.

Predators

We observed nine species of potential avian predators both on and off plots: the Common Raven (n = 193), Loggerhead Shrike (*Lanius ludovicianus*, n = 5), Red-tailed Hawk (*Buteo jamaicensis*, n = 4), Golden Eagle (*Aquila chrysaetos*, n = 4), Burrowing Owl (*Athene cunicularia*, n = 2), Greater Roadrunner (*Geococcyx californianus*, n = 1), Northern Harrier (*Circus cyaneus*, n = 1), American Kestrel (*Falco sparverius*, n = 1), and Prairie Falcon (*Falco mexicanus*, n = 1). Common Ravens composed 91.5% of the observations. We saw more avian predators on the Tortoise Natural Area (75 on plots, 13 off plots) compared with critical habitat (12 on plots, 20 off plots) and private lands (32 on plots, 41 off plots). We found remains of a juvenile tortoise below the perch of a Common Raven off plot at the Tortoise Natural Area. Sightings of Common Ravens were more common on plots in the Tortoise Natural Area and private lands than in critical habitat (Table 5; P = 0.002 and P = 0.018, respectively).

We detected three species of mammalian predators by finding concentrated areas of marking sites, dens, and den complexes on plots: kit fox (*Vulpes macrotis*, n = 22), coyote (*Canis latrans*, n = 15), and badger (*Taxidea taxus*, n = 1); we assigned seven additional observations to canid sign, because it was unclear if the scat was from a coyote or kit fox. Signs of concentrated activity by mammalian predators (i.e., dens, marking sites) also were more common on plots within the Tortoise Natural Area (n = 25) than on plots within critical habitat (n = 11) or private lands (n = 9). By far the most common sign was scat or groups of scat deposited by coyotes and kit foxes (Table 5). We found no remains of

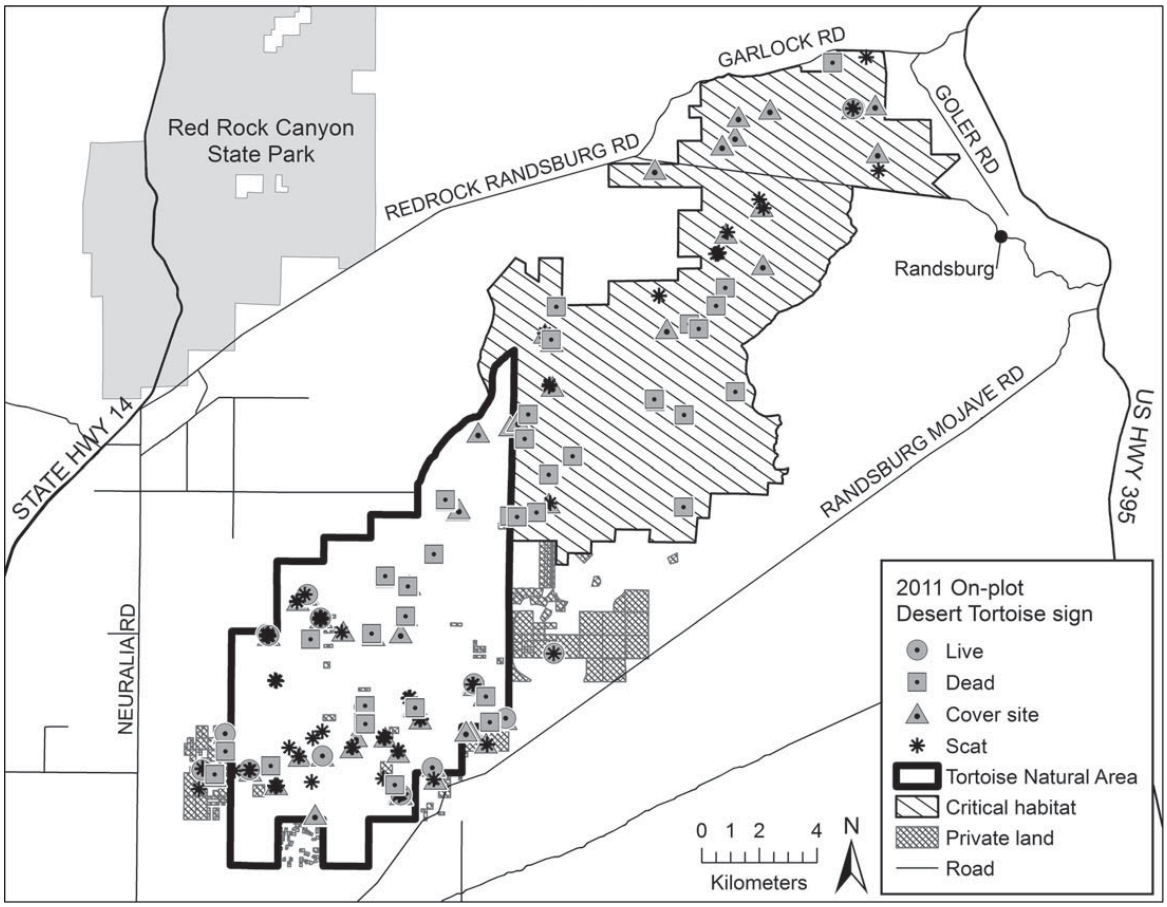

FIG. 3.—Locations of Desert Tortoise sign on the 240 1-ha plots, including live tortoises, remains of dead tortoises, cover sites (burrows and pallets), and scat in three management areas in the western Mojave Desert, eastern Kern County, California.

tortoises in 1222 predator scats or at 26 of the predator dens. We observed one kit fox on a plot and one coyote off plot within the Tortoise Natural Area during the surveys. Although we noted twice as many scat from mammalian predators in the Tortoise Natural Area compared with critical habitat and private lands (Table 5), the differences in scat counts between the three management areas were not significant ($P = 0.15$).

TABLE 5.—Total counts for observations of Common Ravens and mammalian predator scats and five anthropogenic variables in the three types of management areas for Desert Tortoises in the western Mojave Desert, California. Bold font emphasizes a management area with a variable that is significantly higher than the same variable in one or both of the other management areas ($P < 0.05$).

Type of disturbance on plots	Total counts from principal sources of anthropogenic impacts (no. plots affected)		
(Variable names)	Desert Tortoise Research Natural Area	Critical habitat	Private lands
Common Ravens (Ravens)	**88** (40)	32 (24)	**73** (36)
Mammalian predator scat (Mammals)	652 (55)	239 (47)	331 (30)
Sheep scat (Sheep)	1 (1)	0 (0)	**85,208** (77)
Vehicle tracks (Vehicles)	11 (6)	180 (42)	**1407** (73)
Trash, general (Trash)	362 (49)	370 (40)	**1324** (73)
Shooting debris: casings, shells, targets (Firearms)	191 (18)	108 (18)	209 (20)
Mining pits, excavations (Mines)	**6** (4)	**6** (6)	0 (0)

Human Uses

Counts of historic and recent anthropogenic impacts and amounts of area partially or completely denuded of vegetation differed by management area (Table 5). In general, plots on private lands had higher counts and more surface area disturbed by human uses than the other two areas. Total surface area disturbed was 8507 m^2 on the Tortoise Natural Area, 7082 m^2 on critical habitat, and 32,530 m^2 on private lands. Evidence of sheep grazing was almost nonexistent on plots in the Tortoise Natural Area and critical habitat but significantly more prevalent on private land plots ($P < 0.01$). Trash was a common occurrence on plots but counts were highest on private lands ($P = 0.01$), where 91% of plots had trash. On the Tortoise Natural Area, trash was generally old and associated with old homesteads and abandoned mines (broken bottles, rusted cans, old pieces of metal, wood). In critical habitat and on private lands, visitors commonly deposited trash at camp sites, in areas with recreation use, along roadsides, and at edges of urban areas. Counts of debris from shooting (Firearms), another form of trash, were similar on all three management areas ($P = 0.67$); some were very old. Mining was limited to six sites each at the Tortoise Natural Area and critical habitat and was minimal, yet statistically greater than the absence of mining on private lands ($P = 0.03$). With few exceptions, mining consisted of small, shallow pits and bulldozed areas <400 m^2. We considered one pit, covered with boards, as hazardous to tortoises.

Vehicle tracks were least common on the Tortoise Natural Area, although not statistically different from the critical habitat ($P = 0.10$), and significantly more common on private lands ($P < 0.01$; Table 5). The amount of surface area disturbed by vehicle tracks was lowest (467 m^2) on Tortoise Natural Area plots, higher on critical habitat plots (4109 m^2), and highest on private land plots (10,074 m^2). Counts of areas denuded or partially denuded of vegetation by vehicles were lowest in the Tortoise Natural Area and critical habitat ($n = 0$) and highest on private land plots ($n = 297$); total area disturbed was 18,820 m^2 on private lands. One partially denuded area, probably nonvehicle in origin, covered 400 m^2; 12 similar partially or completed denuded areas covering 1736 m^2 occurred on private land plots. Dirt road counts, whether graded or ungraded, were similar on the three management areas; total area disturbed was highest on the Tortoise Natural Area (7640 m^2) and lower on critical habitat and private lands (2863 m^2 and 2320 m^2, respectively). The important difference between the Tortoise Natural Area and other management areas is that dirt roads within Tortoise Natural Area boundaries have received little or no use since 1980. Only landowners with inholdings inside Tortoise Natural Area boundaries can drive to their parcels (a rare event). As a result, shrubs are in the process of colonizing most of these roads.

Models

We began by evaluating relationships between tortoise presence and single predictors. Anthropogenic and predator variables were generally negatively correlated with Desert Tortoise presence, except for Mammals, which was positively correlated; however, the relationships of these single predictors with Desert Tortoises were imprecise, and Sheep was the only predictor with a significant relationship when we considered only one predictor (Table 6). Although no other single predictor had a strong relationship with the Desert Tortoise, when we included all variables together in the full model, Sheep and management area were the only two predictors with a high variance inflation factor (6.9 and 10.7, respectively), which suggests multicollinearity between these variables. After we conducted backward removal model selection, Sheep remained as a significant predictor, with a 21% decrease in odds of occurrence of tortoise sign per twofold increase in Sheep ($P = 0.06$), whereas management area showed differences only with respect to the effects of Mammals within areas (Table 7; Appendix 1). The occurrence of tortoise sign also varied by vegetation association, with box-thorn having the highest probability of occurrence (0.46), about double that of Nevada ephedra and indigo bush (Table 7).

When we modeled the three management areas separately, we found differences in mean numbers of tortoise sign, with the

TABLE 6.—Estimated coefficients from a binomial logistic model on the presence of Desert Tortoise sign (live and dead tortoises and other tortoise sign) based on seven predictor variables (five anthropogenic uses and two types of predator sign). Each coefficient was estimated in isolation by separate models of presence and absence of Desert Tortoise sign, with only management area and vegetation factors included in all models to control for long-term categorical effects. Regression coefficients (β) are presented with SE, *z*-statistic (*z*), *P*-values (*P*), the 90% confidence interval, and the percent change in the odds of Desert Tortoise sign occurrence for a given change in predictor. * = significant at $P < 0.10$.

					Percent change in odds of Desert Tortoise sign		
Predictor variable	β	SE	*z*	*P*	% change	90% interval	Predictor change
Log(Ravens)	−1.056	0.675	−1.56	0.118	−27.2	(−48 to 2)	2× increment in Ravens
Log(Mammals)	0.196	0.332	0.59	0.556	6.1	(−10 to 25)	2× increment in Mammals
Log(Sheep)	−0.726	0.397	−1.83	0.068*	−19.6	(−34 to −2)	2× increment in Sheep scat
Log(Vehicles)	−0.231	0.431	−0.54	0.592	−6.7	(−25 to 15)	2× increment in Vehicle tracks
Log(Trash)	−0.284	0.321	−0.88	0.376	−8.2	(−22 to 8)	2× increment in Trash
Log(Firearms)	−0.017	0.432	−0.04	0.968	−0.5	(−20 to 23)	2× increment in Firearms
Mines	−0.384	0.533	−0.72	0.472	−31.9	(−72 to 64)	1 additional Mine

Tortoise Natural Area having the highest sign (2.2 per plot), followed by critical habitat (0.87) and private lands (0.09–0.43, depending on vegetation association; Table 8; Appendix 1). Desert Tortoises in the Tortoise Natural Area had no positive or negative associations with predators, livestock, or other human-related impacts. In critical habitat, tortoise sign was negatively associated with Vehicles (28% decrease per twofold increase in tracks); in private lands, tortoise sign was negatively associated with Sheep (14% decrease per twofold increase in Sheep). However, numbers of tortoise sign were also positively associated with certain anthropogenic impacts, including Firearms in critical habitat (52% increase per twofold increase in counts of Firearms) and Trash on private lands (26% increase per two-fold increase in Trash), and with Mammals in both critical habitat and private lands (41% and 46% increase, respectively, per twofold increase in mammalian predator sign). In private lands, we found more tortoise sign in the box-thorn vegetation association than in creosote bush ($P = 0.003$); however, we were unable to test the Nevada

TABLE 7.—Estimated probabilities of the presence of Desert Tortoise sign (live and dead tortoises and other sign) in eastern Kern County, California, including three management areas: Tortoise Natural Area, critical habitat, and private lands. Odds are the ratio of probability of presence divided by probability of absence, and change multiplicatively with incremental changes in predictors of binomial logistic models. These estimates and their chi-square tests were calculated by the final binomial logistic model of presence and absence of Desert Tortoise sign after backward removal of nonsignificant variables ($P > 0.10$) from a full model with all predictors. Significance levels: * = $P < 0.10$, ** = $P < 0.05$. Vegetation associations sharing a superscript capital letter were not significantly different ($P > 0.05$).

				Probability and odds of Desert Tortoise sign, or % change in odds of Desert Tortoise sign per increment in predictor	
Predictor variable	df	χ^2	*P*	Estimate (90% interval)	
Vegetation association	3	7.18	0.066*	0.46 (0.35 to 0.56)[A]	Probability, box-thorn
				0.83 (0.54 to 1.30)	Odds
				0.35 (0.26 to 0.45) [AB]	Probability, creosote bush
				0.53 (0.35 to 0.81)	Odds
				0.25 (0.14 to 0.39)[B]	Probability, indigo bush
				0.33 (0.17 to 0.65)	Odds
				0.20 (0.10 to 0.36)[B]	Probability, Nevada ephedra
				0.25 (0.11 to 0.56)	Odds
Log(Sheep)	1	3.53	0.06*	−21% (−3.5 to −36)	% change, per 2× increment in Sheep
Management area	2	0.539	0.764		
Log(Mammals)	1	0.35	0.554		
Management area × log(Mammals)	2	6.11	0.047**		

TABLE 8.—Estimated mean number of Desert Tortoise sign for each management area: Tortoise Natural Area, critical habitat, and private lands in eastern Kern County, California. These estimates and their chi-square tests were calculated for each management area separately by the final Poisson model (quasi-Poisson for the Tortoise Natural Area), after backward removal of nonsignificant variables ($P > 0.10$) from a full model with all predictors. Significance levels: * = $P < 0.10$, ** = $P < 0.05$, *** = $P < 0.010$. Incr = increment; Na = not applicable.

					Mean Desert Tortoise sign, or % change per increment in predictor	
Management area	Variable	df	χ^2	P	Estimate (90% interval)	
Tortoise Natural Area	None				2.2 (1.6 to 2.9)	Mean
Critical habitat					0.87 (0.61 to 1.2)	Mean
	Log(Vehicles)	1	3.91	0.048**	−28% (−3.9 to −45)	% change, per 2× incr in vehicle tracks
	Log(Firearms)	1	5.81	0.016**	52% (17 to 97)	% change, per 2× incr in firearms
	Log(Mammals)	1	9.34	0.002***	41% (18 to 69)	% change, per 2× incr in predator sign
Private lands	Vegetation association	3	12.3	0.006***	Na	Mean, Nevada ephedra
					Na	Mean, indigo bush
					0.09 (0.04 to 0.20)	Mean, creosote bush
					0.43 (0.27 to 0.71)	Mean, boxthorn
	Log(Sheep)	1	3.56	0.059*	−14% (−3.2 to −24)	% change, per 2× incr in sheep
	Log(Trash)	1	3.65	0.056*	26% (3.6 to 53)	% change, per 2× incr in trash
	Log(Mammal)	1	5.27	0.022**	46% (15 to 85)	% change, per 2× incr in mammals

ephedra or the indigo bush vegetation associations for differences because the plots were few and no tortoise sign occurred on them (Tables 3 and 8).

DISCUSSION

The topic of protected areas—their role in conserving biodiversity, maintaining populations of rare and endangered species and habitat integrity, and overall management effectiveness—has been a common theme in conservation science during the last decade (e.g., Leverington et al., 2010; Geldmann et al., 2013; Le Saout et al., 2013). Our study explores the effectiveness of three management strategies for two protected areas, the Tortoise Natural Area and adjacent critical habitat, as well as recently acquired private lands, in delivering positive conservation outcomes for the Desert Tortoise by evaluating the tortoise population, predators and predation, and habitat condition in the context of historic land uses and management plans. Our findings represent a spectrum based on land-use histories and protective measures.

Desert Tortoise Populations

Between the late 1970s and early 1990s, densities of all sizes of tortoises declined precipitously in the western Mojave Desert (Table 1; Berry et al., 1986a, 1986b; Berry and Medica, 1995; Brown et al., 1999). At the Tortoise Natural Area and in Fremont Valley, population declines of >90% were documented. The causes were numerous (USFWS, 1994a). Vandalism, vehicle kills on and off roads, and illegal collecting occurred (e.g., Berry, 1986; Berry et al., 1986a, 1986b). Mycoplasmosis contributed to population declines in the late 1980s and early 1990s (Jacobson et al., 1991; Brown et al., 1999). Hyperpredation by the Common Raven, described ~30 yr ago by Campbell (1983), has been and continues as a source of mortality to juvenile and immature tortoises (Berry et al., 1986a; Boarman, 1993; Boarman and Berry, 1995; Kristan and Boarman, 2003). The estimate of population density, 5.5 (5.4–5.6) subadult and adult tortoises/km^2 for the entire study area during spring of 2011, is similar to and within the densities reported by the USFWS using distance sampling, a technique of estimating densities at a landscape scale for critical habitat in the western Mojave Desert (USFWS, 2010). The USFWS reported annual density estimates for adult tortoises (≥180 mm carapace length, includes subadult tortoises) from 2001 through 2007

for the entire western Mojave Desert: mean figures ranged from 3.8 to 6.1 (3.0–8.5)/km^2. In 2011, in a survey of critical habitat in the Fremont–Kramer management area, the USFWS estimated a density of 3.5 adult tortoises/km^2 (USFWS, 2012). Therefore, densities have remained disturbingly low, compared with those reported for periods 26 to 32 yr earlier using mark–recapture studies in the region, e.g., from 40 to 92 subadult and adult tortoises/km^2 (Berry et al., 1986a, 1986b; Berry and Medica, 1995).

During our study, densities of live tortoises were significantly higher inside the Tortoise Natural Area fence than outside in critical habitat (~6×) or private lands (~4×), and sign counts further corroborate the finding. In contrast, critical habitat and private lands had significantly lower densities of adult tortoises and lower tortoise sign counts by >50%. Overall, in all three management areas, the tortoise population was composed primarily of adults in a relatively even sex ratio. The field team observed live juvenile and immature tortoises on plots inside the Tortoise Natural Area and off plots in critical habitat, indicating that some individuals in these age classes were present.

Crude annual death rates for adult tortoises were lowest in the Tortoise Natural Area (2.8%/yr), followed by private lands (6.3%/yr) and critical habitat (20.4%/yr). The high death rates in critical habitat were of particular concern: shell-skeletal remains composed 25.6% of on-plot tortoise sign, whereas in both the Tortoise Natural Area and private lands, shell-skeletal remains were <12% of the total tortoise sign. When causes of death could be determined, they included vehicles, gunshot, and predation by ravens and mammals. We have estimated that subadults and adults in stable populations of this long-lived species probably had annualized death rates of adult age classes of ~2%/yr in the past (Turner et al., 1987).

Subsidized Predators

Counts of Common Ravens and Mammals were highest in the Tortoise Natural Area, followed in descending order by private lands and critical habitat. The modeled relationships between tortoise abundance and predators didn't follow this pattern, however. The models showed no significant associations between tortoise abundance and Ravens in any management area, whereas the results of modeling effects of Mammals on tortoise abundance varied by management area. The most protected area, the Tortoise Natural Area, had no significant associations, positive or negative, with predators, in contrast to critical habitat and private lands, where the association between tortoise abundance and Mammals was positive. However, more than one-third of live tortoises in the study showed signs of predator attacks by mammals (coyotes, kit fox, and dogs), and many tortoise shell-skeletal remains showed signs of predation by mammals as well as Common Ravens. Of particular concern was the moderate to severe damage to shells of 12 of 34 live tortoises; predators severely reduced or chewed off the gular horn in nine cases. Severe injury to the gular horn is typical of dog attacks, which are more common within 5 km of settlements than in remote areas (Andrea Carlson and K.H. Berry, personal observations). A potential confounding factor is that dead tortoises, killed by predators, are included as part of the total tortoise sign used in the models. Furthermore, both the mammalian predators and Common Ravens have the potential to severely limit recovery of tortoise populations and potentially to drive local populations to extinction (Kristan and Boarman, 2003; Esque et al., 2010).

The explanations for the significant positive relationships between tortoise abundance and Mammals in two of the management areas (critical habitat, private lands) may be complex and involve other, unmeasured factors. Subsidies and attractants for mammals in the form of food, trash, and water may be important. Although standing water does not occur within the management areas, year-round food and water are available at one city, two towns, and two settlements within 4 km of the study area edge. The study area also is in proximity to heavily used recreation areas, each with tens of thousands of visitors per year: Jawbone Canyon and Dove Springs off-highway vehicle areas, Red Rock Canyon State Park, the Spangler Hills off-highway vehicle area, and the El Paso Mountains

(Fig. 1). Trash was available in all three management areas but generally was old in the Tortoise Natural Area.

The higher numbers of predator observations in the Tortoise Natural Area may be related to available prey of wild fauna and cover, as well as lack of human disturbances and interference. Studies by Brooks (1995, 1999) support these observations. Brooks reported a greater abundance and diversity of nocturnal rodents, biomass of seeds, and abundance and richness of bird and lizard species inside the fenced Tortoise Natural Area boundaries than outside. Other scientists have reported positive effects of fenced protected areas on birds and carnivores, e.g., populations of two species of foxes were higher inside a fenced protected area than outside (Lenain et al., 2004). The presence of humans may also be a factor, with protected areas offering an escape or greater physical distance from people (Ikuta and Blumstein, 2003; Gehrt et al., 2009).

Desert Tortoise Habitat and Anthropogenic Impacts

In the models, tortoise sign was negatively correlated with two anthropogenic predictor variables, each in a different management area: counts of livestock scat on private lands and vehicle tracks in critical habitat. Scientists have previously identified these predictor variables as contributing to deterioration of habitat and mortality of tortoises (Busack and Bury, 1974; Berry, 1978; Webb and Stielstra, 1979; Nicholson and Humphreys, 1981; USFWS, 1994a; Bury and Luckenbach, 2002; Berry et al., 2008; Keith et al., 2008). At the time of our survey in 2011, the Tortoise Natural Area had been protected from sheep grazing for 31 yr, since 1980, and from most vehicle use for 38 yr, since 1973. In contrast, sheep grazing ended in critical habitat in 1990, the year the Desert Tortoise was federally listed as threatened (USFWS, 1990). Recreation vehicle use was also intensive in critical habitat until the early 1990s, but unauthorized travel off existing routes has continued for decades. Private lands, unless fenced, have no protection from sheep grazing or vehicle use and thus receive heavy use.

Livestock, particularly sheep, have grazed western Mojave Desert lands for over a century (Wentworth, 1948). They have altered composition of perennial and annual vegetation by preferentially consuming edible forbs, perennial grasses, and shrubs (e.g., species of saltbush, hop-sage, white bur-sage, winter fat, Anderson and Cooper's box-thorn). Damage was most severe in the vicinity of watering sites, whether through stock tanks (e.g., Brooks et al., 2006) or watering trucks. In a study on effects of grazing on annual and perennial vegetation, Brooks et al. (2006) reported significant declines in cover, species richness, and density of perennial plants with increasing proximity to watering sites. The declines in cover were due primarily to declines of the small to mid-sized shrubs, e.g., box-thorn, hop-sage, cheesebush, and Nevada ephedra.

Vehicle use off-highway or off paved roads for recreation or other purposes similarly affects perennial vegetation (e.g., Busack and Bury, 1974; Lathrop, 1983; Bury and Luckenbach, 2002; Brooks and Lair, 2009). Where users concentrate camping and racing activities and where route and track densities are high, the result is partial or complete denudation of perennial shrubs, especially the several species of small shrubs occurring between the larger creosote bushes.

The long-term degradation and loss of perennial shrubs by grazing and vehicles probably account for significantly more plots in the creosote bush association on private lands and critical habitat than in the Tortoise Natural Area. The creosote bush association is the simplest and least diverse of the four vegetation associations in our study area, with only two abundant species compared with 5 to 11 abundant species in the other vegetation associations. In contrast, tortoise sign had the highest probability of occurrence in the box-thorn association, regardless of management area.

Both livestock grazing and vehicle use also have negative effects on availability of native annual forbs, important forage for the tortoise, through consumption, trampling, crushing, and compaction (Berry, 1978; Jennings, 1997, 2002; Oftedal, 2002). Grazing and vehicle travel additionally disturb soils, thereby enhancing opportunities for alien annual plants to thrive at the expense of the natives. For example, cover and richness of native annuals declined with

increasing proximity to livestock watering sites (Brooks et al., 2006), and density of dirt roads was positively correlated with richness of alien annual species and biomass of the alien forb, filaree, *Erodium cicutarium* (Brooks and Berry, 2006).

Grazing and vehicle use more directly affect tortoises by damaging or destroying burrows, reducing canopies of shrubs used for shelter from predators and temperature extremes, and injuring or killing tortoises (Berry, 1978; Nicholson and Humphreys, 1981; Bury and Luckenbach, 2002). Paved and dirt roads, designated off-highway vehicle routes, and tracks also provide access to users who may shoot tortoises (Berry, 1986).

The significant effects of two other human activities were limited to private lands (Trash) and critical habitat (Firearms). The models showed a positive association between trash and Desert Tortoise abundance on private lands. The finding of a positive association differs from other, similar studies. Keith et al. (2008) reported that plots with tortoise sign had lower counts of trash than plots without tortoise sign, and Berry et al. (2006) noted that tortoise mortality was correlated with trash counts. Effects of trash on tortoises may have both positive and negative elements, and the relationship may change over time and location. Trash can be an indicator of heavy human use and habitat deterioration, as well as an attractant to subsidized predators. Trash also may attract tortoises, who may eat foreign objects out of hunger, accidental ingestion, or curiosity (Walde et al., 2007), or, if the object is sufficiently large, use it as cover. Consumption of trash as a cause of illness and death is well known to veterinarians who work with chelonians, whether tortoises, freshwater turtles, or sea turtles (Donoghue, 2006; Wyneken et al., 2006). Some trash, such as partially crushed cans, collects water during rain events, and can be a source of drinking water for wild tortoises. Debris from firearms may have similar positive and negative aspects. The positive association of debris from firearms with abundance of tortoise sign in critical habitat may have ominous implications: 25.6% of tortoise sign was composed of dead tortoises in this management area.

One surface-disturbing activity, mining, was minimal in the study area and held no significant associations with tortoise abundance in the models. Historically, mining was an important land use in the western Mojave Desert, especially in the Rand Mining District (eastern edge of our study area) and the nearby El Paso Mountains from the 1860s to recent times (Starry, 1974; Vredenburg et al., 1981; Chaffee and Berry, 2006). Mining has not been authorized in the Tortoise Natural Area since the Congressional withdrawal from the 1872 mining laws in 1980, but our surveys showed that some hazardous sites remain.

Overall, this study confirms that the Tortoise Natural Area, with higher densities of Desert Tortoises, has benefited from the protective fence and elimination of grazing and vehicle use. Despite the emergence and spread of a chronic, infectious disease (mycoplasmosis) throughout the western Mojave Desert (Jacobson et al., 1991; Homer et al., 1998; Brown et al., 1999), adult tortoise densities at the Tortoise Natural Area were significantly higher than in critical habitat not only in our study area but also throughout the West Mojave Recovery Unit (USFWS, 2010, 2012). The mammalian predators and avian predators also may have benefited from protection in the Tortoise Natural Area on the basis of our counts, as well as results from previous studies (Brooks, 1995, 1999). In our study, models of tortoise sign and presence suggest that relationships with these predator groups were neither positive nor negative. In other studies, vertebrate species have received benefits from fenced protected areas by reducing human disturbances and limiting transmission of wildlife-borne diseases (Ikuta and Blumstein, 2003; Lenain et al., 2004; Ferguson and Hanks, 2012). In a global analysis of the effectiveness of protected area management, Leverington et al. (2010) identified several factors related to overall success in maintaining biodiversity: legal establishment, design, legislation, and boundary marking. One critical question, addressed in the 1970s for the Tortoise Natural Area, was whether posting of signs alone was a sufficient and effective method for marking the boundary and eliminating unauthorized recreational vehicle use and sheep grazing. Because

posting was ineffective, the Bureau of Land Management constructed the hog-wire fence in 1979–1980. Also ineffective was posting of signs to limit recreational vehicle use to existing routes and specific areas in the critical habitat portions of our study area, even after decades of effort by the Bureau of Land Management (e.g., Goodlett and Goodlett, 1992; USBLM, 2002, 2006; US District Court, 2009). As a result, the Bureau of Land Management closed and fenced a portion of the critical habitat in 2002, and this area remained closed with the exception of a year (2008–2009), when the area was reopened. Because of continued noncompliance by off-highway vehicle users, the area was again closed. The many years of sheep grazing and off-highway vehicle use degraded the habitat and therefore are likely contributors to the lower abundance and higher mortality of tortoises in critical habitat in 2011.

Management Applications

Historic and recent human uses affect the functioning of ecosystems (Foster et al., 2003). The three management areas provide examples of how these legacies have become embedded in the sites and how Desert Tortoises, in turn, may have been affected. The legacies are persistent and need to be considered in framing expectations for recovery of the Desert Tortoise and in planning habitat restoration. Other important considerations are the types and levels of protection needed, enforcement of protective measures, and long-term monitoring.

In 1994, the USFWS outlined several activities that were incompatible with recovery of the Desert Tortoise and recommended that these activities be prohibited in recovery areas (USFWS, 1994a). Examples include all vehicle activity off designated roads, uncontrolled dogs out of vehicles, dumping and littering, domestic livestock grazing, and discharge of firearms (except for hunting of big game or upland game birds at specified times). The USFWS recommended emergency closures of dirt roads and routes where human-caused mortality of tortoises was a problem, as well as protective fencing and regular and frequent patrols of law enforcement personnel in areas with unauthorized vehicle use and vandalism. They made specific recommendations for the region where our study occurred, including reducing populations of Common Ravens to limit predation on juvenile Desert Tortoises. A few of these recommendations have been implemented; others remain to be accomplished. The results of our studies provide scientific support for implementing recommendations on livestock grazing, vehicle use off designated roads, and canid and raven predation. Areas such as the Tortoise Natural Area, fenced to prohibit entry of vehicles and livestock, appear to have benefited the Desert Tortoise compared with unfenced areas, if higher tortoise densities and lower mortality rates are used as measures. The recent fencing of parts of critical habitat to reduce unauthorized vehicle use may have similar benefits for Desert Tortoise recovery in the future. The high levels of trauma from canids on live tortoises are likely to inhibit recovery and demonstrate the need for better control of dogs and management of wild canids, at least on a local basis. Dog-proof fencing is an option to consider. Likewise, when Desert Tortoise densities are very low, predation by Common Ravens and mammals inhibits recovery. Government agencies have undertaken control of coyotes and Common Ravens to reduce hyperpredation of Desert Tortoises in limited areas of the Mojave and western Sonoran deserts but not at our study area or in the vicinity (USFWS, 2008a, 2008b). Other causes of death to tortoises, such as shooting and vehicle trauma, still remain to be addressed. The USFWS recovery recommendation on trash and litter has not been implemented on a landscape scale; volunteers collect litter in local areas, however.

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Literature Cited

Arnold, S.F. 1981. Asymptotic validity of procedures under nonnormal distributions. Pp. 141–158 *in* The Theory of Linear Models and Multivariate Analysis. John Wiley and Sons, Canada.

Baldwin, B.G., D.H. Goldman, D.J. Keil, R.W. Patterson, T.J. Rosatti, and D.H. Wilken (Eds.). 2012. The Jepson Manual: Vascular Plants of California. 2nd Ed. University of California Press, USA.

Barreto, H., and F.M. Howland. 2006. The Bootstrap Add-In. Pp. 718–721 in H. Barreto and F.M. Howland (Eds.), Introductory Econometrics: Using Monte Carlo Simulation and Microsoft Excel. Cambridge University Press, USA. Available at http://www3.wabash.edu/econometrics/EconometricsBook/Basic%20Tools/ExcelAddIns/bootstrap.htm. Archived by WebCite at http://www.webcitation.org/6LZgPZ8Qs on 2 December 2013.

Berry, K.H. 1978. Livestock grazing and the desert tortoise. Pp. 505–519 in Transactions 43rd North American Wildlife and Natural Resources Conference. Wildlife Management Institute, USA.

Berry, K.H. 1986. Incidence of gunshot deaths in desert tortoises in California. Wildlife Society Bulletin 14:127–132.

Berry, K.H., and M.M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Diseases 37:427–450.

Berry, K.H., and P. Medica. 1995. Desert tortoises in the Mojave and Colorado deserts. Pp. 73–75 in E.L. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac (Eds.), Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. United States Department of the Interior, National Biological Service, USA.

Berry, K.H., and A.P. Woodman. 1984. Preliminary investigations of shell wear in determining adult age groups in desert tortoises. Appendix 4 in K.H. Berry (Ed.), The Status of the Desert Tortoise (*Gopherus agassizii*) in the United States. Desert Tortoise Council Report to US Fish and Wildlife Service, California, USA. Order No. 11310–0083–81.

Berry, K.H., T. Shields, A.P. Woodman, T. Campbell, J. Roberson, K. Bohuski, and A. Karl. 1986a. Changes in desert tortoise populations at the Desert Tortoise Research Natural Area between 1979 and 1985. Proceedings of the Desert Tortoise Council Symposium 1986:100–123.

Berry, K.H., L.L. Nicholson, S. Juarez, and A.P. Woodman. 1986b. Changes in desert tortoise populations at four study sites in California. Proceedings of the Desert Tortoise Council Symposium 1986:60–80.

Berry, K.H., K. Keith, and T. Bailey. 2008. Status of the desert tortoise in Red Rock Canyon State Park, California. California Fish and Game 94:98–118.

Berry, K.H., J.L. Yee, A.A. Coble, W.M. Perry, and T.A. Shields. 2013. Multiple factors affect a population of Agassiz's Desert Tortoise (*Gopherus agassizii*) in the northwestern Mojave Desert. Herpetological Monographs 27:87–109.

Boarman, W.I. 1993. When a native predator becomes a pest: a case study. Pp. 191–206 in S.K. Majumdar, E.W. Miller, D.E. Baker, E.K. Brown, J.R. Pratt, and R.F. Schmalz (Eds.), Conservation and Resource Management. Pennsylvania Academy of Sciences, USA.

Boarman, W.I., and K.H. Berry. 1995. Common ravens in the southwestern United States, 1968–92. Pp. 73–75 in E.L. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac (Eds.), Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of US Plants, Animals, and Ecosystems. United States Department of the Interior, National Biological Service, USA.

Boyer, T.H., and D.M. Boyer. 2006. Turtles, tortoises, and terrapins. Pp. 78–99 in D.R. Mader (Ed.), Reptile Medicine and Surgery, 2nd Ed. Saunders Elsevier Inc., USA.

Brooks, M.L. 1995. Benefits of protective fencing to plant and rodent communities of the western Mojave Desert, California. Environmental Management 19:65–74.

Brooks, M.L. 1999. Effects of protective fencing on birds, lizards, and black-tailed hares in the western Mojave Desert. Environmental Management 23:387–400.

Brooks, M.L., and K.H. Berry. 2006. Dominance and environmental correlates of alien annual plants in the Mojave Desert, USA. Journal of Arid Environments 67:100–124.

Brooks, M.L., and B.M. Lair. 2009. Ecological effects of vehicular routes in a desert ecosystem. Pp. 168–195 in R.H. Webb, L.F. Fenstermaker, J.S. Heaton, D.L. Hughson, E.V. McDonald, and D.M. Miller (Eds.), The Mojave Desert, Ecosystem Processes and Sustainability. University of Nevada Press, USA.

Brooks, M.L., and J.R. Matchett. 2006. Spatial and temporal patterns of wildfires in the Mojave Desert, 1980–2004. Journal of Arid Environments 67:148–164.

Brooks, M.L., J.R. Matchett, and K.H. Berry. 2006. Effects of livestock watering sites on alien and native plants in the Mojave Desert, USA. Journal of Arid Environments 67:125–147.

Brown, D.R., J.L. Merritt, E.R. Jacobson, P.A. Klein, J.G. Tully, and M.B. Brown. 2004. *Mycoplasma testudineum* sp. nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. International Journal of Systematic and Evolutionary Microbiology 43:1527–1529.

Brown, M.B., I.M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E.R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62:4580–4586.

Brown, M.B., K.H. Berry, I.M. Schumacher, K.A. Nagy, M.M. Christopher, and P.A. Klein. 1999. Seroepidemiology of upper respiratory tract disease in the desert tortoise in the western Mojave Desert of California. Journal of Wildlife Diseases 35:716–727.

Burge, B.L. 1978. Physical characteristics and patterns of utilization of cover sites used by *Gopherus agassizi* in southern Nevada. Proceedings of the Desert Tortoise Council Symposium 1978:80–111.

Bury, R.B., and R.A. Luckenbach. 2002. Comparison of desert tortoise (*Gopherus agassizii*) populations in an unused and off-road vehicle area in the Mojave Desert. Chelonian Conservation and Biology 4:457–463.

Busack, S.D., and R.B. Bury. 1974. Some effects of off-road vehicles and sheep grazing on lizard populations in the Mojave Desert. Biological Conservation 6:179–183.

California Department of Fish and Game. 2010. List of Vegetation Alliances and Associations. Vegetation Classification and Mapping Program. California Department of Fish and Game, USA. Available at http://www.dfg.ca.gov/biogeodata/vegcamp/natural_comm_list.asp. Archived by WebCite at http://www.webcitation.org/6LZggMgzq on 2 December 2013.

California Department of Fish and Wildlife. 2013. State and Federally Listed Endangered and Threatened Animals of California. California Department of Fish and Wildlife. Biogeographic Data Branch, California Natural Diversity Database, USA.

Campbell, T. 1983. Some natural history observations of desert tortoises and other species on and near the Desert Tortoise Natural Area, Kern County, California. Proceedings of the Desert Tortoise Council Symposium 1983:80–88.

Chaffee, M.A., and K.H. Berry. 2006. Abundance and distribution of selected elements in soils, stream sediments, and selected forage plants from desert tortoise habitats in the Mojave and Colorado deserts, USA. Journal of Arid Environments 67:35–87.

County of Kern, California. 1942. Code of Ordinances. Title 7—Animals. Chapter 7.16. Estrays-Grazing areas designated. Available at http://www.co.kern.ca.us/. Archived by WebCite at http://www.webcitation.org/6RHyU4fup on 23 July 2014.

Donoghue, S. 2006. Nutrition. Pp. 251–298 in D.R. Mader (Ed.), Reptile Medicine and Surgery. Saunders Elsevier, Inc., USA.

Esque, T.C., K.E. Nussear, K.K. Drake, A.D. Walde, K.H. Berry, R.C. Averill-Murray, A.P. Woodman, W.I. Boarman, P.A. Medica, J. Mack, and J.S. Heaton. 2010. Effects of subsidized predators, resource variability, and human population density on desert tortoise populations in the Mojave Desert, USA. Endangered Species Research 12:167–177.

Fedriani, J.M., T.K. Fuller, and R.M. Sauvajot. 2001. Does availability of anthropogenic food enhance densities of omnivorous mammals? An example with coyotes in southern California. Ecography 24:325–331.

Ferguson, K., and J. Hanks. 2012. The effects of protected area and veterinary fencing on wildlife conservation in southern Africa. Parks 18.1:49–60.

Foster, D., F. Swanson, J. Aber, I. Burke, N. Brokaw, D. Tilman, and A. Knapp. 2003. The importance of land-use legacies to ecology and conservation. BioScience 53:77–88.

Fox, J., and S. Weisberg. 2011. An {R} Companion to Applied Regression, 2nd Ed. Sage, USA. Available at http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. Archived by WebCite at http://www.webcitation.org/6LZgkcXsR on 2 December 2013.

Gehrt, S.D., C. Anchor, and L.A. White. 2009. Home range and landscape use of coyotes in a metropolitan landscape: conflict or coexistence? Journal of Mammalogy 90:1045–1057.

Geldmann, J., M. Barnes, L. Coad, I.D. Craigie, M. Hockings, and N.D. Burgess. 2013. Effectiveness of terrestrial protected areas in reducing habitat loss and population declines. Biological Conservation 161:230–238.

Goodlett, G.O., and G.C. Goodlett. 1992. Studies of unauthorized off-highway vehicle activity in the Rand Mountains and Fremont Valley, Kern County, California. Proceedings of the Desert Tortoise Council Symposium 1992:163–187.

Hazard, L.C., D.R. Shemanski, and K.A. Nagy. 2009. Nutritional quality of natural foods of juvenile desert tortoises (*Gopherus agassizii*): Energy, nitrogen, and fiber digestibility. Journal of Herpetology 43:38–48.

Hazard, L.C., D.R. Shemanski, and K.A. Nagy. 2010. Nutritional quality of natural foods of juvenile desert tortoises (*Gopherus agassizii*): Calcium, phosphorus and magnesium digestibility. Journal of Herpetology 44:135–147.

Homer, B.L., K.H. Berry, M.B. Brown, G. Ellis, and E.R. Jacobson. 1998. Pathology of diseases in wild desert tortoises from California. Journal of Wildlife Diseases 34:508–523.

Hunter, L.M., M. De J. Gonzalez G., M. Stevenson, K.S. Karish, R. Toth, T.C. Edwards, R.J. Lileholm, and M. Cablk. 2003. Population and land use change in the California Mojave: Natural habitat implications of alternative futures. Population Research and Policy Review 22:373–397.

Ikuta, L.A., and D.T. Blumstein. 2003. Do fences protect birds from human disturbance? Biological Conservation 112:447–452.

Jacobson, E.R. 2007. Infectious Diseases and Pathology of Reptiles: Color Atlas and Text. CRC Press, USA.

Jacobson, E.R., J.M. Gaskin, M.B. Brown, R.K. Harris, C.H. Gardiner, J.L. LaPointe, H.P. Adams, and C. Reggiardo. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). Journal of Wildlife Diseases 27:296–316.

Jacobson, E.R., T.J. Wronski, J. Schumacher, C. Reggiardo, and K.H. Berry. 1994. Cutaneous dyskeratosis in free-ranging desert tortoises, *Gopherus agassizii*, in the Colorado Desert of southern California. Journal of Zoo and Wildlife Medicine 25:68–81.

Jacqmin-Gadda, H., S. Sibillot, C. Proust, J.M. Molina, and R. Thiébaut. 2007. Robustness of the linear mixed model to misspecified error distribution. Computational Statistics and Data Analysis 51:5142–5154.

Jennings, W.B. 1997. Invasions of exotic plants: Implications for the desert tortoise, *Gopherus agassizii*, and its habitat in the western Mojave Desert. Pp. 10–12 in J. Van Abbema (Ed.), Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles—An International Conference. New York Turtle and Tortoise Society WCS Recovery Program, USA.

Jennings, W.B. 2002. Diet selection by the desert tortoise in relation to the flowering phenology of ephemeral plants. Chelonian Conservation and Biology 4:353–358.

Johnson, A.J., A.P. Pessier, J.F.X. Wellehan, and E.R. Jacobson. 2005. Identification of a novel herpesvirus from a California desert tortoise (*Gopherus agassizii*). Veterinary Microbiology 111:107–116.

Johnson, A.J., D.J. Morafka, and E.R. Jacobson. 2006. Seroprevalence of *Mycoplasma agassizii* and tortoise herpesvirus in captive desert tortoises (*Gopherus agassizii*) from the Greater Barstow Area, Mojave

Desert, California. Journal of Arid Environments 67:192–201.

Keith, K., K.H. Berry, and J.F. Weigand. 2008. When desert tortoises are rare: Testing a new protocol for assessing status. California Fish and Game 94:75–97.

Kern County Engineering, Surveying and Permit Services Department. 2011. Kern Parcels. Available at http://www.co.kern.ca.us/gis/downloads.asp. Archived by WebCite at http://www.webcitation.org/6LZgqmojW on 2 December 2013.

Kristan, W.B. III, and W.I. Boarman. 2003. Spatial pattern of risk of common raven predation on desert tortoises. Ecology 84:2432–2443.

Krzysik, A.J. 2002. A landscape sampling protocol for estimating distribution and density patterns of desert tortoises at multiple spatial scales. Chelonian Conservation and Biology 4:366–379.

Lance, V.A., and D.C. Rostal. 2002. The annual reproductive cycle of the male and female desert tortoise: Physiology and endocrinology. Chelonian Conservation and Biology 4:302–312.

Lathrop, E. 1983. The effect of vehicle use on desert vegetation. Pp. 153–166 in R.H. Webb and H.G. Wilshire (Eds.), Environmental Effects of Off-Road Vehicles. Impacts and Management in Arid Regions. Springer-Verlag, USA.

Lenain, D.M., E. Olfermann, and S. Warrington. 2004. Ecology, diet and behavior of two fox species in a large, fenced protected area in central Saudi Arabia. Journal of Arid Environments 57:45–60.

Lenth, R.V. 2013. lsmeans: Least-squares means. R package Version 1.10-01. Available at http://CRAN.R-project.org/package=lsmeans. Archived by WebCite at http://www.webcitation.org/6LZh1fMtU on 2 December 2013.

Le Saout, S., M. Hoffmann, Y. Shi, A. Hughes, C. Bernard, T.M. Brooks, B. Bertzky, S.H.M. Butchart, S.N. Stuart, T. Badman, and A.S.L. Rodrigues. 2013. Protected areas and effective biodiversity conservation. Science 342:803–805.

Leu, M., S.E. Hanser, and S.T. Knick. 2008. The human footprint in the West: A large-scale analysis of anthropogenic impacts. Ecological Applications 18:1119–1139.

Leverington, F., K. Lemos Costa, H. Pavese, A. Lisle, and M. Hockings. 2010. A global analysis of protected area management effectiveness. Environmental Management 46:685–698.

McCullagh, P., and J.A. Nelder. 1989. Generalized Linear Models, 2nd Ed. Chapman and Hall, UK.

Minnich, R.A. 2008. California's Fading Wildflowers: Lost Legacy and Biological Invasions. University of California Press, USA.

Murphy, R.W., K.H. Berry, T. Edwards, A.E. Leviton, A. Lathrop, and J.D. Riedle. 2011. The dazed and confused identity of Agassiz's land tortoise, *Gopherus agassizii* (Testudines, Testudinidae) with the description of a new species, and its consequences for conservation. Zookeys 113:39–71.

Nagy, K.A., B.T. Henen, and D.B. Vyas. 1998. Nutritional quality of native and introduced food plants of wild desert tortoises. Journal of Herpetology 32:260–267.

NOAA (National Oceanic and Atmospheric Administration). 2010–2011. Climatological Data for California. National Climatic Data Center, Asheville, North Carolina. Randsburg Station, Kern County, USA.

Nicholson, L., and K. Humphreys. 1981. Sheep grazing at the Kramer study plot, San Bernardino County, California. Proceedings of the Desert Tortoise Council Symposium 1981:163–194.

Norris, F. 1982. On beyond reason: homesteading in the California Desert, 1885–1940. Southern California Quarterly—Historical Society of Southern California 64:297–312.

Oftedal, O.T. 2002. Nutritional ecology of the desert tortoise in the Mohave and Sonoran deserts. Pp. in 194–241 in T.R. Van Devender (Ed.), The Sonoran Desert Tortoise: Natural History, Biology, and Conservation. University of Arizona Press and the Arizona–Sonora Desert Museum, USA.

Powers, R.L. 1988. South Fork Country. The Arthur H. Clark Company. Fifth printing by Westernlore Press, USA.

Powers, R.L. 2000. Kern River Country. Fourth printing, The Arthur H. Clark Company, USA.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07–0. Available at http://www.R-project.org/ Archived by WebCite at http://www.webcitation.org/6MVfpiCBW on 10 January 2014.

Starry, R.M. 1974. Gold Gamble. Maturango Museum Publication 7. Maturango Museum of Indian Wells Valley, USA.

StatSoft, Inc. 2001. STATISTICA (data analysis software system), Version 6. Available at www.statsoft.com. StatSoft, Inc., USA.

Systat Software, Inc. 2007. SYSTAT 12. Available at http://www.systat.com. Systat Software, Inc., USA.

Turner, F.B., and K.H. Berry. 1984. Methods used in analyzing desert tortoise populations. Appendix in K.H. Berry (Ed.), The Status of the Desert Tortoise (*Gopherus agassizii*) in the United States. Order No. 11310–0083–81. Desert Tortoise Council Report to US Fish and Wildlife Service, California, USA.

Turner, F.B., K.H. Berry, D.C. Randall, and G.C. White. 1987. Population ecology of the desert tortoise at Goffs, California, 1983–1986. Report to Southern California Edison Company, USA.

USBLM (US Department of the Interior, Bureau of Land Management). 1973. Interim Critical Management Plan for Recreational Vehicle Use in the California Desert. United States Department of the Interior, Bureau of Land Management, California Desert Program, USA.

USBLM (US Department of the Interior, Bureau of Land Management). 1980. The California Desert Conservation Area Plan, 1980. United States Department of the Interior, Bureau of Land Management, Desert District, California, USA.

USBLM (US Department of the Interior, Bureau of Land Management). 1993. Final Rand Mountains–Fremont Valley Management Plan. A Sikes Act Plan. United States Department of the Interior, Bureau of Land Management, Ridgecrest Resource Area, California, USA.

USBLM (US Department of the Interior, Bureau of Land Management). 2002. Decision Record and Finding of No Significant Impact for the Interim Closure to

Motorized Vehicle Use of Selected Routes within the Western Rand Mountains Area of Critical Environmental Concern. United States Department of the Interior, Bureau of Land Management, Ridgecrest Field Office, California, USA.

USBLM (US Department of the Interior, Bureau of Land Management). 2006. Record of Decision, West Mojave Plan, Amendment to the California Desert Conservation Area Plan. March 2006. United States Department of the Interior, Bureau of Land Management, California Desert District, USA.

USBLM (US Department of the Interior, Bureau of Land Management). 2012. Geospatial Data Downloads. Available at http://www.blm.gov/ca/gis/. Archived by WebCite at http://www.webcitation.org/6LZhCi1u0 on 2 December 2013.

USBLM (US Department of the Interior, Bureau of Land Management) and California Department of Fish and Game. 1988. A Sikes Act Management Plan for the Desert Tortoise Research Natural Area and Area of Critical Environmental Concern. United States Department of the Interior, Bureau of Land Management, Ridgecrest Office, California, USA.

US District Court. 2009. Order summary judgment motions. Case 3:06-cv-04884-SI. Center for Biological Diversity, et al., Plaintiffs v. BLM. United States District Court for the Northern District of California, California, USA. Available at http://www.biologicaldiversity.org/programs/public_lands/deserts/california_desert_conservation_area/pdfs/WEMO_NECO_case_order_9-28-09.pdf. Archived by WebCite at http://www.webcitation.org/6MVf7ZZHO on 10 January 2014.

US District Court. 2011. Order re: remedy. Case 3:06-cv-04884-SI. Center for Biological Diversity, et al., Plaintiffs v. BLM. United States District Court for the Northern District of California, USA.

USFWS (US Fish and Wildlife Service). 1990. Endangered and threatened wildlife and plants; determination of threatened status for the Mojave population of the desert tortoise. Federal Register 55:12178–12191.

USFWS (US Fish and Wildlife Service). 1994*a*. Desert Tortoise (Mojave Population) Recovery Plan. US Fish and Wildlife Service, Oregon, USA.

USFWS (US Fish and Wildlife Service). 1994*b*. Endangered and threatened wildlife and plants; determination of critical habitat for the Mojave population of the desert tortoise. Federal Register 59:5820–5866.

USFWS (US Fish and Wildlife Service). 2008*a*. Environmental Assessment to Implement a Desert Tortoise Recovery Plan Task: Reduce Common Raven Predation on the Desert Tortoise. Final. United States Fish and Wildlife Service, California, USA.

USFWS (US Fish and Wildlife Service). 2008*b*. Letter dated April 15 (2008-I-00365) from Carl T. Benz to M.A. Bari, National Training Center, Fort Irwin concurring on proposed predator removal program. United States Fish and Wildlife Service, California, USA.

USFWS (US Fish and Wildlife Service). 2010. Mojave Population of the Desert Tortoise (*Gopherus agassizii*), 5-Year Review: Summary and Evaluation. United States Fish and Wildlife Service, Desert Tortoise Recovery Office, Nevada, USA.

USFWS (US Fish and Wildlife Service). 2011. Revised Recovery Plan for the Mojave Population of the Desert Tortoise (*Gopherus agassizii*). United States Fish and Wildlife Service, California, USA.

USFWS (US Fish and Wildlife Service). 2012. Range-wide Monitoring of the Mojave Desert Tortoise (*Gopherus agassizii*): 2011 Annual Report (Draft). United States Fish and Wildlife Service, Desert Tortoise Recovery Office, Nevada, USA.

Vredenburg, L.M., G.L. Shumway, and R.D. Hartill. 1981. Desert Fever: An Overview of Mining in the California Desert. Living West Press, USA.

Walde, A.D., M.L. Harless, D.K. Delaney, and L.L. Pater. 2007. Anthropogenic threat to the desert tortoise (*Gopherus agassizii*): Litter in the Mojave Desert. Western North American Naturalist 67:147–149.

Webb, R.H., and S.S. Stielstra. 1979. Sheep grazing effects on Mojave Desert vegetation and soils. Environmental Management 3:517–529.

Wentworth, E.N. 1948. America's Sheep Trails. Iowa State College Press, USA.

Wyneken, J., D.R. Mader, E.S. Weber, III, and C. Merigo. 2006. Medical care of sea turtles. Pp. 972–1007 in D.R. Mader (Ed.), Reptile Medicine and Surgery. Saunders Elsevier, Inc., USA.

Zimmerman, L.C., M.P. O'Connor, S.J. Bulova, J.R. Spotila, S.J. Kemp, and C.J. Salice. 1994. Thermal ecology of desert tortoises in the eastern Mojave Desert: Seasonal patterns of operative and body temperatures, and microhabitat utilization. Herpetological Monographs 8:45–59.

Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev, and G.M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, USA.

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Appendix I

Coefficients for generalized linear models for Desert Tortoise presence—in all three management areas and for abundance in three separate areas. Colons in the predictor variable represent an interaction effect between two predictors. Coefficients for presence and private lands models were tested using *z*-statistics. Coefficients for the Tortoise Natural Area and critical habitat models were analyzed with quasi-Poisson models and were tested using *t*-statistics based on 78 and 76 df respectively. Regression coefficients (β) are presented with SE, *z*-statistic (*z*), and *P*-values (*P*). Levels of significance: * $P < 0.10$, ** $P < 0.05$, *** $P < 0.010$.

Response variable	Predictor variable	β	SE	*z*, *t*	*P*	
Presence in three areas	Intercept (box thorn and Tortoise Natural Area)	0.934	0.400	2.33	0.020	
	Veg. (creosote bush)	−0.456	0.365	−1.25	0.211	**
	Veg. (indigo bush)	−0.926	0.465	−1.99	0.046	**
	Veg. (Nevada ephedra)	−1.195	0.544	−2.20	0.028	**
	Log(Sheep)	−0.795	0.411	−1.93	0.053	*
	Management area (critical habitat)	−0.888	0.471	−1.88	0.059	*
	Management area (private lands)	−1.043	1.043	−1.00	0.317	
	Log(Mammals)	−0.509	0.442	−1.15	0.250	
	Management area (critical habitat): log(Mammals)	1.504	0.737	2.04	0.041	**
	Management area (private): log(Mammals)	1.644	0.860	1.91	0.056	*
Abundance in TortoiseNatural Area	Intercept	0.772	0.177	4.36	<0.001	***
Abundance in critical habitat	Intercept	−0.396	0.308	−1.29	0.202	
	Log(Vehicles)	−1.075	0.572	−1.88	0.064	*
	Log(Firearms)	1.387	0.526	2.64	0.010	**
	Log(Mammals)	1.152	0.361	3.19	0.002	***
Abundance in private lands	Intercept (box thorn)	−0.491	0.760	−0.65	0.518	*
	Veg. (creosote bush)	−1.567	0.529	−2.96	0.003	
	Veg. (indigo bush)	−17.108	3168	−0.01	0.996	**
	Veg. (Nevada ephedra)	−16.789	2282	−0.01	0.994	
	Log(Sheep)	−0.516	0.248	−2.08	0.037	**
	Log(Trash)	0.764	0.393	1.94	0.052	*
	Log(Mammals)	1.248	0.485	2.57	0.010	**

Kern County, California and to this day, we help manage the DTRNA under a cooperative agreement with the BLM's Ridgecrest field office. We have carefully studied the Draft DRECP. Our review determined that not only was the DTRNA not considered to have any existing legal conservation status, but that of six action alternatives presented, all but one contained Development Focus Areas that directly abutted on, overlapped with, or encompassed most of - the DTRNA. Each and every concern that we raised regarding development on and around the DTRNA was ignored in its entirety. Two scientific surveys conducted in 2011 and 2012 show that there is six times the density of tortoises found within the DTRNA as on adjacent critical habitat. In addition, if we look solely at the densities of adult breeding tortoises we find that there are 3 to 5 times as many at DTRNA as can be found even at USFWS's critical habitats in California. We are requesting permanent legal conservation status for the DTRNA, protecting it from vehicle use, mining, grazing, leasing for oil and gas, renewable energy, and any other use! The DRECP disregards conservation history and conservation status of the DTRNA, contradicting existing management plans and 40 years of commitments by federal and state agencies. Please help protect the DTRNA.

The Fence Experiment at the Desert Tortoise Research Natural Area Interpretive Center: An Evaluation after 33 Years

Kristin H. Berry[1], Tim Shields[2], Julie Yee[3], William Perry[3]:
U.S. Geological Survey, [1]Riverside and [3]Dixon, CA; [2]Haines, AK

In 1978, Kristin Berry, while working at the U.S. Bureau of Land Management, had the opportunity to design and initiate a long-term experiment on the efficacy of protective, permeable fencing for the desert tortoise (*Gopherus agassizii*). The fence was to be constructed in 1979 along the boundary of the Desert Tortoise Research Natural Area (DTRNA), western Mojave Desert, California. The purpose was to protect tortoises and their habitat from sheep grazing and recreational vehicles. The 7.77 km^2 plot was centered on the interpretive kiosk, outhouse, and parking lot and had two components, 4.53 km^2 inside the fence and 3.24 km^2 outside the fence. Between 1979 and 2012, the plot was surveyed for tortoises in spring of 7 years using mark-recapture techniques; in addition to live tortoises, data were collected on shell-skeletal remains, other signs of tortoises, perennial vegetation, predators, and evidence of human use. In 1979, the baseline year, 590 tortoises were registered; no significant differences in density existed inside vs. outside the fence. By 1985 the population had declined in numbers and density, with significant differences between inside and outside the fence. Declines continued over the years both inside and outside the fence, reaching the lowest point in 2002 with a count of 47 total individuals. In 2012, the last survey, total counts were up (N = 73), and densities were significantly higher inside than outside the fence (>3 times). Densities outside the fence closely approximated estimates developed by the U.S. Fish and Wildlife Service using distance sampling in nearby critical habitat. We explore changes in distribution and other demographic attributes of the tortoises through the decades, as well as potential factors affecting recovery at the Desert Tortoise Research Natural Area.

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Original Contribution

Integrating Gene Transcription-Based Biomarkers to Understand Desert Tortoise and Ecosystem Health

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Abstract: Tortoises are susceptible to a wide variety of environmental stressors, and the influence of human disturbances on health and survival of tortoises is difficult to detect. As an addition to current diagnostic methods for desert tortoises, we have developed the first leukocyte gene transcription biomarker panel for the desert tortoise (*Gopherus agassizii*), enhancing the ability to identify specific environmental conditions potentially linked to declining animal health. Blood leukocyte transcript profiles have the potential to identify physiologically stressed animals in lieu of clinical signs. For desert tortoises, the gene transcript profile included a combination of immune or detoxification response genes with the potential to be modified by biological or physical injury and consequently provide information on the type and magnitude of stressors present in the animal's habitat. Blood from 64 wild adult tortoises at three sites in Clark County, NV, and San Bernardino, CA, and from 19 captive tortoises in Clark County, NV, was collected and evaluated for genes indicative of physiological status. Statistical analysis using a priori groupings indicated significant differences among groups for several genes, while multidimensional scaling and cluster analyses of transcription C_T values indicated strong differentiation of a large cluster and multiple outlying individual tortoises or small clusters in multi-dimensional space. These analyses highlight the effectiveness of the gene panel at detecting environmental perturbations as well as providing guidance in determining the health of the desert tortoise.

Keywords: *Gopherus agassizii*, mRNA, immune function, physiological health, reptile

Introduction

Desert tortoises (*Gopherus agassizii*) occur throughout the Mojave Desert and are subjected to a myriad of ecological and environmental stressors. Increased human development has led to habitat loss, increased predation, contaminant and pathogen exposure, and altered wildfire regimes. These stressors have been suggested as synergistic threats to desert tortoise populations, attributed frequently to population declines (Luckenbach 1982; Christopher et al. 2003; Tracy et al. 2004; Esque et al. 2010; USFWS 2011a). Desert tortoises are slow to respond to environmental stressors and seldom display clinical signs of disease, which is typical of other long-lived reptiles (Christopher et al. 2003). Understanding how tortoises respond to these environmental stressors is key to supporting conservation and management of habitat needed for this threatened species.

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Tortoises are susceptible to a wide variety of environmental stressors, and the influence of human disturbances on health and survival of tortoises is difficult to detect. In addition to current diagnostic methods for desert tortoises (e.g., Brown et al. 2002; Ritchie 2006), we propose a genetic biomarker panel, enhancing the ability to identify specific environmental conditions potentially linked to declining animal health. Despite the urgent need for ecological studies on reptile health and physiology, the development of molecular genetic tools for research in reptiles has been slow, and most are adaptations from studies in other taxa. Applying contemporary gene transcript analysis to identify genomic response to environmental stress or disease has the potential to transform studies of reptile ecology (Burczynski et al. 2000; Bartosiewicz et al. 2001). Advanced technologies, based upon and developed from biomedical models of human physiology and disease, aid researchers with cutting-edge diagnostic tools for both domestic and wildlife veterinary applications (Burczynski et al. 2000; Bartosiewicz et al. 2001; Bowen et al. 2007, 2012; Miles et al. 2012; Sitt et al. 2008). Gene-based analyses of desert tortoises afford the opportunity for minimally invasive assessments of physiologic state in response to intrinsic and extrinsic factors, not only in individuals or populations, but potentially at landscape scales (Acevedo-Whitehouse and Duffus 2009).

Gene transcript analysis may improve the assessment of desert tortoises by detecting the earliest observable signs of physiological perturbation, as gene transcripts are typically evident prior to clinical manifestations to environmental stressors (McLoughlin et al. 2006). Consequently, application of quantitative gene transcript analysis technology will likely provide an invaluable addition to current approaches for monitoring potential physiological impairment (McLoughlin et al. 2006). Herein, we describe the development of quantitative real-time polymerase chain reaction (qPCR) assays to measure differential transcript levels of multiple genes in the desert tortoise. The genes described are fundamental to immune function (Cray et al. 2009; Zhou et al. 2008, 2011; Li et al. 2011), responses to pathogens (Kibenge et al. 2005; Zhou et al. 2008), nutritional and thermal stress (Otero et al. 2005; Iwama et al. 1999; Tsan and Gao 2004), shell formation (Chen et al. 2012), xenobiotic metabolism (Walsh et al. 2010; Oesch-Bartlomowicz and Oesch 2005), and tumorigenesis (Zhou et al. 2008) (Table 1). Measurement of differential transcription of a selected suite of genes potentially can provide an early warning of compromised health and related environmental stressors in free-ranging animals.

Methods

Study Animals

Health evaluations were conducted on wild desert tortoises (n = 64, 22F:42M) between May and October 2011 on Bureau of Land Management public lands at Hidden Valley and Piute Valley in Clark County, Nevada and at Ft. Irwin in San Bernardino, CA, USA. Habitat at all sites was dominated by Mojave desertscrub vegetation (Turner and Brown 1982) consisting of creosote bush/white bursage [*Larrea tridentata/Ambrosia dumosa*] plant association with many other shrubs, grasses, and annual plants present. We also evaluated 19 captive adult tortoises (11F:8M) at the Desert Tortoise Conservation Center in Clark County, NV, USA. These animals were deemed unhealthy based on visual examination by specialized veterinarians. Each captive tortoise presented multiple clinical signs of potential illnesses associated with long-term weight loss and reduced or under-conditioned body scoring. These tortoises were identified by veterinarians for euthanasia and as such were not screened serologically at the time of our sampling for known bacterial or viral infections. These tortoises were neither under our care nor management, and we could not control decisions about their care or disease screening. Their inclusion in the data set was merely exploratory to determine how these ill captives compare to the wild populations.

Tissue Collection

Blood samples (1.0 mL) were collected from each tortoise via subcarapacial venipuncture (Hernandez-Divers et al. 2002) using a 3.8-cm, 23-gage needle and 3 cc syringe coated with sodium heparin. An aliquot (0.5 mL) of whole blood was placed immediately into an RNeasy Animal Protect™ collection tube (Qiagen, Valencia, CA) and frozen at −20°C. The remaining blood (0.5 mL) was transferred to lithium heparin microtainers and stored on ice for no longer than 4 h. Plasma was separated using centrifugation with a centrifugal force of 1318×g. All tissue samples were stored at –70°C. Aliquots of plasma (0.01 mL) were screened for antibody presence to *Mycoplasma agassizii* and *M. testudineum* using an enzyme-linked immunoassay

Table 1. Documented Function of 11 Genes Identified in Free-Ranging Desert Tortoises

Gene	Gene function
CaM	Calmodulin (CaM) is a small acidic Ca^{2+}-binding protein, with a structure and function that is highly conserved in all eukaryotes. CaM activates various Ca^{2+}-dependent enzyme reactions, thereby modulating a wide range of cellular events, including metabolism control, muscle contraction, exocytosis of hormones and neurotransmitters, and cell division and differentiation (Chen et al. 2012). CaM has also been reported to be a pivotal calcium metabolism regulator in the shell formation (Li et al. 2004)
AHR	The arylhydrocarbon receptor (AHR) responds to classes of environmental toxicants including polycyclic aromatic hydrocarbons, polyhalogenated hydrocarbons, dibenzofurans, and dioxin (Oesch-Bartlomowicz and Oesch 2005). Depending upon the ligand, AHR signaling can modulate T-regulatory (T_{REG}) (immune-suppressive) or T-helper type 17 (T_H17) (pro-inflammatory) immunologic activity (Quintana et al. 2008; Veldhoen et al. 2008)
Mx1	The Mx1 gene responds to viral infection (Tumpey et al. 2007). Vertebrates have an early strong innate immune response against viral infection, characterized by the induction and secretion of cytokines that mediate an antiviral state, leading to the upregulation of the MX-1 gene (Kibenge et al. 2005)
HSP 70	The heat shock protein 70 is produced in response to thermal or other stress (Iwama et al. 1999; Tsan and Gao 2004). In addition to being expressed in response to a wide array of stressors (including hyperthermia, oxygen radicals, heavy metals, and ethanol), heat shock proteins act as molecular chaperones (De Maio 1999). For example, heat shock proteins aid the transport of the AHR/toxin complex in the initiation of detoxification (Tanabe et al. 1994)
SAA	Serum Amyloid A (SAA), an acute phase protein, serves as a core part of the innate immunity involving physical and molecular barriers and responses (Cray et al. 2009). Upon infection and inflammation or tissue damage and stress, SAA is induced by pro-inflammatory signals, and is a major indicator of bacterial infection, especially at early stage, in reptiles (Zhou et al. 2008, 2011)
MyD 88	Myeloid differentiation factor 88 (MyD88) is one of the key adaptor proteins to signal transduction that triggers downstream cascades involved in innate immunity. MyD88 might possess an important role in defense against microbial infection in Chinese soft-shelled turtles similar to that in mammals (Li et al. 2011)
CD9	CD9 is a molecular facilitator, provides co-stimulation to naïve T lymphocytes, regulates the aggregation of MHC-II molecules, and triggers antigen presentation. Upregulation of turtle CD9 was shown in response to bacterial infection (Zhou et al. 2008)
SOD	Superoxide dismutase (SOD)—Superoxide dismutases are a class of enzymes that catalyze dismutation of superoxide into oxygen and hydrogen peroxide and function as important antioxidant defense molecules (Walsh et al. 2010). Oxidative stress itself can lead to or result from certain inflammatory conditions (Walsh et al. 2010)
ATF	Similar to inflammatory responses in mammals, ATF can mediate inflammatory responses in reptiles. The upregulation of ATF in reptiles indicates involvement in bacterial infection (Zhou et al. 2008)
CL	Cathepsin L, an acute phase protein, plays a major role in antigen processing, tumor invasion and metastasis, bone resorption, and turnover of intracellular and secreted proteins involved in growth regulation (Zhou et al. 2008). Turtle cathepsin L may be involved in anti-bacterial immune response (Zhou et al. 2008)
LEP	Leptin links nutritional status with neuroendocrine and immune functions. Initially thought to be a satiety factor that regulates body weight by inhibiting food intake and stimulating energy expenditure, leptin is a hormone whose multiple effects include regulation of endocrine function, reproduction, and immunity (Otero et al. 2005)

(ELISA; Wendland et al. 2007). Plasma samples (n = 24 wild tortoises) were randomly selected and evaluated for additional clinicopathologic variables such as serum amyloid A (SAA; Belgrave et al. 2013), haptoglobin (Cray and Belgrave 2013), and electrophoresis (using Helena electrophoresis system; Cray et al. 2011). Protein electrophoresis includes fraction values for prealbumin, albumin, α_1 globulins, α_2 globulins, β-globulins, γ-globulins, albumin to globulin ratio (A:G), and total protein. Additionally, sloughed oral epithelial cells were collected using sterile oral swabs, and screened for Testudinid herpesvirus 2 using polymerase chain reaction (PCR) (Jacobson et al. 2012).

RNA Extraction

Blood samples were placed directly into RNeasy Protect Animal Blood Tubes (Qiagen, Valencia, CA) and then frozen at −20°C until extraction of RNA (Bowen et al.

2012). Rapid RNA degradation and induced transcription of certain genes after blood draws have led to the development of methodologies for preserving the RNA transcription profile immediately after blood is drawn. The RNeasy Protect Animal Blood Tube contains a blend of RNA stabilizing reagents that protect RNA molecules from degradation by RNases and prevents further induction of gene transcription. The RNA from blood in RNeasy Protect Animal Blood Tubes was isolated according to manufacturer's standard protocols except each sample was initially split in two to account for the nucleated red blood cells. The extracted RNA was stored at −80°C until analysis. All RNA was checked for quality on a nanodrop 2000 and achieved A260/A280 ratios of approximately 2.0 and A260/A230 ratios of less than 1.0.

cDNA Synthesis

A standard cDNA synthesis was performed on 2 μg of RNA template from each animal. Reaction conditions included 4 U reverse transcriptase (Omniscript, Qiagen, Valencia, CA), 1 μM random hexamers, 0.5 mM each dNTP, and 10 U RNase inhibitor, in RT buffer (Qiagen, Valencia, CA). Reactions were incubated for 60 min at 37°C, followed by an enzyme inactivation step of 5 min at 93°C, and then stored at −20°C until further analysis.

PCR Primers

Degenerate primers were designed based upon multi-species alignments (GenBank) as previously described by Bowen et al. (2007). Briefly, degenerate primer pairs were developed for the desert tortoise and were used on cDNA from three randomly selected tortoise samples. Degenerate primer pairs were designed to amplify 11 genes of interest and one ribosomal housekeeping gene (Bowen et al. 2007). PCR amplifications using these primers were performed on 20 ng of each cDNA sample in 50 μL volumes containing 20–60 pmol of each primer, 40 mM Tris–KOH (pH 8.3), 15 mM KOAc, 3.5 mM Mg $(OAc)_2$, 3.75 μg/mL bovine serum albumin (BSA), 0.005% Tween-20, 0.005% Nonidet-P40, 200 μM each dNTP, and 5U of Advantage® 2 *Taq* polymerase (Clontech, Palo Alto, CA). The PCR was performed on an MJ Research PTC-200 thermal cycler (MJ Research, Watertown, MA) and consisted of 1 cycle at 94°C for 3 min, and then 40 cycles at 94°C for 30 s, at 60°C for 30 s, and 72°C for 2 min, with a final extension step of 72°C for 10 min. The products of these reactions were electrophoresed on 1.5% agarose gels and resulting bands visualized using ethidium bromide staining. Definitive bands representing PCR products of a predicted base pair size of the targeted gene were excised from the gel, and extracted and purified using a commercially available nucleic acid-binding resin (Qiaex II Gel extraction kit, Qiagen, Valencia, CA).

Nucleotide sequences of both strands were determined by dideoxy nucleotide methods using an automated sequencer (Model 373; Applied Biosystems, Foster City, CA). Nucleotide sequences of the PCR products were analyzed using Align™ and Contig™ sequence alignment software programs (Vector NTI™; Informax Inc, North Bethesda, MD) and compared to known sequences using the NCBI BLAST program (Altschul et al. 1990), and the IMGT/HLA database (Robinson et al. 2001). Primer pairs appropriate for real-time PCR were designed based on the elucidated desert tortoise sequences for each gene (Table 2).

Real-Time PCR

Real-time PCR systems for the individual, tortoise-specific housekeeping gene (18S), and genes of interest (Table 1) were run in separate wells. Briefly, 1 μL of cDNA was added to a mix containing 12.5 μL of QuantiTect SYBR Green® Master Mix [5 mM Mg^{2+}] (Qiagen, Valencia, CA), 0.5 μL each of forward and reverse sequence-specific primers, 0.5 μL of uracil-*N*-glycosylase (Invitrogen, Carlsbad, CA), and 10.0 μL of RNase-free water; total reaction mixture was 25 μL. The reaction mixture cDNA samples for each gene of interest and 18S were loaded into 96-well plates in duplicate and sealed with optical sealing tape (Applied Biosystems, Foster City, CA). Reaction mixtures containing water, but no cDNA, were used as negative controls; thus approximately three to four individual tortoise samples were run per plate.

Amplifications were conducted on a 7300 Real-time Thermal Cycler (Applied Biosystems, Foster City, CA). Reaction conditions were as follows: 50°C for 2 min, 95°C for 15 min, 45 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 31 s, and an extended elongation phase at 72°C for 10 min. Reaction specificity was monitored by melting curve analysis using a final data acquisition phase of 60 cycles of 65°C for 30 s and verified by direct sequencing of randomly selected amplicons (Bowen et al. 2007). Cycle

Table 2. Desert Tortoise-Specific Quantitative Real-Time Polymerase Chain Reaction Primers Used in the Analysis of Free-Ranging Tortoises

Gene	Forward primer	Reverse primer	Amplicon length (bp)
AHR	atgcttaccgaagacagcac	ctcctcatcctgctgaatac	119
CAL	agacagcgaggaggagatc	tcatcatctgtcagcttctca	122
CD9	gccattgaagtagctgttgc	gctggctgattcctcttatc	106
MDF88	gtccaagagatgatcagaga	catcttcctacaccgtctct	134
MX-1	tggacactaaggttcagagt	cacgtggcaccacttctg	128
HSP 70	gccttcacsgacaccgag	aggcgcttggcatcgaaga	87
SAA	gacatgcgggaagcgaattata	gccaggccctctttgg	83
Leptin	caccaactaaagattcagcac	gtggagtagtcattgatgtgt	145
CL	gtcagtgtggatcttgttnggca	tcatntccttttgggatgtccac	127
SOD	tcaatcctaatggcaaaaacca	cagcaatcacattgccaagatc	79
18S	actcaacacgggaaacctca	aaccagacaaatcgctccac	124
ATF	agtgttggcaaaggtgaaggga	gcacataacttctactctgactacc	70

See Table 1 for interpretation of gene abbreviations.

threshold (C_T) crossing values, the measure of gene transcription for the genes of interest, were normalized to the 18S housekeeping gene.

Clinical Health Assessments

Health evaluations were performed using recommended clinical procedures (USFWS 2011b; Lamberski et al. 2012). Evaluations included the animal's general posture, respiration, and detailed visual inspection of the face (with specific attention to the eyes, periocular tissue, nares, mouth, tongue, and oral mucosa), skin, and shell for any clinical or physical abnormality in association with the animal's weight, behavior, activity, and body condition (USFWS 2011b). Clinical signs associated with physiological changes in desert tortoises often include periocular or conjunctival swelling or sunken eyes, mucous and serous ocular and nasal discharge, occluded eroded or asymmetrical nares, abnormal respiration, and weight loss. Abnormal respiration includes shortness of breath, uncomfortable awareness of breathing effort, and wheezing. For this study, tortoises categorized as having *only* "sunken eyes" (often an indicator of dehydration or emaciation) or "asymmetrical nares" (which may be a developmental anomaly or indicator of previous respiratory diseases) were not considered clinically compromised.

The body condition of each tortoise was scored using a value ranging from 1 to 9 to provide a relative index of the muscle mass, size, and attitude or behavior (USFWS 2011b; Lamberski et al. 2012). Body condition scoring (BCS) is commonly used for domestic animals and wildlife to estimate body condition in animal herds by quantifying the muscle mass and fat deposits in relation to skeletal features. In particular, BCS indicators for tortoises include the amount of muscle tissue and fat deposits around the sagittal crest and limbs, in addition to the relative amount of muscle and fat tissue around the limbs and inguinal areas of exposed body tissue. For desert tortoises, BCSs are generally ranked in three categories: under-conditioned (1–3), acceptable or good condition (4–6), and over-conditioned (7–9).

Statistical Analyses

We analyzed qPCR data using normalized values (housekeeping gene threshold crossing subtracted from the gene of interest threshold crossing); the lower the normalized value, the more the transcripts present. A change in normalized value of 2 is approximately equivalent to a fourfold change in the amount of the transcript. Commonly used parametric tests to distinguish differential gene transcription among populations were based partly on the assumption that the values being compared were sampled from normally distributed populations. We tested the assumption that transcript levels of our genes of interest were log-normally distributed, in order to use such tests for detection of disease or environmental stressors (McLoughlin et al. 2006). Geometric means and 95% upper and lower confidence limits were computed, and Anderson–Darling and Shapiro–Wilk tests for normality were performed for all genes (NCSS, Statistical and Power Analysis Software, UT, USA). We used

nonparametric statistical analyses because C_T measures of gene transcription provided by qPCR were not normally distributed for 10 of the 11 genes of interest. First, we used conventional mean responses per classification group (Piute Valley, Hidden Valley, Ft. Irwin, and captive-impaired) with data assessed for statistical significance between classification ranks using Kruskal–Wallis with Dunns' Multiple Comparison Tests (NCSS, 2007, Kaysville, Utah). Then we conducted multivariate, multidimensional scaling analysis (MDS) in conjunction with cluster analysis for statistical and graphical representation of individual desert tortoises clustered by similarity in transcription and not by pre-defined groups such as location (Primer v6 software, Plymouth, UK). Statistical comparisons of individuals grouped by clusters were made using SIMPROF (Primer v6), which is a similarity profile permutation test for significance among a priori, unstructured clusters of samples. Transcription responses of sex by sites were compared using ANOSIM (Primer v6), a nonparametric analog to a 2-way ANOVA. Statistical significance was based on P values ≤ 0.05.

Results

Clinical Conditions

Of the 64 free-ranging animals sampled, 11 (17.2%) were found to have clinically significant anomalies, with 7 (10.9%) having more than one anomaly. Anomalies included periocular edema, conjunctival edema and hyperemia due to inflammation, recession of periocular tissue, ocular and nasal discharge (both serous and mucoid), and occluded and eroded nares with associated reduction in activity. All wild tortoises had middle ranged BCSs (range 4–6), indicating adequate muscle and fat deposits relative to skeletal features such as the sagittal crest. ELISA test results for *M. agassizii* were 9 of 64 positive (14.0%) and 12 positive for *M. testudenium* (18.8%). PCR test results for Testudinid Herpesvirus 2 were negative for all tortoises.

Means and ranges, respectively, of additional laboratory analyses for 24 desert tortoises were as follows: total protein (g/dL): 3.18, 0.2–6.0; A/G: 0.30, 0.16–0.49; prealbumin (gpe): 0.09, 0.01–0.19; albumin (g/dL): 0.57, 0.03–0.95; α_1-globulins: 0.48, 0.03–1.22; α_2-globulins: 0.55, 0.03–1.15; β-globulins: 1.20, 0.05–2.82; γ-globulins: 0.27, 0.02–0.50; SAA (mg/L): 23.23, 0.17–77.01. Electrophoresis results were non-remarkable; however, standard reference ranges for this species do not exist for most variables.

Gene Transcription Profiling

Desert tortoise-specific qPCR primers for the 11 genes of interest and housekeeping gene are defined in Table 2. All genes had unimodal distributions, but only one gene, serum amyloid A (SAA), had transcript values following a normal distribution ($P = 0.24$). Additionally, the 95% confidence intervals were independent of the mean normalized values, indicating that the dynamic ranges did not depend upon a gene's transcript level, as all primer efficiencies were approximately equal.

Statistical analysis using a priori groupings (i.e., Piute Valley, Hidden Valley, Ft. Irwin, and captive-impaired) indicated significant differences among groups for several genes (Table 3). Three genes were transcribed at significantly higher levels in the captive-impaired tortoises than in other groups (MX1, CD9, and MyD88). Two genes were transcribed at significantly lower levels in captive-impaired tortoises than in other groups (ATF and leptin). Significant differences in gene transcription were also identified among free-ranging tortoise groups; transcription patterns consistent with a stronger anti-inflammatory and anti-bacterial response existed in tortoises from Fort Irwin in comparison with tortoises from Hidden Valley. Transcription patterns consistent with an increased bacterial response in tortoises from Piute Valley were evidenced in comparison with tortoises from Hidden Valley (Table 3).

MDS and cluster analyses of transcription C_T values indicated strong differentiation of a large cluster and multiple outlying individual tortoises or small clusters in multidimensional space (Fig. 1). There was little to no indication of separation or clusters by sites (Fig. 1). In addition, transcription responses did not differ significantly between sexes ($P > 0.69$) across all sites or among sites by sex ($P > 0.19$). Cluster analysis and SIMPROF identified primarily significant differences ($P < 0.001$–0.024 and one at $P < 0.05$) among 18 distinct clusters, 17 of those consisting of 1–9 individual tortoises, and one cluster consisting of 26 individuals. We used the geometric mean C_T of the largest cluster as the benchmark to qualitatively compare the remaining clusters, with a conservative focus on genes in clusters that were 3 or more C_T difference from genes in the benchmark cluster (Table 4). The 64 free-ranging tortoises had normal BCS, and only 17.2% had visual signs of clinically significant anomalies; thus it stands to reason that the largest grouping would represent animals generally in good health. Examining transcription by individual genes (Table 1), the data were not highly variable. For convenience, we arrange the clusters into

Table 3. Geometric Mean Normalized (to the 18S Housekeeping Gene in Each Animal) Cycle Threshold (C_T) Transcription Values for Targeted Genes (see Table 1) in Desert Tortoises Sampled at Piute Valley, Hidden Valley, Ft. Irwin and Captive Desert Tortoises

	Location			
	Captive-impaired	Piute valley	Hidden valley	Ft. Irwin
n:	19	17	8	39
SAA	16.58 (15.58, 17.63)	16.14 (15.05, 17.31)	15.98 (12.60, 20.27)	16.76 (15.94, 17.63)
HSP70	11.93 (11.00, 12.94)	12.20 (11.16, 13.34)	12.21 (11.20, 13.31)	12.48 (11.99, 12.99)
MX1	15.58[a] (14.47, 16.77)	18.35[b] (17.52, 19.23)	16.06 (12.54, 20.58)	16.82[b] (15.75, 17.96)
CD9	12.64[a] (11.97, 13.35)	11.31[a] (9.39, 13.62)	14.35[b] (12.85, 16.03)	13.02 (12.10, 14.02)
SOD	9.56 (8.86, 10.31)	9.49 (8.41, 10.70)	10.43 (9.40, 11.58)	10.50 (9.75, 11.31)
AHR	13.95 (12.88, 15.10)	14.54 (13.92, 15.19)	14.55 (11.50, 18.41)	14.17 (13.71, 14.66)
MyDA88	15.43[a] (14.75, 16.14)	15.56 (14.94, 16.20)	16.91[b] (15.19, 18.81)	15.61 (15.19, 16.03)
CaM	9.56 (9.18, 9.95)	9.55 (8.78, 10.40)	10.20 (8.85, 11.74)	9.53 (9.00, 10.10)
ATF	11.17[a] (9.88, 12.62)	10.28 (8.81, 12.00)	12.98[a] (9.46, 17.82)	8.74[b] (7.70, 9.91)
CL	16.68 (15.33, 18.16)	15.96 (14.81, 17.21)	19.38[a] (14.45, 26.00)	15.00[b] (14.25, 15.79)
Leptin	14.15[a] (12.90, 15.51)	12.97 (11.71, 14.36)	13.71 (11.31, 16.62)	12.37[b] (11.71, 13.06)

Note that the *smaller* the mean value, the *higher* the level of transcription. Unique letters indicate significant difference. An absence of letters indicates no significant difference

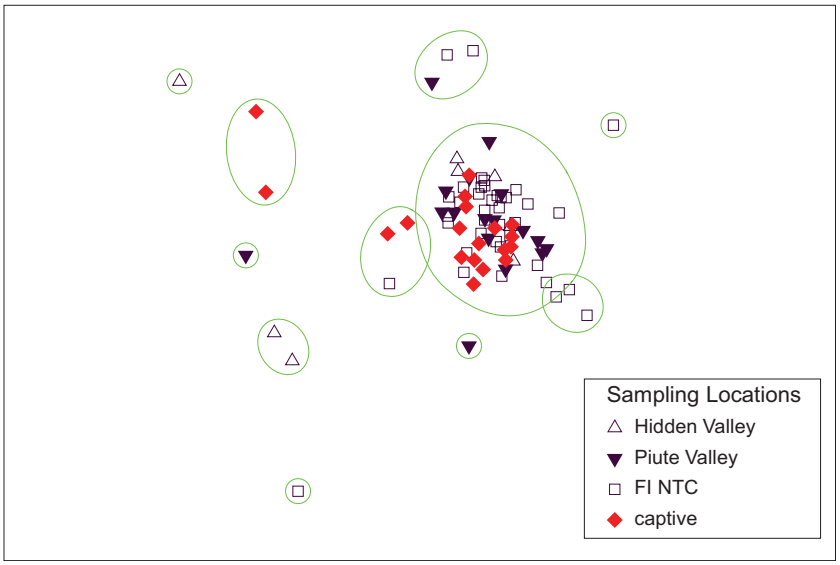

Figure 1. Multivariate, nonparametric, multidimensional scaling of gene transcription profiles (see Table 1) of free-ranging desert tortoises. Interpretive cluster analysis and SIMPROF (similarity profile permutation test, Primer v6) indicated significant ($P < 0.001$–0.007 for 16 clusters and $P < 0.05$ for one cluster) separation among all clusters depicted by *circles*. Shown is two-dimensional (stress = 0.09), but three-dimensional (stress = 0.04) representation shows less overlap of *circles* among clusters.

three groups (A, B, and C) based on similarity of transcript profile.

Group A: Transcripts in clusters 2 and 3 were <3 C_T difference from those in the benchmark, cluster 1. These two clusters differed because overall transcription was slightly higher (0.3–2.4 C_T difference) across all genes compared to the benchmark (Table 4).

Group B and C: Transcription of the other 42 tortoises separated among 15 clusters was variable, but within group patterns were evident. Group B (14 tortoises) comprised 7 clusters or individual outliers (4–10 in Table 4) and had 4–7 genes with 3–11 C_T difference from the benchmark. Transcription patterns between individual tortoises and among clusters within this group were highly variable. For example, an individual tortoise identified in Cluster #4 (Table 4) had elevated transcription for all genes evaluated, whereas the individual tortoise identified in cluster # 5 had elevated transcription for SAA, HSP70, CD9, SOD, AHR, MyD88, and CaM, but had downregulated the transcription for MX1, ATF, CL, and Leptin.

Table 4. Geometric Mean C_T Values for Each Cluster

Cluster	*n*	saa	HSP70	MX1	CD9	SOD	AHR	MDF88	CaM	ATF	CL	Leptin
Group A	41											
1 (dataset standard)	26	18.02	12.82	18.87	13.89	10.80	15.08	15.95	10.29	10.88	15.55	13.69
2	9	17.51	11.96	17.43	11.97	9.21	13.78	14.90	8.61	8.68	14.39	12.70
3	6	15.71	12.68	16.50	12.69	9.88	13.75	15.20	9.60	10.53	15.49	12.33
Group B	14											
4	1	15.75	9.40	13.09	7.02	6.79	11.42	13.44	6.52	3.69	12.08	11.27
5	1	14.18	8.00	20.59	5.36	6.14	13.27	13.46	6.92	12.41	16.86	14.71
6	1	9.06	11.03	8.18	24.00	24.00	10.06	19.06	19.06	15.61	18.88	11.17
7	1	20.70	15.40	20.87	16.59	13.78	14.56	16.00	10.02	2.61	10.60	5.85
8	5	14.31	11.34	15.53	10.93	7.99	13.24	14.12	7.63	7.65	13.27	11.37
9	3	22.24	17.36	21.97	18.87	16.67	17.68	18.67	10.93	11.40	18.17	13.37
10	2	10.38	10.82	10.31	14.85	11.46	10.58	19.08	11.27	18.19	28.69	12.23
Group C	28											
11	6	16.16	12.60	14.85	12.92	9.58	12.02	14.99	9.14	7.75	14.99	13.05
12	2	15.91	11.36	19.96	9.56	7.80	14.41	14.94	8.46	6.66	13.73	13.11
13	3	14.96	12.41	14.51	11.47	9.99	15.26	16.54	9.89	13.21	21.58	14.70
14	4	17.08	9.50	18.21	11.76	7.81	17.95	18.17	10.86	21.31	23.90	22.05
15	2	15.38	11.50	12.94	7.87	8.51	12.63	14.50	8.09	3.98	13.77	11.97
16	3	16.06	12.40	17.09	12.55	9.78	13.76	14.94	9.79	7.70	15.30	8.61
17	7	14.87	11.98	13.38	12.27	10.12	13.96	14.84	8.92	11.11	16.17	12.83
18	1	19.87	15.67	18.20	14.80	13.41	16.33	17.54	12.24	9.18	16.70	10.01

Statistical analyses of transcription C_T values indicated strong differentiation of a large cluster and multiple smaller clusters in multidimensional space (Fig. 1). Cluster analysis and SIMPROF identified primarily significant difference ($P < 0.001$–0.024 and one at $P < 0.05$) among 18 distinct clusters, 17 of those consisting of 1–9 individual tortoises, and the largest cluster consisting of 26 individuals. We used the geometric mean C_T values of the largest cluster (Cluster 1) as a benchmark or dataset standard to qualitatively compare the remaining clusters, with focus on genes that had 3 or more C_T value difference from genes in this cluster.

The 28 tortoises in group C separated across 8 clusters (11–17 in Table 4). Fifteen tortoises had transcription values of 3 genes that expressed a 3–4 C_T difference, and 13 tortoises had just 1–2 significantly different genes but most of these were 5–6 C_T different from the benchmark. The 15 tortoises also had elevated transcription of MX1, AHR, and ATF (6 tortoises), elevated CD9, SOD, and ATF (2 tortoises), and elevated SAA, MX1, but low (6 C_T value difference) CL (3 tortoises), or elevated HSP70 and low transcription of ATF, CL, and leptin (4 tortoises). The other 13 tortoises clustered by elevated MX1 and CD9 (2 tortoises), ATF and leptin (3), SAA and MX1 (7), or only elevated leptin (1).

Transcription of MX1 was elevated (i.e., 3–11 C_T value difference from the benchmark) in more (8 out of 18) clusters than any other gene. Transcription of SAA was elevated in 6 clusters (and low in another cluster) and was most closely associated with elevated C_T of MX1 in those 6 clusters. Transcription of ATF was high in 6 clusters and low in 3 other clusters. Transcriptions of the genes CD9 and SOD were elevated in 4 clusters, with CD9 elevated in an additional cluster. The gene AHR was elevated in 4 clusters, and the remaining genes (HSP70, CaM, CL, leptin, MyD88) were elevated in 3 or fewer clusters with no apparent association of increased (or decreased) transcription with other genes.

Clinical health matrices analyzed by clusters did not correlate with gene transcription profiles. The 19 captive adult tortoises deemed unhealthy generally did not cluster by gene transcription pattern when considering all genes combined. However, two-thirds of these animals were associated with clusters other than the benchmark or the 2 clusters with pattern similar to the benchmark. Proportionately, fewer (11%) were associated with the benchmark than any other cluster where found (17–71%) (Table 4).

Discussion

We describe the first leukocyte gene transcript data for desert tortoises. Acquisition of a blood leukocyte transcript profile has the potential to identify stressed or diseased animals prior to the onset of clinical signs (Bowen et al. 2012; Stott and McBain 2012). This gene transcript profile included a combination of immune response or physiological defense-associated genes with the potential to be modified by biological or physical injury and consequently provide information on the type and magnitude of stressor present in the animal's environment. Variable transcript patterns may be indicative of the presence of environmental or immunological stressors. Our findings suggest increased biotic or abiotic stimuli in these tortoises' environments that triggered increased immune or cellular detoxification responses. The pattern across genes and among geographic groups of animals indicates that some of these environmental effects were location specific. These patterns probably reflect site-specific environmental perturbations. Additionally, gene transcript profiles support clinical diagnoses for captive tortoises.

Significant transcript profile differences existed among a priori groupings (i.e., Piute Valley, Hidden Valley, Ft. Irwin, and captive-impaired). This is likely reflective of different environmental conditions in these locations. The three genes transcribed at significantly higher levels in the captive-impaired tortoises than in other groups (MX1, CD9, and MyD88) may be indicative of response to viral, bacterial, and microbial infection, respectively. These animals were held in captivity and were selected for euthanasia due to their poor clinical conditions. The increased transcription of these three genes could simply be associated with animals in poor condition or it could potentially be associated with microbial spread in captive conditions. The two genes transcribed at significantly lower levels in captive-impaired tortoises than in other groups (ATF and leptin) are indicative of an anti-inflammatory response and poor nutrition, respectively. Significant differences in gene transcription were also identified among free-ranging tortoise groups, indicating differential environmental pressures present in each area.

A key requirement for translation of gene transcript biomarker technology to clinical applications is the establishment of a normal or "reference" range of values, distinguishing between natural variation in gene transcript levels among healthy subjects and changes among those with compromised health (McLoughlin et al. 2006). In desert tortoises, determining a normal baseline for clinically healthy tortoises proved challenging. The transcript results taken as a whole weakly correlated with standard clinical assessments of health and may be more sensitive indicators of physiological perturbations (McLoughlin et al. 2006). Standard clinical assessments of health, limited pathogen diagnostics, and generalized definitions and terminology associated with both may be inadequate to accurately reflect the health status of such wildlife species (Christopher et al. 2003). Due to the reduced metabolic state and activity level of poikilothermic organisms, clinical signs of health or disease conditions may be slow to emerge (Lykakis and Cox 1968; Christopher et al. 2003) even when transcriptional responses have begun.

The earliest detectable signs of physiologic imbalance are altered levels of gene transcripts, and those are often associated with immune function (McLoughlin et al. 2006). Measurements of the functional immune states as elucidated by gene transcription could potentially add valuable information to current health assessment matrices. To analyze our transcript results, we used the largest cluster of tortoises identified by transcript analysis, a subset of those deemed clinically healthy, as our benchmark with which to compare other tortoises varying transcript patterns and clinical health conditions.

Of particular note, in order of prevalence, transcription of MX1, SAA, ATF, and to a lesser extent CD9, SOD, and AHR was elevated in 4 or more clusters of tortoises. The association of MX1 with SAA, and also ATF and SOD has been linked with bacterial or viral infections in other species (Wiens and Vallejo 2010; Zou et al. 2013). These findings correlate well with observations of potential pathogen-related health issues in desert tortoises across their range (Jacobson et al. 1995; Sandmeier et al. 2013). Elevated AHR has been linked to organic contamination, and though not as prevalent as the pathogen indicator genes, this finding was noteworthy in typically remote natural areas. One possible explanation is that Ft. Irwin and the surrounding areas are largely military bases with active artillery and bombing ranges. Our findings of elevated transcription did not signify that these tortoises were experiencing ill health and could be more indicative of a properly functioning immune response rather than an immune-compromised system. However, the potential for elevated environmental stressors in the habitats of these tortoises requires investigation. Costs associated with

mounting an immune or detoxification response create a delicate balance between a protective immune phenotype (transcription pattern in this case) and a potential misallocation of resources. Trade-offs among components of the vertebrate immune defenses themselves are common and occur when one portion of an organism's immune system is upregulated while another portion is downregulated (Pedersen and Babayan 2011). Failure to acknowledge or measure these trade-offs may lead to erroneous conclusions.

There is a dearth of knowledge regarding protective transcription phenotypes of non-model organisms. Identification of the ideal protective immune phenotype can only be measured through long-term sampling or experimental manipulations (Pedersen and Babayan 2011). Thus, continued development of gene transcription research is essential for the development of future diagnostic capabilities and improved understanding of environmental effects on sensitive wildlife species such as the desert tortoise. To date, a full clinical, morphological, and transcript workup on tortoises has not been permitted as this species is protected under the Endangered Species Act; however, information gleaned from known healthy and unhealthy tortoises would help create a baseline from which to understand transcriptional responses.

Fourteen known wild tortoises were identified in this study that appeared most indicative of environmental stressors that taxed their immune or detoxification systems. Health assessments of these and also other animals in the study would further refine identification of conditions that may link directly to elevated transcription of multiple but specific genes. Study focused on the habitats of these animals to determine factors that could stimulate transcription would also be useful. Finally, our study was by no means a comprehensive evaluation of gene transcription as a useful tool for assessing desert tortoises' state of health. Future studies are necessary to identify gene responses specific to known injurious stimuli, and to increase the efficacy of the diagnostic capability by inclusion of additional immune or detoxification genes.

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References

Acevedo-Whitehouse K, Duffus LJ (2009) Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B* 364:3429–3438

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410

Bartosiewicz M, Penn S, Buckpitt A (2001) Applications of gene arrays in environmental toxicology: fingerprints of gene regulation associated with cadmium chloride, benzo(a)pyrene, and trichloroethylene. *Environmental Health Perspectives* 109:71–74

Belgrave RL, Dickey MM, Arheart KL, Cray C (2013) Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *Journal of the American Veterinary Medical Association* 243:113–119

Bowen L, Schwartz J, Aldridge B, Riva F, Miles AK, Mohr FC, Stott JL (2007) Differential gene expression induced by exposure of captive mink to fuel oil: a model for the sea otter. *EcoHealth* 4:298–309

Bowen L, Miles AK, Murray M, Haulena M, Tuttle J, Van Bonn W, Adams L, Bodkin JL, Ballachey B, Estes J, Tinker MT, Keister R, Stott JL (2012) Gene transcription in sea otters (*Enhydra lutris*); development of a diagnostic tool for sea otter and ecosystem health. *Molecular Ecology Resources* 12:67–74

Brown DR, Schumacher IM, McLaughlin GS, Wendland LD, Brown MB, Klein PA, Jacobson ER (2002) Application of diagnostic tests for mycoplasmal infections of desert and gopher tortoises, with management recommendations. *Chelonian Conservation and Biology* 4:497–507

Burczynski ME, McMillian M, Ciervo J, Li L, Parker JB, Dunn RT II, Hicken S, Farr S, Johnson MD (2000) Toxicogenomics-based discrimination of toxic mechanism in HepG2 human hepatoma cells. *Toxicological Sciences* 58:399–415

Chen Z, Wang H, Matsumura K, Qian P (2012) Expression of calmodulin and myosin light chain kinase during larval settlement of the barnacle *Balanus Amphitrite*. *PLoS ONE* 7:e31337

Christopher MM, Berry KH, Henen BT, Nagy KA (2003) Clinical disease and laboratory abnormalities in free-ranging desert tortoises in California (1990-1995). *Journal of Wildlife Diseases* 39:35–36

Cray C, Belgrave RL (2013) Haptoglobin quantitation in serum samples from clinically normal and clinically abnormal horses. *Journal of Equine Veterinary Science* 34(2):337–340

Cray C, Zaias J, Altman NH (2009) Acute phase response in animals: a review. *Comparative Medicine* 59:517–526

Cray C, Rodriguez M, Decker LS, Arheart KL (2011) Differences in protein fractions of avian plasma among three commercial electrophoresis systems. *Journal of Avian Medicine and Surgery* 25(2):102–110

De Maio A (1999) Heat shock proteins: facts, thoughts, and dreams. *Shock* 11:1–12

Esque TC, Nussear KE, Drake KK, Walde AD, Berry KH, Averill-Murray RC, Woodman AP, Boarman WI, Medica PA, Mack J, Heaton JS (2010) Effects of human population density, resource variability, and subsidized predators on desert tortoise populations in the mojave desert. *Endangered Species Research* 12(2):167–177. doi:10.3354/esr00298

Hernandez-Divers SM, Hernandez-Divers SJ, Wyneken J (2002) Angiographic, anatomic and clinical technique descriptions of a subcarapacial venipuncture site for chelonians. *Journal of Herpetological Medicine and Surgery* 12(2):32–37

Iwama GK, Mathilakath MV, Forsyth RB, Ackerman PA (1999) Heat shock proteins and physiological stress in fish. *American Zoologist* 39:901–909

Jacobson ER, Brown MB, Schumacher IM, Collins BR, Harris RK, Klein PA (1995) Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. *Chelonian Conservation and Biology* 1:279–284

Jacobson ER, Berry KH, F-X J, Wellehen JR, Origgi F, Childress A, Braun J, Schrenzei M, Yee J, Rideout B (2012) Serological and molecular evidence for Testudinid herpesvirus 2 in wild Agassiz's desert tortoises, *Gopherus agassizii*. *Journal of Wildlife Diseases* 48(3):747–757

Kibenge MJT, Munir K, Kibenge FSB (2005) Constitutive expression of Atlantic salmon Mx1 protein in CHSE-214 cells confers resistance to infectious salmon anaemia virus. *Virology Journal* 2:75

Lamberski N, Braun J, Witte C, Christopher M, Field K, Averill-Murray R, Keener L, Robbins P, Johnson J, Covert A, Walsh A, Rideout B (2012) Identifying key clinical signs and validating body condition scores to minimize disease spread and maximize individual survival during desert tortoise translocations. In: *Proceedings of the Joint Annual Meeting for Wildlife Disease Association and European Association of Wildlife Diseases*, Lyon, France

Li S, Xie L, Zhang C, Zhang Y, Gu M, et al. (2004) Cloning and expression of a pivotal calcium metabolism regulator: calmodulin involved in shell formation from pearl oyster (*Pinctada fucata*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 138:235–243

Li X, Zhu B, Chen N, Hu H, Chen J, Zhang X, Li J, Fang W (2011) Molecular characterization and functional analysis of MyD88 in Chinese soft-shelled turtle *Trionyx sinensis*. *Fish Shellfish Immunology* 30:33–38

Luckenbach RA (1982) Ecology and management of the desert tortoise (*Gopherus agassizii)* in California. In: *North American Tortoises: Conservation and Ecology*, Bury RB (editor), Washington, DC: USDOI-FWS, Wildlife Research Report 12, pp 1–37

Lykakis JJ, Cox FEG (1968) Immunological response of the toad, *Xenopus laevis*, to the antigens of the ciliate, *Tetrahymena pyriformis*. *Immunlogy* 15:429–437

McLoughlin K, et al. (2006) Limited dynamic range of immune response gene expression observed in healthy blood donors using RT-PCR. *Molecular Medicine* 12:185–195

Miles AK, Bowen L, Ballachey B, Bodkin JL, Murray M, Keister RA, Stott JL (2012) Variations of transcript profiles between sea otters (*Enhydra lutris*) from Prince William Sound, Alaska, and clinically normal reference otters. *Marine Ecology Progress Series* 451:201–212

Oesch-Bartlomowicz B, Oesch F (2005) Phosphorylation of cytochromes P450: first discovery of a posttranslational modification of a drug-metabolizing enzyme. *Biochemical and Biophysical Research Communications* 338:446–449

Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gomez-Reino JJ, Gualillo O (2005) Leptin, from fat to inflammation: old questions and new insights. *FEBS Letters* 579:295–301

Pedersen AB, Babayan SA (2011) Wild immunology. *Molecular Ecology* 20:872–880

Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL (2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 453:46–47

Ritchie B (2006) Virology. In: *Reptile Medicine and Surgery*, Mader DR (editor), *2nd ed.* St. Louis, MO: Saunders Elsevier, pp 391–417

Robinson J, Waller M, Parham P, Bodmer JG, Marsh SGE (2001) IMGT/HLA database—a sequence database for the human major histocompatibility complex. *Nucleic Acids Research* 29:210–213

[USFWS] U.S. Fish and Wildlife Service (2011) *Revised Recovery Plan for the Mojave Population of the Desert Tortoise (Gopherus agassizii*), Sacramento, CA: U.S. Fish and Wildlife Service, Pacific Southwest Region

[USFWS] U.S. Fish and Wildlife Service (2011) *Health Assessment Procedures for the Desert Tortoise (Gopherus agassizii*): A Handbook Pertinent to Translocation, Reno: Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service

Sandmeier FC, Tracy CR, Hagerty BE, DuPre S, Mohammadpour H, Hunter K (2013) Mycoplasmal upper respiratory tract disease across the range of the threatened Mojave desert tortoise: associations with thermal regime and natural antibodies. *EcoHealth* 10:63–71

Sitt T, Bowen L, Blanchard MT, Smith BR, Gershwin LJ, Byrne BA, Stott JL (2008) Quantitation of leukocyte gene expression in cetaceans. *Developmental and Comparative Immunology* 32:1253–1259

Stott JL, McBain JF (2012) Fowler's zoo and wild animal medicine: current therapy. In: *Longitudinal Monitoring of Immune System Parameters of Cetaceans and Application to Their Health Management*, Chap. 63, Eric Miller R, Fowler M, (editors), 7th ed., Davis: University of California, pp 482–489

Tanabe S, Iwata H, Tatsukawa R (1994) Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Science of the Total Environment* 154:163–177

Tracy CR, Averill-Murray RC, Boarman WI, Delehanty DJ, Heaton JS, McCoy ED, Morafka DJ, Nussear KE, Hagerty BE, Medica PA (2004) *Desert Tortoise Recovery Plan Assessment*, Reno: Technical Report. U.S. Fish and Wildlife Service

Tsan M, Gao B (2004) Cytokine function of heat shock proteins. *American Journal of Physiology-Cell Physiology* 286:C739–C744

Tumpey TM, Szretter KJ, Van Hoeven N, Katz JM, Kochs G, Haller O, Garcia-Sastre A, Staecheli P (2007) The Mx1 gene protects mice against the pandemic 1918 and highly lethal human H5N1 influenza viruses. *Journal of Virology* 81:10818–10821

Turner RM, Brown DE (1982) 154.1 Sonoran desertscrub. In: *Biotic Communities of the American Southwest-United States and Mexico*, Brown DE (editor), Desert Plants 4, pp 181–221

Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 453:106–109

Walsh CJ, Leggett SR, Carter BJ, Colle C (2010) Effects of brevetoxin exposure on the immune system of loggerhead sea turtles. *Aquatic Toxicology* 97:293–303

Wendland LD, Zacher LA, Klein PA, Brown DR, Demcovitz D, Littell R, Brown MB (2007) Improved enzyme-linked immunosorbent assay to reveal *Mycoplasma agassizii* exposure: a valuable tool in the management of environmentally sensitive tortoise populations. *Clinical and Vaccine Immunology* 14:1190–1195

Wiens GD, Vallejo RL (2010) Temporal and pathogen-load dependent changes in rainbow trout (*Oncorhynchus mykiss*) immune response traits following challenge with biotype 2 *Yersinia ruckeri*. *Fish and Shellfish Immunology* 29:639–647

Zhou X, Guo QL, Dai HP (2008) Identification of differentially expressed immune-relevant genes in Chinese soft-shelled turtle (*Trionyx sinensis*) infected with *Aeromonas hydrophila*. *Veterinary Immunology Immunopathology* 125:82–91

Zhou X, Wang L, Feng H, Guo Q, Dai H (2011) Acute phase response in Chinese soft-shelled turtle (*Trionyx sinensis*) with *Aeromonas hydrophila* infection. *Developmental and Comparative Immunology* 35:441–451

Zou W, Chen D, Xiong M, Zhu J, Lin X, Wang L, Zhang J, Chen L, Zhang H, Chen H, Chen M, Jin M (2013) Insights into the increasing virulence of the swine-origin pandemic H1N1/2009 influenza virus. *Sciences Report* 3:1601

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Mitigation-driven translocations: are we moving wildlife in the right direction?

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Despite rapid growth in the field of reintroduction biology, results from scientific research are often not applied to translocations initiated when human land-use change conflicts with the continued persistence of a species' population at a particular site. Such mitigation-driven translocations outnumber and receive more funding than science-based conservation translocations, yet the conservation benefit of the former is unclear. Because mitigation releases are economically motivated, outcomes may be less successful than those of releases designed to serve the biological needs of species. Translocation as a regulatory tool may be ill-suited for biologically mitigating environmental damage caused by development. Evidence suggests that many mitigation-driven translocations fail, although the application of scientific principles and best practices would probably improve the success rate. Lack of transparency and failure to document outcomes also hinder efforts to understand the scope of the problem. If mitigation-driven translocations are to continue as part of the growing billion-dollar ecological consulting industry, it is imperative that the scale and effects of these releases be reported and evaluated.

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The use of translocations – the human-mediated, intentional movement of living organisms from one area to another as a wildlife management tool – has risen exponentially over the past few decades (Seddon *et al.* 2007; IUCN/SSC 2013). Indeed, numbers of translocations are predicted to increase even more rapidly if assisted colonization is adopted as a tool for climate-change mitigation (Hoegh-Guldberg *et al.* 2008; IUCN/SSC 2013). Although the field of reintroduction biology is moving steadily forward through the application of scientific principles (Seddon *et al.* 2007), many wildlife translocations have evaded academic scrutiny and have not been subject to a common set of widely accepted standards. These translocations attempt to reduce animal mortality caused by human activities (eg development), by relocating individuals away from project sites. This motive for relocating animals is quite different from need-based strategies to augment or restore animal populations through conservation-driven translocations. While the motives may differ, the techniques used and scrutiny applied should be similar (IUCN/SSC 2013). Henceforth, we refer to translocations initiated to reduce animal deaths as a result of development activities as "mitigation-driven translocations" (Panel 1). Many countries have regulatory frameworks that cover such translocations. Legal requirements, driven in part by public perception and pressure, also provide the primary funding mechanism for such activities, requiring that developers relocate protected species from affected land. This raises the question of whether such animals are simply being spared a socially unacceptable death via bulldozer only to perish out of view at a release site (eg "phased destruction"; Jackson *et al.* 1983), or whether the translocations are a useful tool, wisely applied to minimize the effects of humans' actions on imperiled wildlife. We contend that mitigation-driven translocations, while well-financed, are often inappropriately executed, poorly documented, and unquestioningly used without regard to larger, more strategic conservation goals.

In a nutshell:

- The number of mitigation translocations – the movement of animals out of the path of development projects – appears to be increasing rapidly and likely far exceeds the number of conservation-driven translocations
- Mitigation-driven translocations all too often fail to follow accepted scientific best practices and are poorly documented, providing few opportunities to apply lessons learned and to improve the conservation efficacy of similar projects in the future
- A new approach to mitigation-driven translocations rejects "tit-for-tat" translocations as a way to mediate impacts at a development site and instead substitutes a more strategic allocation of resources in a scientifically responsible and conservation-relevant fashion

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Scope of the issue

Mitigation-driven translocations have risen sharply over the past 10–15 years (Miller *et al.* 2014), perhaps more so

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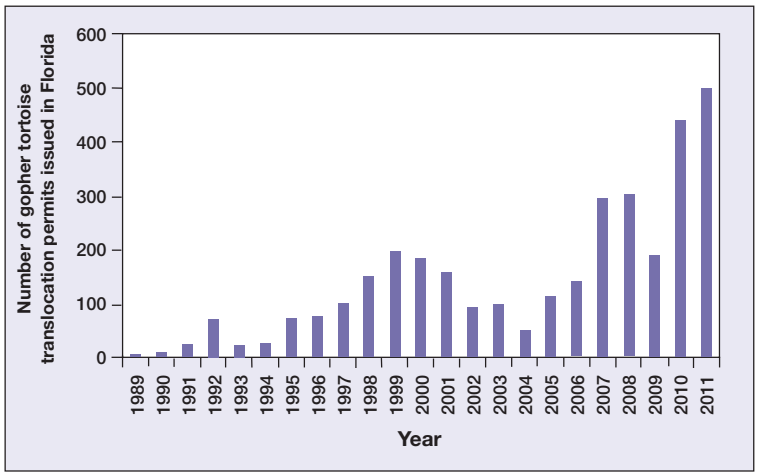

Figure 1. *Gopher tortoise (*Gopherus polyphemus*) translocation permits issued in the US state of Florida (1989–2011). Data from Florida Fish and Wildlife Conservation Commission, courtesy of S Power and J Berish.*

than conservation-based releases, and are often generously funded. It is currently impossible to ascertain the level of investment in these activities, but the global ecological consultancy market has an estimated value of between US$1.6 billion and US$4.8 billion (Hill and Arnold 2012). Although translocations may represent only a small proportion of consultancy services, the amount spent on mitigation-driven translocations easily dwarfs that allocated to conservation-focused translocation programs carried out by government and nonprofit groups. In Australia, an estimated AU$14.14 million (US$11.85 million) has been spent on mitigation-based translocations for a single species of frog (green and golden bell frog *Litoria aurea*) over the past 15 years, as compared with an estimated AU$3.29 million (US$2.76 million) devoted to conservation-based translocations for all other amphibian species combined during the same time period. In the UK, the cost of mitigating development-related impacts on great crested newts (*Triturus cristatus*) approaches £100 000–£200 000 (US$157 000–US$314 000) per project, an order of magnitude higher than the expenditure on other conservation actions for the same species (Lewis 2012). In the US, one solar energy project expected to spend US$22 million on activities related to the translocation of Mojave desert tortoises (*Gopherus agassizii*) from the development site (Desmond 2012); fortunately, a monitoring plan was included in this project's approval process. In Brazil, purportedly vast monetary sums are dedicated to relocating animals from the sites of large hydroelectric projects; such actions are legally required but essentially undocumented (Teixeira *et al.* 2007).

The numbers of animals moved from project sites can be astounding. In one particular case, almost 24 000 reptiles (290 adders [*Vipera berus*], 400 grass snakes [*Natrix natrix*], 17 000 common lizards [*Zootoca vivipara*], and 6000 slow worms [*Anguis fragilis*]) as well as 300 water voles (*Arvicola amphibius*) and over 350 great crested newts (*T cristatus*) were relocated for a port development project in the UK (Leggett 2011; Williams 2011). In Australia, almost 20 000 tadpoles and more than 350 post-metamorphic green and golden bell frogs were translocated to five locations as part of a single mitigation-based translocation program (McFadden *et al.* 2008); there are many other examples at similar or greater scales taking place. Since 1989, more than 70 000 individual gopher tortoises (*Gopherus polyphemus*), which are the focus of hundreds of mitigation-driven projects in the southeastern US, have been permitted for translocation in the state of Florida alone (Florida Fish and Wildlife Conservation Commission, unpublished data). The Florida Fish and Wildlife Conservation Commission, one of the few government agencies worldwide that is tracking wildlife translocations, has seen a marked increase in translocation permits issued for gopher tortoises during the past 5 years (Figure 1).

Panel 1. What is a mitigation-driven translocation?

We use the term "mitigation-driven" to characterize translocations that are implemented in response to legislation or governmental regulation, with the intent of reducing a development project's effects on animals or plants inhabiting the site. These translocations are therefore, by nature, reactions to immediate anthropogenic threats to species under governmental protection, such as those listed under the US Endangered Species Act. The regulatory intent is clearly to mitigate the population-level effects of the loss of individuals of the protected species and their habitat. However, we argue that the language and enforcement of these regulations often do not address this intended outcome effectively. The word mitigation is often used to describe an attempt to offset or balance out the unavoidable negative effects of a project by performing positive actions elsewhere; yet that is only one aspect of mitigation. Internationally, the Business and Biodiversity Offsets Programme recognizes a "mitigation hierarchy" that includes avoidance, minimization, rehabilitation/restoration, and offset (http://bbop.forest-trends.org/pages/mitigation_hierarchy). This hierarchy is mirrored in similar terms in environmental assessment mitigation and offset policies around the world. Regulation in the US defines mitigation as being inclusive of minimizing project impacts (US Code of Federal Regulations, Title 40, Chapter V, Part 1508, Section 1508.20, "Mitigation"). Under these regulations, translocations primarily serve as minimization (presumed reductions in the number of deaths that will occur as a direct result of the project). Without additional regulatory requirements to monitor the fate of translocated individuals, to design studies that improve translocation strategies for future attempts, and to ensure that relocated individuals contribute to the establishment of viable populations at the receiving site, the regulatory intent of mitigating population-level detrimental effects is little more than wishful thinking.

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Global lack of documentation

The outcomes of mitigation-driven translocations are often poorly documented. Despite the large numbers of animals translocated from the pathway of development, little attention is paid to whether the numbers removed represent a substantial proportion of the animals actually present at the site, or whether the translocated individuals survive to establish or augment a viable population at the release site. These issues can be particularly problematic for species that are hard to detect. In the UK, for example, one study involving a cryptic species of lizard (the slow worm *A fragilis*) showed that the removal of more than 100 individuals did not substantially deplete the population (Platenberg and Griffiths 1999). Claims that considerable numbers of animals can be removed from sites over the course of a few days or weeks seem untenable in many cases, and may reflect issues with detectability rather than actual numerical declines.

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Figure 2. *Black rhinoceros (*Diceros bicornis*) populations have benefited greatly from metapopulation management through translocation. Excellent record-keeping by government biologists and private partners has enabled the resulting data to be analyzed and applied for management.*

A wider understanding of mitigation-driven translocations is currently hindered by limited access to data. Even in wealthy nations such as Australia, the UK, and the US, record-keeping for mitigation-driven translocations is almost always inadequate, inaccessible, or nearly nonexistent. In Australia, state-based governance of development proposals discourages the creation of a national registry of approved developments and their conditions of consent (to development). State-based registries keep records of only the largest approved developments, and there is no example in Australia of a systematic registry that records the details of translocations that occur in a mitigation context. In the UK, the quantity and accessibility of records depends on the protection afforded to the species. For highly protected species, there is a legal requirement to submit a report to the relevant government agency. However, enforcement of this requirement has been limited; one-half of the case studies on file lack any type of report (Edgar *et al.* 2005; Lewis 2012). When reports are submitted, less than 10% contain informative population monitoring data (Lewis 2012). Although the US Fish and Wildlife Service (USFWS) issues permits for mitigation-based translocations of species protected under the US Endangered Species Act, there is no comprehensive system to track results. Furthermore, very few US state-based agencies monitor this issue, and in the rare cases when such translocations are tracked, it is often for only one or two key species (eg the Florida Fish and Wildlife Conservation Commission monitoring of gopher tortoise translocations).

Determining the effectiveness of mitigation-based translocations is often complicated by the absence of governmental tracking and monitoring. Moreover, the failure of developed nations to implement best practices for documenting translocations means that there are no model systems to emulate. The United Arab Emirates, for example, is currently experiencing extremely rapid development and is requiring translocations of flora and fauna for mitigation (Gardner and Howarth 2009), but the country's natural resource managers lack templates to follow. The utility of good record-keeping systems lies not only in documenting whether translocations are working properly, but also in evaluating the conditions and methodologies under which success is optimized. For instance, detailed record-keeping on black rhinoceros (*Diceros bicornis*; Figure 2) translocations by the South African governmental agencies administering a conservation-driven translocation program enabled a large, retrospective meta-analysis that identified key ecological and management factors predicting success (Linklater *et al.* 2011, 2012).

Poor implementation

The success rate of scientifically responsible translocations, outside the mitigation context, is approximately 26–46% (Griffith *et al.* 1989; Linnell *et al.* 1997; Germano and Bishop 2009), depending on the definition of success and the taxonomic group involved. Data for mitigation-based translocations may not exist or are difficult to obtain, either because post-release monitoring was not required or because the data are "buried" in largely inaccessible reports to regulatory agencies (but see Platenberg and Griffiths 1999; Esque *et al.* 2010). Yet in some cases conservation practitioners have been able to

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access such data, thereby providing insights into possible success rates for this underreported class of translocations.

On the west coast of New Zealand, thousands of endangered carnivorous land snails were translocated at a cost of over NZ$2 million (US$1.56 million) in order to make way for an opencast coal mine (TerraNature 2006). While monitoring occurred during the first 18 months, the death rate of tracked snails was 30%; population models suggested that such high mortality rates would cause population collapses for this long-lived and slow-to-breed species (Morris 2010). In California, populations of several species of kangaroo rats (*Dipodomys* spp), involving thousands of individuals, have been relocated through mitigation-driven translocations, but until recently, most of these efforts were unsuccessful, with no individuals surviving after 1 year post-release (Shier and Swaisgood 2012). By contrast, a scientifically based translocation program, sensitive to the ecological and behavioral requirements of the species, has yielded high survival and reproductive rates and the establishment of five new populations (Shier and Swaisgood 2012). This study raised an issue that was not immediately evident to wildlife managers: namely that translocating members of solitary, aggressive species alongside familiar neighbors affects establishment and post-release fitness. Without clear empirical evidence from carefully designed experiments, this knowledge would not have found its way into management strategies for the species. Other examples of poor translocation outcomes abound, albeit without scientific conservation-driven counterparts for comparison (Panel 2).

A parallel approach to mitigation-driven translocations may be found in the welfare-motivated relocations of nuisance wildlife, which also benefit little from the latest scientific innovations in conservation-driven translocations. Although rarely documented, what studies are available typically exhibit poor success rates (Massei *et al.* 2010), with mortality rates as high as 97% for gray squirrels (*Sciurus carolinensis*; Adams *et al.* 2004). By contrast, Frair *et al.* (2007) took an experimental approach to translocating North American elk (*Cervus elaphus*) from areas with high levels of human–animal conflict and releasing them into areas where the elk would have conservation value, thereby avoiding the need for lethal control. In a carefully designed experiment conducted in partnership with regional governmental agencies in Canada, these authors demonstrated empirically that, somewhat unexpectedly, releasing elk into high-quality foraging areas was associated with lower survival due to increased predation. This study again underscores the important role of experimentation in translocation biology. Mitigation-driven translocations represent an excellent opportunity to test hypotheses and, over time, devise optimal protocols based on clear empirical evidence. Unfortunately, this opportunity for improving knowledge is rarely taken.

The flaws in mitigation-driven translocations cannot

Panel 2. Case studies of mitigation-driven translocations

Mitigation-driven translocations are affecting a wide range of taxonomic groups worldwide (Figure 3). Although most cases lack adequate monitoring and reporting, the large and growing number of development projects should require scientists, managers, and policy makers to assess the use of this tool and its impacts on the wildlife it is meant to protect.

(a) Burrowing owls (*Athene cunicularia*) are routinely subjected to "passive relocation" in North America, where the birds are evicted from their burrows as a form of mitigation to remove them from harm's way before the land clearing begins. Although consultants are often paid lucrative fees to perform the relocation, post-release monitoring is rarely considered. It is therefore not known whether eviction ultimately leads to the same fate as being killed on site, or what effects the relocated owls might have on the social dynamics of individuals already residing at the site where they settle.

(b) In the UK, the great crested newt (*Triturus cristatus*) is frequently found at development sites, resulting in the licensing of hundreds of mitigation projects each year. Although millions of pounds sterling are spent on great crested newt mitigation in the UK, it is unclear whether the actions undertaken have been effective (Lewis 2012).

(c) Due to coal mining on public lands on New Zealand's west coast, over 6500 individuals of two species of large endemic and endangered land snails (*Powelliphanta* spp) have been translocated over the past 10 years (Morris 2010). Several of the translocated populations have already failed. More than 800 of these snails died while temporarily taken into captivity (TerraNature 2006).

(d) In the US, several mitigation-driven translocations of the endangered Stephens' kangaroo rat (*Dipodomys stephensi*) resulted in 100% mortality, while a science-based translocation program developed by testing behavioral and ecological hypotheses succeeded in establishing several reproducing populations (Shier and Swaisgood 2012)

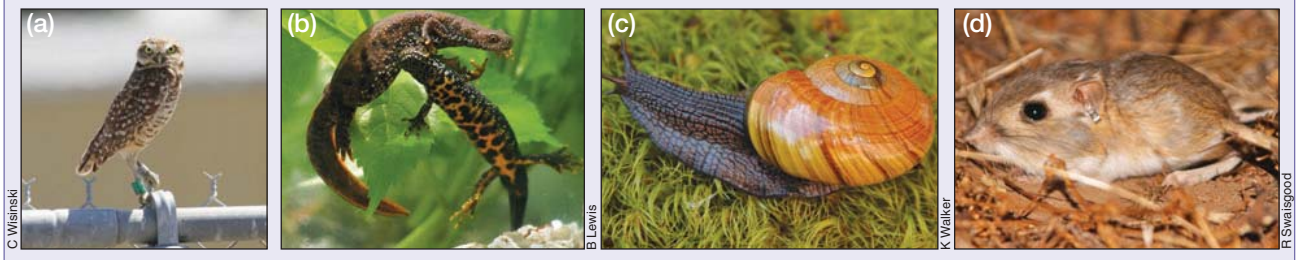

Figure 3. *Examples of species affected by mitigation translocations: (a) burrowing owl (*Athene cunicularia*); (b) great crested newt (*Triturus cristatus*); (c) land snails (*Powelliphanta*); (d) Stephens' kangaroo rat (*Dipodomys stephensi*).*

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be assigned solely to poor scientific preparation, as these initiatives are subject to constraints that are absent in conservation-driven translocations. For instance, the timing of the required actions is often driven by the developer's schedule, rather than by a sound ecological assessment of the length of time necessary to remove a population and re-establish it elsewhere. This is particularly problematic for habitat-limited but widespread species, where lack of suitable unoccupied sites within the species' range led to releases into occupied habitat with unknown consequences for the resident and receiving populations.

A misguided conservation strategy

While government agencies largely fail to document mitigation-driven translocations, the scientific community has likewise failed to raise this as a concern. Indeed, such activities are essentially ignored by the scientific community (though see Richard-Hansen *et al.* 2000; Edgar *et al.* 2005; Kyek *et al.* 2007; Esque *et al.* 2010; IUCN/SSC 2013). In a textbook on reintroduction biology, Seddon *et al.* (2012) outlined reasons for translocations; although several types of wildlife translocations – including conservation-driven efforts – were described in detail, there was little mention of mitigation-driven translocations. Meanwhile, the relative success of scientific conservation-driven translocations is being used to justify the use of mitigation-driven translocations globally.

Mitigation translocations often represent a misguided conservation strategy. In 1983, scientists warned that while translocating red-cockaded woodpeckers (*Picoides borealis*) from development sites in the southeastern US might be possible, it should not be considered as a simple or appropriate mitigation strategy (Jackson *et al.* 1983). Three decades later, the same concerns and considerations are still relevant, but are ignored by the majority of the conservation community and the involved regulatory agencies. Mitigation-driven translocations are, by our definition, supply driven, meaning that they are initiated in response to a supply of animals that must be removed from harm's way. Conservation translocations are demand driven, being implemented to establish viable populations in suitable unoccupied or under-occupied habitat with the aim of conserving the species. By contrast, the majority of mitigation-driven translocations serve no conservation purpose, despite regulatory intent. The current "tit-for-tat" approach – requiring direct alleviation of impacts specifically linked to the animals and plants at the affected site – virtually ensures a piecemeal, ad hoc approach to species conservation. Other goals for species recovery may then go unaddressed and unfunded, and the project may be unjustifiable as a conservation tool even if the translocated individuals survive. Indeed, translocations may do more harm than good if release sites are not carefully chosen. If animals are released into areas already near their ecological carrying capacity, then the survival of released animals may come at the cost of higher mortality among members of the resident population, potentially exacerbated by social disruption or disease transmission (Aiello *et al.* 2014). If survival and establishment are low, as we suspect is the case for many species, then translocation will have negligible conservation value, despite a suitable release site having been selected.

We contend that there is a large gap between the regulatory intent of mitigation-driven translocations and their implementation: moving individual animals away from a development site without addressing the bigger conservation picture. Part of the problem is conceptual: how does one define and assign metrics to "success" in such projects (Miller *et al.* 2014)? Establishment of a viable population from the relocated founders is not usually the primary goal of mitigation-driven releases, although it is the implied regulatory or legislative intent (Panel 1). However, there have been many failures to meet even the less lofty aim of ensuring survival of animals in the short term. Regrettably, most current regulatory regimes aim to protect individuals but fail to conserve populations, and therefore do neither.

Endangered species conservation has long been underfunded; only US$176 million was allocated to endangered species in the USFWS's 2012 budget (Corn 2012). More conservation dollars are not likely to be forthcoming, and it is imperative to use what little there is more wisely. Mitigation-driven translocations, funded by developers rather than taxpayers, should conform to best-practice standards for conservation science. It should be the responsibility of the developers, their consultants, and the regulatory agencies to demonstrate the effectiveness of translocation as a tool to achieve conservation outcomes that are consistent with the regulatory intent. This process should be transparent, with clear goals for each translocation and data made freely available for public scrutiny. If current regulations and practices do not uphold these standards, they should be revised. When translocation as a tool is ill-suited to offsetting the impacts of a planned development on a protected species, then the regulatory framework should be flexible enough to allow other, more strategic approaches, regardless of whether they entail the loss of some individuals. Under these circumstances, better use of development mitigation dollars can be realized if applied to achieve range-wide strategic conservation priorities for the affected species.

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References

Adams LW, Hadidian J, and Flyger V. 2004. Movement and mortality of translocated urban–suburban grey squirrels. *Anim Welfare* **13**: 45–50.

Aiello CM, Nussear KE, Walde AD, *et al.* 2014. Disease dynamics during wildlife translocations: disruptions to the host population and potential consequences for transmission in desert tortoise contact networks. *Anim Conserv* **17(S1)**: 27–39.

Corn ML. 2012. Fish and Wildlife Service: FY2013 appropriations and policy. Congressional Research Service Report R42466.

Desmond J. 2012. Ivanpah and the DOE loan guarantee program. Bright Source Energy. www.brightsourceenergy.com/ivanpah-and-the-doe-loan-guarantee-program#.UytlovldU61. Viewed 6 May 2014.

Edgar PW, Griffiths RA, and Foster JP. 2005. Evaluation of translocation as a tool for mitigating development threats to great crested newts (*Triturus cristatus*) in England, 1990–2001. *Biol Conserv* **122**: 45–52.

Esque TC, Nussear KE, Drake KK, *et al.* 2010. Effects of subsidized predators, resource variability, and human population density on desert tortoise populations in the Mojave Desert, USA. *Endang Species Res* **12**: 167–77.

Frair JL, Merrill EH, Allen JR, and Boyce MS. 2007. Know thy enemy: experience affects elk translocation success in risky landscapes. *J Wildlife Manage* **71**: 541–54.

Gardner AS and Howarth B. 2009. Urbanisation in the United Arab Emirates: the challenges for ecological mitigation in a rapidly developing country. *BioRisk* **3**: 27–38.

Germano JM and Bishop PJ. 2009. Suitability of amphibians and reptiles for translocation. *Conserv Biol* **23**: 7–15.

Griffith B, Scott JM, Carpenter JW, and Reed C. 1989. Translocation as a species conservation tool: status and strategy. *Science* **245**: 477–80.

Hill D and Arnold R. 2012. Building the evidence base for ecological impact assessment and mitigation. *J Appl Ecol* **49**: 6–9.

Hoegh-Guldberg O, Hughes L, McIntyre S, *et al.* 2008. Assisted colonization and rapid climate change. *Science* **321**: 345–46.

IUCN/SSC (International Union for Conservation of Nature/Species Survival Commission). 2013. Guidelines for reintroductions and other conservation translocations. Version 1.0. Gland, Switzerland: IUCN/SSC.

Jackson JA, Schardien BJ, and Miller PR. 1983. Moving red-cockaded woodpecker colonies: relocation or phased destruction? *Wildlife Soc B* **11**: 59–62.

Kyek M, Maletzky A, and Achleitner S. 2007. Large scale translocation and habitat compensation of amphibian and reptile populations in the course of the redevelopment of a waste disposal site. *Z Feldherpetologie* **14**: 175–90.

Leggett D. 2011. Environmental management and monitoring of dredging and reclamation. Dredging International. www.ciria.com/emsagg/Seminar_London_Gateway.htm. Viewed 19 Aug 2014.

Lewis B. 2012. An evaluation of mitigation actions for great crested newts at development sites (PhD dissertation). Canterbury, UK: University of Kent.

Linklater WL, Adcock K, Du Preeze P, *et al.* 2011. Guidelines for large herbivore translocation simplified: black rhinoceros case study. *J Appl Ecol* **48**: 493–502.

Linklater WL, Gedir JV, Law PR, *et al.* 2012. Translocations as experiments in the ecological resilience of an asocial megaherbivore. *PLoS ONE* **7**: e30664.

Linnell JD, Aanes R, Swenson JE, *et al.* 1997. Translocation of carnivores as a method for managing problem animals: a review. *Biodivers Conserv* **6**: 1245–57.

Massei G, Quy RJ, Gurney J, and Cowan DP. 2010. Can translocations be used to mitigate human–wildlife conflicts? *Wildlife Res* **37**: 428–39.

McFadden M, Duffy S, Harlow P, *et al.* 2008. A review of the green and golden bell frog *Litoria aurea* breeding program at Taronga Zoo. *Aus Zool* **34**: 291–96.

Miller KA, Bell T, and Germano JM. 2014. Understanding publication bias in reintroduction biology by assessing translocations of New Zealand's herpetofauna. *Conserv Biol* **28**: 1045–56.

Morris R. 2010. An unfortunate experiment. *Forest and Bird* **337**: 14–18.

Platenberg RJ and Griffiths RA. 1999. Translocation of slow-worms (*Anguis fragilis*) as a mitigation strategy: a case study from south-east England. *Biol Conserv* **90**: 125–32.

Richard-Hansen C, Vie J-C, and de Thoisy B. 2000. Translocation of red howler monkeys (*Alouatta seniculus*) in French Guiana. *Biol Conserv* **93**: 247–53.

Seddon PJ, Armstrong DP, and Maloney RF. 2007. Developing the science of reintroduction biology. *Conserv Biol* **21**: 303–12.

Seddon PJ, Strauss WM, and Innes J. 2012. Animal translocations: what are they and why do we do them? In: Ewen JG, Armstrong DP, Parker KA, *et al.* (Eds). Reintroduction biology: integrating science and management. West Sussex, UK: Wiley-Blackwell.

Shier DM and Swaisgood RR. 2012. Fitness costs of neighborhood disruption in translocations of a solitary mammal. *Conserv Biol* **26**: 116–23.

Teixeira CP, de Azevedo CS, Mendel M, *et al.* 2007. Revisiting translocation and reintroduction programmes: the importance of considering stress. *Anim Behav* **73**: 1–13.

TerraNature. 2006. High risk of extinction from *Powelliphanta* snail translocation. www.terranature.org/snailTranslocation.htm. Viewed 6 May 2014.

Williams CE. 2011. From Shell Haven to Wiltshire – notes on the UK's biggest mass reptile translocation. *ARG Today* **9**: 2–4.

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Research Article

The Effects of Homing and Movement Behaviors on Translocation: Desert Tortoises in the Western Mojave Desert

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ABSTRACT Translocation of threatened or vulnerable species is a tool increasingly used for conservation and management. However, in some species, homing and movement behaviors may undermine the success of translocation efforts. For the federally protected Agassiz's desert tortoise (*Gopherus agassizii*), translocation is a strategy used to manage declining populations, yet homing behavior in this species is poorly understood. To explore homing behavior and movement patterns after translocation, we radio tracked 80 tortoises during a 2-phase experimental translocation. Phase 1 included 40 tortoises that were translocated, then monitored for a period of 37 days (21 Sep–28 Oct 2009), and phase 2 included a different group of 40 tortoises that were translocated and then monitored for 186 days (13 Apr–20 Oct 2010). In both phases, we assigned tortoises randomly to 1 of 3 treatment groups: translocated (displaced 2, 5, or 8 km from their source location), handling control, or control. After translocation, 20% of the translocated tortoises were able to navigate to their source location, and translocation distance had an effect on their ability to navigate home. We found 44% of tortoises in the 2-km translocated group returned home; 1 tortoise in the 5-km group, and no tortoises in the 8-km translocated group returned. The time required to reach home ranged from 5 to 37 days for the 2-km group, and 34 days for the 5-km group. We deemed tortoises to have homed successfully if they returned to their source location within 37 days of translocation as this reflected the duration of phase 1 and allowed for a balanced comparison between the 2 phases. We found that translocated tortoises moved at least 1.5 times more overall than the control groups, with some individuals moving >10 km from the translocation site. These patterns persisted even after accounting for seasonal and sex differences in distance traveled. By identifying homing behaviors and quantifying post-translocation movement patterns, this experiment addressed a key data gap in tortoise behavior that may limit the efficacy of tortoise translocation efforts. Our results point to the need to account for behavioral responses of tortoises to minimize risk to translocated individuals and maximize the success of translocation projects. © 2014 The Wildlife Society.

KEY WORDS desert tortoise, Fort Irwin, *Gopherus agassizii*, homing, Mojave Desert, National Training Center, reintroduction, translocation.

Wildlife and resource managers are frequently tasked with maintaining or promoting population growth in species of conservation concern based on best available information. In some cases, success of a proposed management action may be limited by current knowledge of the behavioral characteristics and ecology of an organism. As a result, incorporating and accounting for behavioral responses to management strategies have been suggested as a key component to improving the success of management and conservation actions (Buchholz 2007, Caro 2007). Understanding behavioral responses such as movement patterns, changes in habitat use, or altered thermoregulatory behaviors, to specific management actions has served to improve and refine management strategies and protocols (Martins et al. 2012, Nussear et al. 2012, Abele et al. 2013, Heer et al. 2013).

Reintroductions and translocations, the human-mitigated movement of organisms from one area to release in another (International Union for Conservation of Nature 2013), are conservation management tools that provide a unique opportunity to explore these behavioral responses. Although accounting for behavior has been recognized as an important element to successful wildlife management, many reintroduction and translocation projects have occurred without understanding or consideration for behavioral responses, potentially limiting the success of translocation efforts (Letty

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et al. 2007, Sheean et al. 2012), where success may be measured by survivorship (Troy et al. 2013), breeding success (King et al. 2013), integration into an existing population (Scillitani et al. 2012), or restoration of key ecological functions (Griffiths et al. 2010). For example, a study of northern water snakes (*Nerodia sipedon sipedon*) found thermoregulatory behaviors were impaired in translocated individuals, leading to management recommendations that included matching pre- and post-translocation habitat conditions, releasing individuals into enclosures, and enriching environmental conditions for captive snakes prior to translocation (Roe et al. 2010).

Translocation, which is used to establish, re-establish, augment, or mitigate populations in decline, has yielded varied results across a broad range of taxa. Translocations have been used with fishes (Sheller et al. 2006, Vincenzi et al. 2012), birds (Reynolds et al. 2012, White et al. 2012), mammals (Van Houtan et al. 2009, Scillitani et al. 2012, Shier and Swaisgood 2012), and herpetofauna (Nelson et al. 2002, Nussear et al. 2012). For reptiles and amphibians, translocations have had limited success for some species, with survival rates of translocated animals ranging from 14% to 42% (Griffith et al. 1989, Dodd and Seigel 1991, Fischer and Lindenmayer 2000, Germano and Bishop 2009). For example, a translocation study of Gila monsters (*Heloderma suspectum*) found individuals translocated less than 1 km returned to their point of capture, and those translocated greater than 1 km demonstrated high rates of movement with increased risk of predation, thermoregulatory costs, and mortality (Sullivan et al. 2004). Similarly, studies of timber rattlesnakes (*Crotalus horridus*; Reinert and Rupert 1999) and eastern box turtles (*Terrapene carolina*; Hester et al. 2008) both found decreased survival and increased movement post-translocation relative to individuals in the resident population.

A recent review of 91 herpetofauna translocations reported the leading causes of translocation failure (defined as failure to establish a self-sustaining population) were homing behavior, the ability to return to the place of origin, and large movements away from translocation sites (Germano and Bishop 2009). Although the mechanisms underlying these responses are poorly understood, there are a number of putative proximate factors, including stress, disease, displacement by conspecifics, avoidance of predators, habitat preference, or homing (Bertolero et al. 2007, Field et al. 2007, Teixeira et al. 2007). Ultimately these increased movements may lead to an increase in mortality (Sullivan et al. 2004, Field et al. 2007, Berry et al. 2009), increased predation risk (Bertolero et al. 2007, Esque et al. 2010), or increased exposure to disease (Wendland et al. 2010). Furthermore, post-translocation movement responses overlay existing patterns that often vary by sex (Tuberville et al. 2005, Harless et al. 2009, Nussear et al. 2012), season (Zimmerman et al. 1994, Eubanks et al. 2003), or weather and climate conditions (Duda et al. 1999, Zylstra et al. 2013). Although the success rates of herpetofaunal translocations have improved in recent years, a general lack of knowledge concerning the factors responsible for unsuccessful translocations still remains (Germano and Bishop 2009).

Increasing land use pressure is one of the primary drivers of translocations of desert species, including the desert tortoise (*Gopherus agassizii*). Ranging across the southwest United States and northwest Mexico, the desert tortoise is a species in decline despite conservation efforts (U.S. Government Accountability Office 2002, USFWS 2011). The Sonoran population (*G. morafkai*) was recently separated from the federally protected Mojave population, found northwest of the Colorado River (Murphy et al. 2011). Listed as threatened in 1990 (USFWS 1990), habitat loss (Doak et al. 1994, Heaton et al. 2008, Darst et al. 2013), disease (Brown et al. 1994, Homer et al. 1998), and predation (Bjurlin and Bissonette 2004, Boarman et al. 2006, Berry et al. 2013), have worked synergistically to erode existing populations across the entire range of both species. The revised recovery plan for the Mojave population of the desert tortoise acknowledges a large number of threats to this species, and in an effort to help recover and manage tortoise populations, translocation has been identified as a key management strategy in response to habitat loss and changes in land-use (USFWS 2011).

Recent land-use changes in the Mojave Desert have included renewable energy developments proposed at an unprecedented rate (Lovich and Ennen 2011), with United States Bureau of Land Management (BLM) processing approximately 70 solar development applications covering over 2,200 km^2 of public lands in California, Nevada, and Arizona, as of March 2014 (BLM 2014). Another land use pressure comes from expansion of military training grounds. The Marine Corps Air Ground Combat Center near Twentynine Palms, California, has approved an expansion of over 600 km^2 and is anticipated to affect over 600 adult tortoises (Department of the Navy 2013). A 2008 land expansion of the National Training Center, Fort Irwin (NTC; Public Law 107–314 2002) near Barstow, California, annexed 545 km^2 of adjacent lands, supporting an estimated 2,000 desert tortoises (Heaton et al. 2008). In an effort to protect this population, more than 500 tortoises were translocated from the NTCs southern expansion area to nearby translocation sites in April 2008.

Using the NTC as a case study, we developed an experimental approach to understand the prevalence of homing and movement behavior on desert tortoise translocation and to explore whether desert tortoises exhibit homing behavior or other behavioral responses to translocation. Desert tortoises have demonstrated a high degree of site fidelity (O'Connor et al. 1994, Harless et al. 2009) and are hypothesized to have homing abilities (Berry 1974, Field et al. 2007), suggesting some degree of spatial awareness, but neither the mechanism nor the extent of these behaviors have been studied. Our study explores the variables that may influence homing behavior and the impact that homing and related behaviors may have on tortoise survival post-translocation. This translocation experiment highlights the factors that might limit translocation success in this and other reptile species of conservation concern.

STUDY AREA

We conducted this study on approximately 90 km^2 of the western expansion area on the NTC, a 2,500-km^2 army training facility (Fig. 1). This expansion area is bounded to the north and east by active training areas of the NTC and the Naval Air Weapons Station, China Lake, and to the south and west by land primarily managed by the BLM. The study site was historically used by both the military (California-Arizona Maneuver Area, established in 1944) and the public until 2001 when the land was transferred to the NTC. The area is representative of natural Mojave creosote scrub desert habitat with minimal development and anthropogenic disturbance.

METHODS

Homing and Movement

To locate and mark tortoises on the landscape, we conducted extensive tortoise surveys at 10-m spacing on the western expansion area commencing in April 2008. As part of the survey, tortoises were weighed, measured for midline carapace length (MCL), fitted with a radio transmitter (Holohil Systems Limited, Carp, Ontario, Canada; Boarman et al. 1998), and given a preliminary health assessment. A separate research team further assessed the tortoises through a comprehensive field examination for clinical signs of health and diseases (Berry and Christopher 2001). Blood samples were submitted to the University of Florida for enzyme-linked immunosorbent assay (ELISA) testing to identify the infectious pathogens *Mycoplasma agassizii* and *M. testudineum* (K. Berry, United States Geological Survey, personal communication; Wendland et al. 2007, Jacobson and Berry 2012). We used a subset of 80 tortoises (40 males and 40 females) from this initial survey over 2 experimental phases under United States Fish and Wildlife Service research permit TE 218901. Phase 1 was conducted for 37 days, from 21 September to 28 October 2009, and phase 2 was conducted for 186 days, from 13 April to 20 October 2010; both phases included 40 tortoises each, and we translocated tortoises only once. Phase 1 was limited to 37 days because the study was designed to return tortoises to their capture location prior to winter brumation, which usually occurs by the end of October (Nussear et al. 2007). Selected tortoises were adults, (MCL >209 mm), and tested negative for exposure to *M. agassizii* and *M. testudineum*, with 4 exceptions that were of unknown disease status (K. Berry, personal communication). We randomly placed the 80 individuals into 3 treatment groups (translocated, handling control, and control) in the following male/female ratios: phase 1: translocated (12/11), handling control (4/5), control (5/3); and phase 2: translocated (12/12), handling control (4/4), control (4/4).

The tortoises in the translocated treatment were located using radio telemetry, weighed, measured, given a rapid assessment for recent trauma or signs of disease, soaked in water for 20 minutes to hydrate them, placed in a secure box in a vehicle, and transported to their release site 2 km, 5 km, or 8 km away from their capture location upon initiation of the experiment. Upon release, we placed tortoises in the shade of a creosote shrub and observed them from a distance

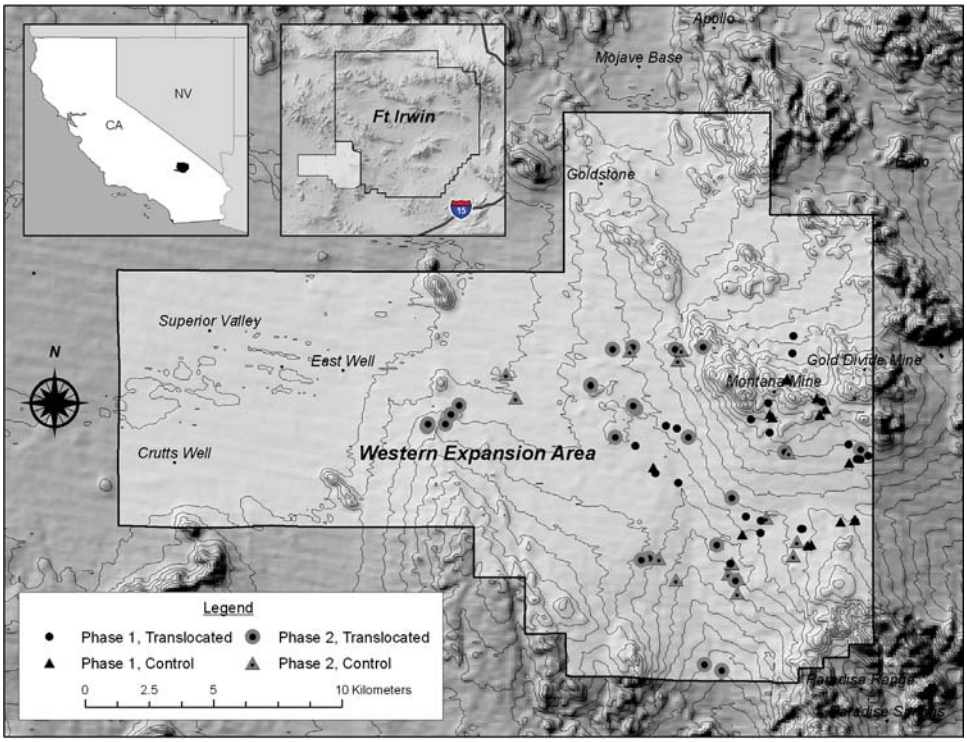

Figure 1. Map of the Western Expansion Area on the National Training Center, Fort Irwin near Barstow, California, USA. We radio-tracked 47 translocated and 33 control desert tortoises over 2 phases, 21 September–28 October 2009, and 13 April–20 October 2010. Tortoise initial capture locations for translocated (black circles for phase 1, grey circles for phase 2) and control (black triangles for phase 1 and grey triangles for phase 2) animals are indicated on the map.

of approximately 10 m, for 20 minutes. We chose release sites randomly within areas of suitable habitat, and both the capture and release areas were in creosote scrub habitat. We did not place tortoises within 50 m of previously known tortoises or active tortoise burrows. We chose this range of experimental distances based on the topography of the study area which was constrained by mountains, dry lake beds, fence line boundaries, and paved roadways. These translocation distances included sites that were in or near their home range (2 km), and sites outside of the tortoises' home ranges (8 km). In this region, desert tortoise home ranges average 16 ha for females and 44 ha for males (Harless et al. 2009). To ensure translocated tortoises were likely to be moved out of their core activity areas, we calculated the maximum linear distance (in meters) across a minimum convex polygon (MCP) activity range of 54 resident tortoises that had been monitored for 13–29 months immediately prior to the commencement of our experiment by a separate research group. This maximum linear distance ranged 309.2–2,368.7 m for females, and 400.5–1,724.9 for males. We calculated MCP and distances using Hawth's tools (Beyer 2004) in ArcGIS 9.3 (ESRI, Redlands, CA; Appendix A).

The handling group served to control for the effect of handling the tortoises during translocation and had 2 treatments: 8 tortoises were handled by researchers at their burrow for less than 1 hour (weighed, measured, and given a health assessment), and 9 tortoises were handled for up to 3 hours (weighed, measured, given a health assessment, soaked in water for 20 minutes, placed in a vehicle and transported), then returned to their initial capture site. These handling times reflected our estimated minimum and maximum times for processing a tortoise during this experiment. Control group tortoises had a radio transmitter attached at least 6 months prior to the commencement of the experiment and otherwise, were not handled at all. We eventually combined handling control and control groups for all analyses (see Results).

We radiotracked tortoises in all treatment groups 2–7 times per week, using hand-held radio receivers (R-1000 Communication Specialist Inc., Orange, CA) and a Yagi-Uda directional hand-held antenna (AFAntronics, Champaign, IL). We had no interruptions in our tracking efforts due to equipment failure. At each tracking event, we recorded geographic location (Universal Transverse Mercator, UTM) with Garmin GPSMap76Cx and Garmin GPSMap76CSx units (Garmin Inc., Olathe, KS), which were calibrated daily and had an estimated error of 3–6 m. We used ambient temperature data collected from the weather station at the Barstow-Daggett airport, located approximately 45 km from our study site (Weather Underground 2011). We categorized the temperatures into 3 blocks: block $1 \leq 20^{\circ}$C, 20°C $<$ block $2 < 32^{\circ}$C, and block $3 \geq 32^{\circ}$C where reduced tortoise activity levels roughly corresponded with lower temperatures in block 1 and higher temperatures in block 3. Upon conclusion of the 2 experimental phases (28 Oct 2009 and 20 Oct 2010), we returned all translocated tortoises to their capture location, and monitored them for a week to ensure their well-being. All tortoises were in good condition upon conclusion of our experiment, and these animals continued to be monitored monthly at a minimum by a separate research group.

Statistical Analysis

We evaluated movement behavior using 4 metrics: 1) the ability of tortoises to find their way home, where home was any location within 500 m of their original capture location; 2) directionality, assessing if the animal traveled in the direction of their capture location and how direct their path was; 3) the total distance traveled, calculated as the sum of the straight line distances between radio tracking point locations over time; and 4) net displacement, calculated as the straight-line distance between the tortoises' initial release point and the capture location on day 37 for both experimental periods. To allow for balanced comparisons, we conducted analyses for homing ability, directionality, total distance traveled, and net displacement between the first 37 days of both experimental phases (21 Sept–28 Oct 2009 and 13 April–20 May 2010) using SYSTAT (SYSTAT Software Inc. San Jose, CA). We conducted an additional analysis of total distance traveled on the full phase 2 data set to capture movement patterns across different temperatures, and over time.

To assess ability to home, we used Pearson's chi-square and Cochrane's test of linear trend for ordered data to determine if translocated distance affected the number of tortoises that returned to their original location. We explored directionality using 2 metrics of circular statistics: angular dispersion (r), a measure of how direct the movement path was, and average directionality (θ), the mean angle of travel (Zar 1999). We standardized the mean angle of travel to 0 for all tortoises so it was not influenced by the relative position of the release versus the capture location. Tortoises moving in the opposite direction of home with no angular concentration would have θ and r values of 180 and 0 respectively, whereas those exhibiting perfect homing ability would have θ and r values of 0 and 1.0. We used Pearson's correlation to determine how average θ and r were related to one another. We used analysis of covariance (ANCOVA) to analyze average θ, where the factors were experimental phase (1 or 2) and distance (2, 5, or 8 km), and the covariate was average r. We used a 2-sample t-test to investigate differences in θ and r between tortoises that arrived home and those that did not.

We used general linear models (GLM) to determine whether there were differences of total distance traveled (square root transformed) or net displacement (log transformed) within the first 37 days after translocation between the translocated and control groups for both phases. We used the independent variables experimental treatment and sex, and we found no significant interaction between them. To further analyze the phase 2 data set, we used a generalized linear mixed model (GLMM) to test total distance traveled (square root transformed) for differences among treatment, sex, and temperature blocks using the PROC GLMMIX command with a variance component structure (SAS Institute Inc. Cary, NC). Our full model included the fixed effects of treatment, sex, temperature block, with all

interactions, and the random effects of tortoise identification number and week, to account for repeated measures of individual animals over time. To test differences across significant fixed effects, we used post-hoc least squares mean Tukey-Kramer pairwise comparisons. We applied a Pearson chi-square to further investigate categorical differences in net-displacement (as being greater or less than 1 km) between translocated and control groups. We set significance levels to $\alpha < 0.05$.

RESULTS

We found no difference in total distance traveled or net displacement between the 2 handling control regimes (distance: $F_{1,\ 15} > 1.337$ $P > 0.266$; displacement: $F_{1,\ 15} > 0.541$ $P > 0.474$) or between our handling control and control groups ($F_{1,\ 31} > 1.404$ $P > 0.245$); therefore, we combined these 2 treatments for all analyses, and categorized them all as controls.

Homing Movements

We found a statistically significant number of tortoises navigated home among our 3 distance groups. In total, 9 out of 47 tortoises returned home, 5 in phase 1 ($n = 23$; 2 km: 4/10; 5 km: 1/7; 8 km: 0/6) and 4 in phase 2 ($n = 24$; 2 km: 4/8; 5 km: 0/8; 8 km: 0/8). Eight of these were in the 2-km distance group, and 1 was in the 5-km distance group (phase 1: $\chi^2 = 3.76$, $P = 0.052$; phase 2: $\chi^2 = 7.2$, $P = 0.007$). The time required to reach home ranged from 5–37 days for the 2-km distance, and 34 days for the 5-km distance group. Although not categorized as homing in this analysis, in phase 2 we had 1 female tortoise in the 8-km distance group navigate to within 670 m of her previously known location, 20 days post-translocation. No tortoises returned home after day 37, despite phase 2 continuing for 186 days, and we observed no mortality throughout the duration of both experimental phases.

Directionality and Angular Dispersion in Movement

We found a negative correlation between average directionality (θ) and average angular dispersion (r), where low θ was associated with a high r value ($r = -0.434$, $P = 0.002$). We found θ was predicted by distance ($F_{2,\ 42} = 8.526$, $P = 0.001$), experimental phase ($F_{1,\ 42} = 5.416$, $P = 0.025$), and r ($F_{1,\ 42} = 10.970$, $P = 0.002$), where translocated tortoises that arrived home traveled in both the correct direction of home, and with less angular dispersion (e.g., in straighter paths). We found translocated tortoises that homed had a lower θ and higher r values than translocated tortoises that did not (r: $t_{11.708} = -3.497$, $P = 0.005$, θ: $t_{12.675} = 4.345$, $P = 0.001$; Figs. 2, 3).

Total Distance Moved

We found an effect of both treatment (phase 1: $F_{3,\ 35} = 21.946$, $P < 0.001$; phase 2: $F_{3,\ 35} = 3.782$, $P = 0.019$) and sex (phase 1: $F_{1,\ 35} = 9.416$, $P < 0.004$; phase 2: $F_{1,\ 35} = 11.255$, $P = 0.002$) in both experimental phases where translocated tortoises moved more than controls, and male tortoises moved more than female tortoises with no interaction between treatment and sex. Tukey's post hoc test showed different means across treatment groups ($P < 0.05$) where in phase 1, the 2-km, 5-km, and 8-km distance treatments all moved more than the control treatment, and in phase 2, only 5-km and 8-km distance treatments moved more than the control group (means of total distance traveled, phase 1: 2 km = 3,192 m, 5 km = 7,589 m, 8 km = 5,436 m, control = 1,361 m; phase 2: 2 km = 6,920 m, 5 km = 12,750 m, 8 km = 11,293 m, control = 6,994 m). Our analysis of the phase 2 data using a GLMM found an effect of temperature block, sex, and the 2-way interactions of temperature block by sex, and temperature block by treatment, where males moved farther than females regardless of temperature block (block 1: $t_{64} = 5.26$, $P < 0.001$; block 2: $t_{64} = 6.38$, $P < 0.001$; block 3: $t_{64} = 3.62$, $P = 0.008$), and translocated tortoises moved more than control tortoises only at the mid-range ambient temperatures (block 2: $t_{64} = 6.20$, $P < 0.001$; Fig. 4).

Net Displacement

We found a difference in net displacement among groups, with the translocated groups displacing longer distances than the control groups in both phase 1 ($F_{3,\ 35} = 9.242$, $P < 0.001$) and phase 2 ($F_{3,\ 35} = 6.624$, $P = 0.001$; Fig. 5). We found no difference between sexes ($P > 0.05$) in either phase. We further analyzed the net displacement data to consider whether there were categorical differences in net displacement distance between tortoises that moved and the tortoises that did not move. We found a difference in both phase 1 ($\chi^2_3 = 13.737$, $P < 0.003$) and phase 2 ($\chi^2_3 = 21.845$, $P < 0.001$) where the translocated treatment had proportionally more tortoises that moved greater than 1 km.

DISCUSSION

Translocation has been identified as a key, and often preferred, management strategy for desert tortoises in response to habitat loss and changes in land-use (USFWS 2011). However, choosing appropriate translocation sites for desert tortoises is challenging, and must take into account population densities, disease status of both recipient and donor populations, present and future anthropogenic influences, predator densities, and habitat structure. Assuming that the goal is to keep translocated individuals away from their home range of origin, our data suggest that moving tortoises a short distance, <2 km, is unlikely to result in successful translocation. Relocating tortoises short distances may have advantages, such as keeping tortoises in or near their home range or within a similar habitat type, and increasing the probability of maintaining social and genetic ties with neighboring tortoises (Berry 1986). However, based on our results, this strategy may increase the likelihood of a tortoise returning home and thus undermine this management strategy unless an effective barrier fence is in place. Furthermore, homing tortoises are more likely to encounter fence line boundaries built to exclude tortoises from their site of origin during some translocation efforts (D. Hinderle, San Diego State University, personal observation). Tortoise exclusion fencing may increase vulnerability to predation, mortality, or thermal stress, and such physical obstacles have been shown to limit dispersal, impede gene flow, and/or increase mortality in other taxa (Aresco 2005*a, b*; Clark et al. 2010).

In an effort to minimize successful homing, an alternative may be to move tortoises more than 2 km from their source location during translocation efforts. Although longer distance translocations may reduce the likelihood of individuals returning to their source location, we found some evidence that homing may occur with translocation distances >2 km; 1 tortoise returned home from 5 km away, and another, who was moved 8 km away, navigated to within 670 m of home. Our results also point to another cost of long distance translocations; the data suggest that increased total movement and net displacement may correspond to increased translocation distance, although this trend was not statistically significant. In areas where tortoises are translocated >5 km from their original site, increased movements and net displacement could dramatically heighten vulnerability to predation, mortality, disease, and aggressive conspecific interactions (Berry et al. 2009, Germano and Bishop 2009), and may increase the likelihood of encountering an anthropogenic landscape, including fence lines, roads, or developed areas (Sullivan et al. 2004).

Regardless of the distance moved, we found that all translocated tortoises moved longer distances and had greater net displacement than the control group within the first 5 weeks of translocation, and increased movements persisted over time in more than 80% of the translocated tortoises (32/38 translocated tortoises, excluding those that navigated to their capture location). This measureable behavioral response to translocation may challenge translocation efforts irrespective of whether a tortoise exhibits homing. Such increased movements may influence ability to breed successfully (Tuberville et al. 2011), affect survivorship (Tuberville et al. 2008) or have physiological consequences (Moulherat et al. 2014).

Our study also indicates that males and females exhibit different responses to translocation, consistent with other translocation research (Field et al. 2007, Nussear et al. 2012). Although we found the ability to navigate home was the same for males and females, given the importance of females to population growth (Doak et al. 1994), female homing may have a disproportionate impact on the population if this behavior were to increase mortality in females, although this was not observed during the short duration of this study. In terms of total movement, males moved more than females in all treatment groups, a finding consistent with males having larger home ranges (O'Connor et al. 1994, Harless et al. 2009). In the phase 2 experimental period, males consistently moved more than females across all temperature blocks, but this was most pronounced at temperatures between 20–32°C, which was also the temperature range where translocated tortoises moved more than control animals. Our data indicates this effect of sex across all temperatures is primarily driven by the increased movement of males from August through October, and is consistent with peak spermatogenesis and mating (Rostal et al. 1994, Lance and Rostal 2002). Such increased movement may cause males to disperse into areas of disease or heavy anthropogenic use at a higher rate than females, and suggests that the success of translocation efforts may vary seasonally. However, even with characteristic seasonal variability, translocated animals moved more than the control groups, indicating translocation may elicit atypical movement in this species irrespective of season. There also appeared to be a critical distance between 2 km and 5 km where tortoises were no longer able to locate home, and repeating this experiment with a suite of translocation distances between 1 km and 5 km would help identify these critical distances and aid to inform future translocations.

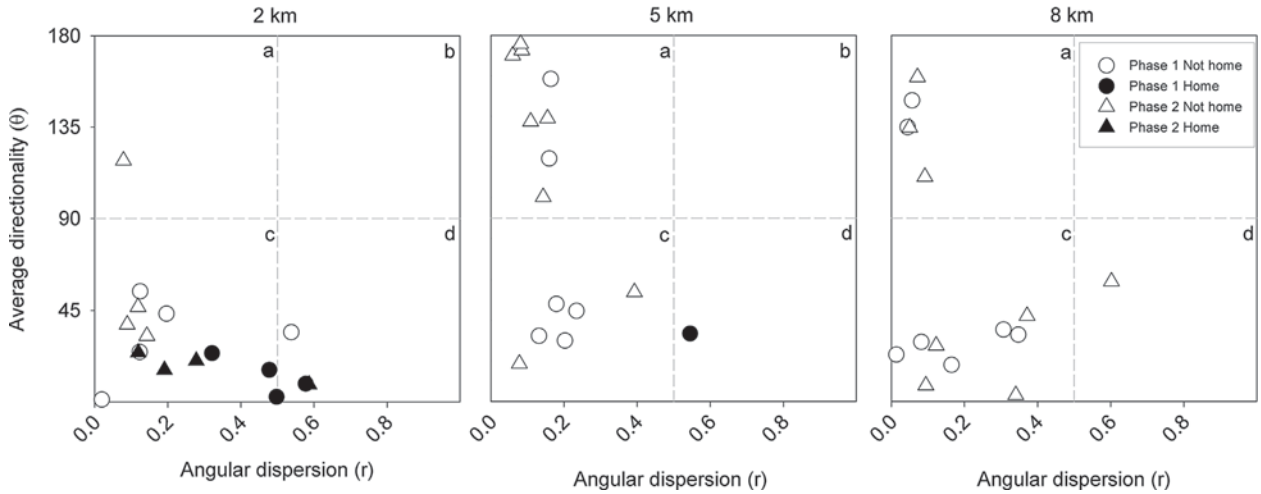

Figure 2. Directionality (θ) and angular dispersion (*r*) of desert tortoises in 3 translocation distance groups occurring at the National Training Center, Fort Irwin near Barstow, California, USA, in 2009 and 2010. Tortoises able to navigate home had higher *r* values and lower θ values than tortoises not able to navigate home. Tortoises moving in the opposite direction of home with no angular concentration would have θ and *r* values of 180 and 0, respectively. Tortoises exhibiting perfect homing ability would have θ and *r* values of 0 and 1.0, respectively. Quadrants generally correspond with different movement patterns, where tortoises in quadrant (a) traveled in the wrong direction and were not concentrated in their movement bearings; (b) moved in the wrong direction toward a concentrated bearing; (c) exhibited some directionality; and (d) demonstrated homing, with both directionality and minimal angular dispersion. Solid symbols indicate tortoises that arrived home (defined as within 500 m of their capture location within 37 days of experimental translocation). Open symbols indicate tortoises that did not return home. Phase 1 occurred from 21 September to 28 October 2009, and phase 2 occurred from 13 April to 20 October 2010.

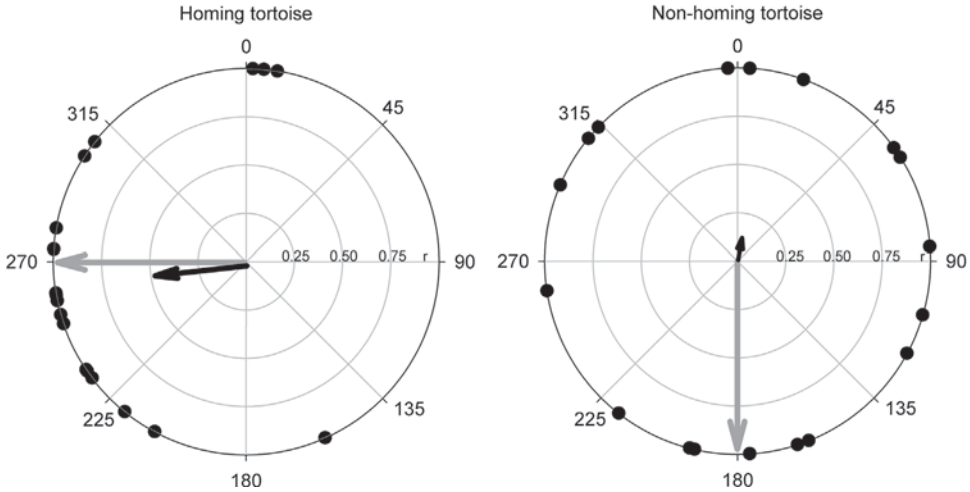

Figure 3. Examples of orientation of 2 translocated desert tortoises during an experiment on National Training Center, Fort Irwin, California, USA in 2009; 1 that returned home in 18 days, and another that did not. Both were female tortoises in the 2-km distance group in phase 1 of the experiment. Movement bearings are indicated on the outside of the circle, and angular dispersion (r) values are indicated by the open concentric rings. Solid circles represent desert tortoise movement bearings from release site to final location; direction and length of black arrow indicates mean angle of travel and angular dispersion (r), respectively; and grey arrow indicates homeward direction. The tortoise that returned home had a mean angle of travel of 266°, similar to the homeward direction of 270°, and minimal angular dispersion ($r = 0.49$). The tortoise that did not return home had a homeward direction of 180°, a mean angle of travel of 7°, and angular dispersion of $r = 0.08$.

As with all in situ experiments, uncontrolled factors may have influenced our results. In this study, neither behavioral, nor genetic data were available to account for how existing social structure may have affected the behavior of translocated tortoises. Translocations are known to disrupt social structures (Bertolero et al. 2007, Haydon et al. 2008) and translocation distance possibly served as a proxy for social structure. Individuals translocated greater than 2 km away may have exhibited increased movement in response to different and unfamiliar conspecifics rather than the new environment. Tortoises in this experiment may have been particularly habituated to human activity and presence, as all tortoises had been previously handled. Although we did not detect obvious differences between the control and handling treatment groups, we cannot discount potential effects of human activity and presence. We also were unable to account for or measure the impact of translocation on the resident tortoises in the translocated sites. As with translocated individuals, resident tortoises at recipient sites may be indirectly affected by translocation and experience higher rates of disease transmission or increased likelihood of conspecific aggression, both of which may have negative effects on survival (Haydon et al. 2008, Wendland et al. 2010).

Translocation is becoming a common instrument for conservation, mitigation, management, and restoration in many ecosystems and across many taxa (Seddon et al. 2007, Teixeira et al. 2007, Germano and Bishop 2009). The synergistic effects of disease (Brown et al. 1994, Christopher et al. 2003), habitat loss (Boarman and Sazaki 2006), predation (Esque et al. 2010) and the likely effect of climate change (Weltzin et al. 2003, Seager et al. 2007, Lovich et al.

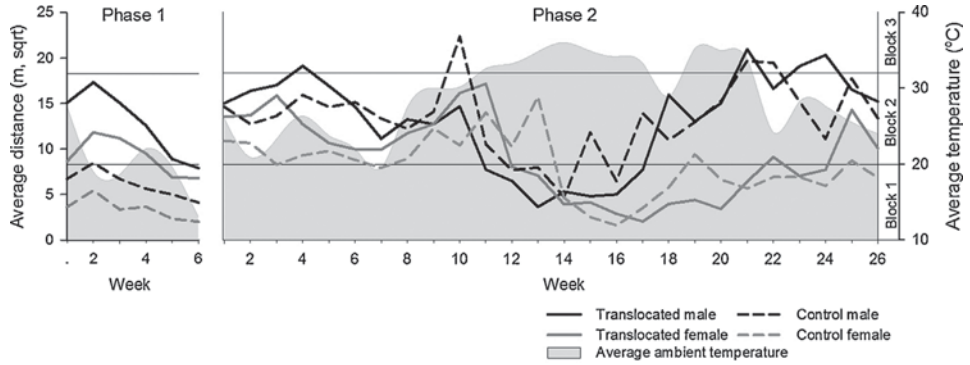

Figure 4. Mean distance (meters, square root-transformed) traveled by tortoises during a homing experiment in Fort Irwin, California. We experimentally translocated desert tortoises from their capture location and monitored their movements and ability to travel home. Phase 1 began 21 September 2009 (left panel) and lasted 37 days ($n = 35$), and phase 2 began 13 April 2010 (right panel) and lasted 186 days ($n = 36$). The average weekly ambient temperature (°C) shown in the grey profile, was obtained from the weather station at the Barstow-Daggett airport, located approximately 45 km from the study site. Temperatures blocks 1 and 3 roughly correspond with reduced tortoise activity. Tortoises in the phase 1 and phase 2 experiments were different individuals, and this graph only includes individuals who did not return home.

2014) have substantially influenced desert tortoises and the population continues to decline despite over 2 decades of federal protection. Translocations are employed to meet a range of management goals, including population re-establishment (Macmillan 1995), moderating habitat-use conflicts (Sullivan et al. 2004), and mitigating pending threats (Guyot and Clobert 1997, Heaton et al. 2008), as in the case of the Fort Irwin Expansion project. A better understanding of the long-term consequences of these, and other behavioral responses to translocation and the impact these responses may have on individual survival rates of translocated individuals is essential to improve the likelihood of success of this strategy for the desert tortoise and other reptiles at risk of extirpation.

MANAGEMENT IMPLICATIONS

Curtailing impacts that would require the translocation of desert tortoises is critical to conserve desert tortoises and their habitat. If translocation is required, our study indicates its success may be impeded by homing behavior and large movements after translocation assuming the goal is for tortoises to remain within the recipient site. Results from this experiment provide strong evidence that some desert tortoises in the NTC region exhibit homing behavior, and, if translocated, they are more likely to return home when their recipient site is less than 2 km away from their original home range. We found all tortoises that were able to get home, did so within 37 days, and that their trajectories towards home were generally straighter than those that did not get home. We also found increased movements persisted over time and therefore recipient sites should be large enough to support a translocated population with movement patterns and net displacement distances which, based on our findings, may be substantial. We recommend that tortoises should be monitored more closely during the first weeks or months post-translocation for homing behaviors and potentially fence-walking, should their original home range be excluded by a physical barrier.

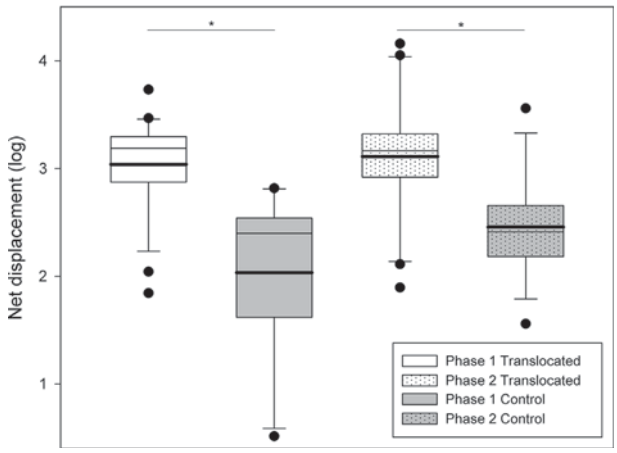

Figure 5. Mean net displacement distance (meters; $\log_{10}$ transformed) traveled by desert tortoises after experimental translocation at National Training Center, Fort Irwin, California, in 2009 and 2010. The boundary of the box closest to 0 indicates the 25th percentile, the thin line within the box marks the median, and thick line within the box marks the mean, the boundary of the box farthest from 0 indicates the 75th percentile, whiskers indicate 90th and 10th percentile, and outliers beyond are shown as points. A significant difference in net displacement between groups within each phase is indicated with an *.

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LITERATURE CITED

Abele, S. L., A. J. Wirsing, and D. L. Murray. 2013. Precommercial forest thinning alters abundance but not survival of snowshoe hares. Journal of Wildlife Management 77:84–92.

Aresco, M. J. 2005*a*. The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biological Conservation 123:37–44.

Aresco, M. J. 2005*b*. Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a north Florida lake. Journal of Wildlife Management 69:549–560.

Berry, K. H. 1974. Desert tortoise relocation project: Status report for 1972. California Division of Highways, Ridgecrest, California, USA.

Berry, K. H. 1986. Desert tortoise (Gopherus agassizii) relocation: implications of social behavior and movements. Herpetologica 42:113–125.

Berry, K. H., and M. M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Diseases 37:427–450.

Berry, K. H., T. Gowan, and J. Mack. 2009. Health and survival of 158 tortoises translocated from Fort Irwin: year 1 of the health research program. Page 4 *in* Proceedings of the 34th annual meeting and symposium. The Desert Tortoise Council, 20–22 February 2009, Mesquite, Nevada, USA.

Berry, K. H., J. L. Yee, A. A. Coble, W. M. Perry, and T. A. Shields. 2013. Multiple factors affect a population of Agassiz's desert tortoise (Gopherus agasizii) in the northwestern Mojave Desert. Herpetological Monographs 27:87–109.

Bertolero, A., D. Oro, and A. Besnard. 2007. Assessing the efficacy of reintroduction programmes by modelling adult survival: the example of Hermann's tortoise. Animal Conservation 10:360–368.

Beyer, H. L. 2004. Hawth's Analysis Tools for ArcGIS. http://www.spatialecology.com/htools. Accessed 15 Jan 2011.

Bjurlin, C. D., and J. A. Bissonette. 2004. Survival during early life stages of the desert tortoise (*Gopherus agassizii*) in the South-Central Mojave Desert. Journal of Herpetology 38:527–535.

Boarman, W., T. Goodlett, G. Goodlett, and P. Hamilton. 1998. Review of radio transmitter attachment techniques for turtle research and recommendations for improvement. Herpetological Review 29:26–33.

Boarman, W. I., M. A. Patten, R. J. Camp, and S. J. Collis. 2006. Ecology of a population of subsidized predators: common ravens in the central Mojave Desert, California.. Journal of Arid Environments 67:248–261.

Boarman, W. I., and M. Sazaki. 2006. A highway's road-effect zone for desert tortoises (*Gopherus agassizii*). Journal of Arid Environments 65:94–101.

Brown, M. B., I. M. Schumacher, P. A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62:4580–4586.

Buchholz, R. 2007. Behavioural biology: an effective and relevant conservation tool. Trends in Ecology & Evolution 22:401–407.

Caro, T. 2007. Behavior and conservation: A bridge too far? Trends in Ecology & Evolution 22:394–400.

Christopher, M. M., K. H. Berry, B. T. Henen, and K. A. Nagy. 2003. Clinical disease and laboratory abnormalities in free-ranging desert tortoises in California (1990–1995). Journal of Wildlife Diseases 39:35–56.

Clark, R. W., W. S. Brown, R. Stechert, and K. R. Zamudio. 2010. Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. Conservation Biology 24:1059–1069.

Darst, C. R., P. J. Murphy, N. W. Strout, S. P. Campbell, K. J. Field, L. Allison, and R. C. Averill-Murray. 2013. A strategy for prioritizing threats and trcovery actions for at-risk species. Environmental Management 51:786–800.

Department of the Navy. 2013. Record of decision for land acquisition and airspace establishment to support large-scale marine air ground task force live-fire and maneuver training at the Marine Corps Air Ground Combat Center, Twentynine Palms, CA. Department of the Navy, Twentynine Palms, California, USA.

Doak, D., P. Kareiva, and B. Kleptetka. 1994. Modeling population viability for the desert tortoise in the western Mojave Desert. Ecological Applications 4:446–460.

Dodd, C. K., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles-Are they conservation strategies that work? Herpetologica 47:336–350.

Duda, J. J., A. J. Krzysik, and J. E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. Journal of Wildlife Management 63:1181–1192.

Esque, T. C., K. E. Nussear, K. K. Drake, A. D. Walde, K. H. Berry, R. C. Averill-Murray, A. P. Woodman, W. I. Boarman, P. A. Medica, J. Mack, and J. S. Heaton. 2010. Effects of subsidized predators, resource variability, and human population density on desert tortoise populations in the Mojave Desert, USA. Endangered Species Research 12:167–177.

Eubanks, J. O., W. K. Michener, and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). Herpetologica 59:311–321.

Field, K. J., C. R. Tracy, P. A. Medica, R. W. Marlow, and P. S. Corn. 2007. Return to the wild: translocation as a tool in conservation of the desert tortoise (*Gopherus agassizii*). Biological Conservation 136:232–245.

Fischer, J., and D. B. Lindenmayer. 2000. An assessment of the published results of animal relocations. Biological Conservation 96:1–11.

Germano, J. M., and P. J. Bishop. 2009. Suitability of amphibians and reptiles for translocation. Conservation Biology 23:7–15.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool-status and strategy. Science 245:477–480.

Griffiths, C. J., C. G. Jones, D. M. Hansen, M. Puttoo, R. V. Tatayah, C. B. Muller, and S. Harris. 2010. The use of extant non-indigenous tortoises as a restoration tool to replace extinct ecosystem engineers. Restoration Ecology 18:1–7.

Guyot, G., and J. Clobert. 1997. Conservation measures for a population of Hermann's tortoise *Testudo hermanni* in southern France bisected by a major highway. Biological Conservation 79:251–256.

Harless, M. L., A. D. Walde, D. K. Delaney, L. L. Pater, and W. K. Hayes. 2009. Home range, spatial overlap, and burrow use of the desert tortoise in the west Mojave Desert. Copeia 2:378–389.

Haydon, D. T., J. M. Morales, A. Yott, D. A. Jenkins, R. Rosatte, and J. M. Fryxell. 2008. Socially informed random walks: incorporating group dynamics into models of population spread and growth. Proceedings of the Royal Society B: Biological Sciences 275:1101–1109.

Heaton, J. S., K. E. Nussear, T. C. Esque, R. D. Inman, F. M. Davenport, T. E. Leuteritz, P. A. Medica, N. W. Strout, P. A. Burgess, and L. Benvenuti. 2008. Spatially explicit decision support for selecting translocation areas for Mojave desert tortoises. Biodiversity and Conservation 17:575–590.

Heer, P., J. Pellet, A. Sierro, and R. Arlettaz. 2013. Evidence-based assessment of butterfly habitat restoration to enhance management practices. Biodiversity and Conservation 22:239–252.

Hester, J. M., S. J. Price, and M. E. Dorcas. 2008. Effects of relocation on movements and home ranges of eastern box turtles Journal of Wildlife Management 72:772–777.

Homer, B. L., K. H. Berry, M. B. Brown, G. Ellis, and E. R. Jacobson. 1998. Pathology of diseases in wild desert tortoises from California. Journal of Wildlife Diseases 34:508–523.

International Union for Conservation of Nature [IUCN]. 2013. Guidelines for reintroductions and other conservation trnaslocations. Version 1.0. IUCN/SSC, Gland, Switzerland.

Jacobson, E. R., and K. H. Berry. 2012. *Mycoplasma testudineum* in free-ranging desert tortoises, *Gopherus agassizii*. Journal of Wildlife Diseases 48:1063–1068.

King, R. S., J. L. Espenshade, S. K. Kirkpatrick-Wahl, M. K. Lapinski, I. Malekan, and J. M. Ricket. 2013. Whooping crane *Grus americana* chick mortality and management intervention. Wildlife Biology 19:420–428.

Lance, V. A., and D. C. Rostal. 2002. The annual reproductive cycle of the male and female desert tortoise: physiology and endocrinology. Chelonian Conservation and Biology 4:302–312.

Letty, J., S. Marchandeau, and J. Aubineau. 2007. Problems encountered by individuals in animal translocations: Lessons from field studies. Ecoscience 14:420–431.

Lovich, J. E., and J. R. Ennen. 2011. Wildlife conservation and solar energy development in the desert southwest. United States. Bioscience 61:982–992.

Lovich, J. E., C. B. Yackulic, J. Freilich, M. Agha, M. Austin, K. P. Meyer, T. R. Arundel, J. Hansen, M. S. Vamstad, and S. A. Root. 2014. Climatic variation and tortoise survival: Has a desert species met its match. Biological Conservation 169:214–224.

Macmillan, S. 1995. Restoration of an extirpatied red-sided garter snake (*Thamnophis sirtalis parietalis*) population in the interlake region of Manitoba. Canada. Biological Conservation 72:13–16.

Martins, I., M. L. Arosa, R. S. Ceia, S. Parejo, J. A. Ramos, and C. Damgaard. 2012. The winter energetics of the Azores bullfinch and the implications for the restoration of its native laurel forest habitat. Ecological Modelling 231:80–86.

Moulherat, S., V. Delmas, T. Slimani, E. El Mouden, T. Louzizi, F. Lagarde, and X. Bonnet. 2014. How far can a tortoise walk in open habitat before overheating? Implications for conservation. Journal for Nature Conservation 22:186–192.

Murphy, R. W., K. H. Berry, T. Edwards, A. E. Leviton, A. Lathrop, and J. D. Riedle. 2011. The dazed and confused identity of Agassiz's land tortoise, *Gopherus agassizii* (Testudines, Testudinidae) with the description of a new species, and its consequences for conservation. ZooKeys 113:39–71.

Nelson, N. J., S. N. Keall, D. Brown, and C. H. Daugherty. 2002. Establishing a new wild population of tuatara (*Sphenodon guntheri*). Conservation Biology 16:887–894.

Nussear, K. E., C. R. Tracy, P. A. Medica, D. S. Wilson, R. W. Marlow, and P. S. Corn. 2012. Translocation as a conservation tool for Agassiz's desert tortoises: Survivorship, reproduction, and movements. Journal of Wildlife Management 76:1341–1353.

Nussear, K. E., T. C. Esque, D. F. Haines, and C. R. Tracy. 2007. Desert tortoise hibernation: Temperatures, timing, and environment. Copeia 378–386.

O'Connor, M. P., L. C. Zimmerman, D. E. Ruby, S. J. Bulova, and J. R. Spotila. 1994. Home range size and movements by desert tortoises, *Gopherus agassizii*, in the eastern Mojave Desert. Herpetological Monographs 8:60–71.

Public Law 107–314. 2002. Bob Stump national defense authorization act for fiscal year 2003. Incorporates by reference Title XXIX Fort Irwin military lands withdrawl act of 2001. U.S. Congress, Washington, D.C., USA.

Reinert, H. K., and R. R. Rupert. 1999. Impacts of translocation on behavior and survival of timber rattlesnakes, *Crotalus horridus*. Journal of Herpetology 33:45–61.

Reynolds, M. H., J. S. Hatfield, L. P. Laniawe, M. S. Vekasy, J. L. Klavitter, P. Berkowitz, L. H. Crampton, and J. R. Walters. 2012. Influence of space

use on fitness and the reintroduction success of the Laysan teal. Animal Conservation 15:305–317.

Roe, J. H., M. R. Frank, S. E. Gibson, O. Attum, and B. A. Kingsbury. 2010. No place like home: an experimental comparison of reintroduction strategies using snakes. Journal of Applied Ecology 47:1253–1261.

Rostal, D. C., V. A. Lance, J. S. Grumbles, and A. C. Alberts. 1994. Seasonal reproductive cycle of the desert tortoise (*Gopherus agassizii*) in the eastern Mojave Desert. Herpetological Monographs 8:72–82.

Scillitani, L., E. Sturaro, A. Menzano, L. Rossi, C. Viale, and M. Ramanzin. 2012. Post-release spatial and social behaviour of translocated male Alpine ibexes (*Capra ibex ibex*) in the eastern Italian Alps. European Journal of Wildlife Research 58:461–472.

Seager, R., M. F. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H. P. Huang, N. Harnik, A. Leetmaa, N. C. Lau, C. H. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science 316:1181–1184.

Seddon, P. J., D. P. Armstrong, and R. F. Maloney. 2007. Developing the science of reintroduction biology. Conservation Biology 21:303–312.

Sheean, V. A., A. D. Manning, and D. B. Lindenmayer. 2012. An assessment of scientific approaches towards species relocations in Australia. Austral Ecology 37:204–215.

Sheller, F. J., W. F. Fagan, and P. J. Unmack. 2006. Using survival analysis to study translocation success in the Gila topminnow (*Poeciliopsis occidentalis*). Ecological Applications 16:1771–1784.

Shier, D. M., and R. R. Swaisgood. 2012. Fitness costs of neighborhood disruption in translocations of a solitary mammal. Conservation Biology 26:116–123.

Sullivan, B. K., M. A. Kwiatkowski, and G. W. Schuett. 2004. Translocation of urban Gila monsters: a problematic conservation tool. Biological Conservation 117:235–242.

Teixeira, C. P., C. S. De Azevedo, M. Mendl, C. F. Cipreste, and R. J. Young. 2007. Revisiting translocation and reintroduction programmes: the importance of considering stress. Animal Behaviour 73:1–13.

Troy, R. J., P. S. Coates, J. W. Connelly, G. Gillette, and D. J. Delehanty. 2013. Survival of mountain quail translocated from two distinct source populations. Journal of Wildlife Management 77:1031–1037.

Tuberville, T. D., E. E. Clark, K. A. Buhlmann, and J. W. Gibbons. 2005. Translocation as a conservation tool: site fidelity and movement of repatriated gopher tortoises (*Gopherus polyphemus*). Animal Conservation 8:349–358.

Tuberville, T. D., T. M. Norton, B. D. Todd, and J. S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: a comparison of newly released and previously established animals. Biological Conservation 141:2690–2697.

Tuberville, T. D., T. M. Norton, B. J. Waffa, C. Hagen, and T. C. Glenn. 2011. Mating system in a gopher tortoise population established through multiple translocations: apparent advantage of prior residence. Biological Conservation 144:175–183.

U.S. Bureau of Land Management [BLM]. 2014. U.S. BLM Homepage. http://www.blm.gov/wo/st/en/prog/energy/solar_energy.html. Accessed 30 Mar 2014.

U.S. Government Accountability Office. 2002. Endangered species: research strategy and long term monitoring needed for the Mojave desert tortoise recovery program. U.S. Government Accountability Office-03–23, Washington, D.C., USA.

U.S. Fish and Wildlife Service [USFWS]. 1990. Endangered and threatened wildlife and plants: Determination of threatened status for the Mojave population of the desert tortoise. U.S. Fish and Wildlife Service, Ventura, California, USA.

U.S. Fish and Wildlife Service [USFWS]. 2011. Revised recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*). U.S. Fish and Wildlife Service, Pacific Southwest Region, Sacramento, California, USA.

Van Houtan, K. S., J. M. Halley, R. van Aarde, and S. L. Pimm. 2009. Achieving success with small, translocated mammal populations. Conservation Letters 2:254–262.

Vincenzi, S., A. J. Crivelli, D. Jesensek, and G. A. De Leo. 2012. Translocation of stream-dwelling salmonids in headwaters: Insights from a 15-year reintroduction experience. Reviews in Fish Biology and Fisheries 22:437–455.

Weather Underground. 2011. Weather Underground internet services team. Daily ambient temperature for Barstow-Daggett airport, California. http://www.wunderground.com/history/airport/KDAG/2011/01/15/MonthlyHistory.html#calendar. Accessed 15 Jan 2011.

Weltzin, J. F., M. E. Loik, S. Schwinning, D. G. Williams, P. A. Fay, B. M. Haddad, J. Harte, T. E. Huxman, A. K. Knapp, G. H. Lin, W. T. Pockman, M. R. Shaw, E. E. Small, M. D. Smith, S. D. Smith, D. T. Tissue, and J. C. Zak. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. Bioscience 53:941–952.

Wendland, L. D., J. Wooding, C. L. White, D. Demcovitz, R. Littell, J. D. Berish, A. Ozgul, M. K. Oli, P. A. Klein, M. C. Christman, and M. B. Brown. 2010. Social behavior drives the dynamics of respiratory disease in threatened tortoises. Ecology 91:1257–1262.

Wendland, L. D., L. A. Zacher, P. A. Klein, D. R. Brown, D. Demcovitz, R. Littell, and M. B. Brown. 2007. Improved enzyme-linked immunosorbent assay to reveal *Mycoplasma agassizii* exposure: a valuable tool in the management of environmentally sensitive tortoise populations. Clinical and Vaccine Immunology 14:1190–1195.

White, T. H., N. J. Collar, R. J. Moorhouse, V. Sanz, E. D. Stolen, and D. J. Brightsmith. 2012. Psittacine reintroductions: Common denominators of success. Biological Conservation 148:106–115.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey, USA.

Zimmerman, L. C., M. P. O'Connor, S. J. Bulova, J. R. Spotila, S. J. Kemp, and C. J. Salice. 1994. Thermal ecology of desert tortoises in the eastern Mojave Desert: seasonal patterns of operative and body temperatures, and microhabitat utilization. Herpetological Monographs 8:45–59.

Zylstra, E. R., R. J. Steidl, C. A. Jones, and R. C. Averill-Murray. 2013. Spatial and temporal variation in survival of a rare reptile: A 22-year study of Sonoran desert tortoises. Oecologia 173:107–116.

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APPENDIX A. Demographic and activity area calculations for desert tortoises on the western expansion area.Tortoise unique identifying number (ID), sex, midline carapace length (MCL), number of months radio tracked, area (m^2), perimeter (m) and maximum linear distance (m) across the minimum convex polygon (MCP) of 54 desert tortoises on the western expansion area in 2009 and 2010. We calculated the minimum convex polygon from all monthly locations to establish activity areas of resident tortoises on the western expansion area and completed analysis using Hawth's tools in ArcGIS 9.3.

Tortoise ID	Sex	MCL (mm)	Months tracked	MCP area ($m^{2)}$	Maximum distance (m)
1	Female	210	20	44,526.2	309.2
2	Male	266	24	543,258.7	400.5
3	Male	236	22	16,686.0	432.0
4	Female	232	23	52,734.7	475.1
5	Female	235	22	60,552.9	482.1
6	Female	246	19	64,377.9	490.7
7	Male	226	22	114,202.8	504.4
8	Female	221	21	97,278.6	512.6
9	Male	268	24	69,354.2	524.9
10	Male	223	18	76,582.9	526.4
11	Female	229	25	138,696.1	548.2
12	Female	234	23	81,397.4	564.3
13	Female	215	22	80,116.6	579.4
14	Female	251	23	65,634.1	610.8
15	Male	270	20	151,278.7	617.9
16	Female	252	20	116,554.4	627.5
17	Male	270	16	70,347.0	646.2
18	Female	240	16	142,849.3	654.0
19	Female	242	20	94,510.0	658.1
20	Female	232	22	149,995.0	661.9
21	Male	288	20	221,063.6	668.6
22	Male	285	13	151,959.6	688.3
23	Male	225	15	85,623.5	693.6
24	Male	264	20	149,990.8	694.9
25	Male	242	20	231,530.1	704.7
26	Female	253	22	165,283.9	737.1
27	Male	276	21	77,498.7	743.6
28	Male	260	20	277,941.8	758.0
29	Male	288	21	271,448.3	768.4
30	Male	278	22	40,037.7	775.2
31	Male	260	21	110,918.7	782.1
32	Male	277	21	132,092.2	810.2
33	Female	237	17	114,594.1	834.7
34	Female	227	21	119,056.2	846.9
35	Male	272	20	235,180.6	853.6
36	Male	259	20	171,232.7	861.1
37	Male	260	22	282,741.2	890.7
38	Male	281	20	98,583.9	925.0
39	Male	211	22	278,810.0	969.0
40	Male	276	21	451,632.3	1,032.3
41	Male	254	29	276,322.7	1,040.7
42	Female	244	21	146,881.1	1,053.3
43	Male	271	16	238,365.7	1,066.7
44	Female	213	19	88,202.0	1,083.6
45	Female	226	24	109,268.6	1,188.6
46	Male	258	20	227,815.0	1,240.7
47	Female	242	23	415,687.9	1,278.5
48	Female	239	22	134,395.1	1,449.3
49	Female	219	17	295,719.8	1,515.5
50	Female	254	23	735,967.3	1,542.7
51	Female	219	15	464,834.5	1,611.9
52	Male	276	15	195,589.5	1,648.8
53	Male	267	17	1398,071.4	1,724.9
54	Female	214	17	1583,987.0	2,368.7

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Relative abundance and demographic structure of Agassiz's desert tortoise (*Gopherus agassizii*) along roads of varying size and traffic volume

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ABSTRACT

Roads are recognized as important contributors to wildlife population declines and are thought to pose greatest risk to vagile species with large home ranges and long generation times. We examined variation in the relative abundance and demographic structure of Agassiz's desert tortoise (*Gopherus agassizii*) near roads that varied in traffic volume. We found that the abundance of tortoise sign (scat, tracks, pallets, burrows, and live and dead individuals) varied with traffic volume and distance from the road depending on traffic volume. The relative abundance of tortoise sign was greatest along roads with low traffic volume (<1 vehicle/day) compared to roads with intermediate (30–60 vehicles/day) and high (320–1100 vehicles/day) traffic volumes. Additionally, tortoise sign had lower relative abundances at least 200 m from roads with the highest traffic volumes. We found that the frequency of live tortoise encounters decreased with increasing traffic volumes. Tortoise size also correlated significantly with traffic volume, such that tortoises near the highest traffic volume road were smallest. Finally, along the highest traffic road we found greater proportions of juvenile tortoises than along either of the other traffic volume roads. Our results indicate that roads may decrease tortoise populations via several possible mechanisms, including cumulative mortality from vehicle collisions and reduced population growth rates from the loss of larger reproductive animals. Here, we provide evidence that a reptile with a slow life history is susceptible to road presence and that the effect increases with traffic volume.

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1. Introduction

Roads are ubiquitous and pose a significant threat to biodiversity globally (Benitez-Lopez et al., 2010; Eigenbrod et al., 2008; Fahrig and Rytwinski, 2009; Jacobs and Houlahan, 2012). They affect wildlife through numerous mechanisms that can include mortality from vehicle collisions, and loss, fragmentation, and alteration of habitat (Marsh et al., 2008; Trombulak and Frissell, 2000). The types and magnitude of the effects may vary depending on the behaviors of the species (Andrews et al., 2005). For example, species that frequently use or cross roads are likely to be affected directly by increased mortality from vehicle collisions. Alternatively, species that avoid crossing roads may be more susceptible to indirect mechanisms, such as habitat fragmentation, as a consequence of road avoidance, and alteration of nearby physical conditions. Though roads comprise only 1% of surface area, an estimated 19% of the total land within the United States is ecologically affected by roads due to indirect effects that extend 100–800 m beyond the physical footprint of the road (Forman, 2000). The ecologically affected areas along roads, otherwise known as "road-effect zones", are those in which a change in wildlife abundance, demography, or behavior is observed. Given the extensive area affected by roads and the numerous mechanisms through which roads can affect local populations, there is a clear need to develop predictive measures for the contribution of roads to population declines and to develop effective mitigation strategies.

Currently, roads are expected to pose the greatest risk to species that are highly vagile, have large home ranges, large body mass, low reproductive rates, and long generation times (Carr and Fahrig, 2001; Gibbs and Shriver, 2002; Karraker and Gibbs, 2011; Rytwinski and Fahrig, 2011, 2012). Road effects may be particularly damaging to species with low reproductive rates and long generation times because such species have a low intrinsic ability to recover from population declines (Gibbs and Shriver, 2002; Rytwinski and Fahrig, 2012). Although the above patterns have been seen in many mammals (Rytwinski and Fahrig, 2011), there are few

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studies supporting their application to other vertebrates. In fact, studies of amphibians have found the opposite pattern, identifying small, early maturing species as those more susceptible to road mortality (Rytwinski and Fahrig, 2012). Ultimately, however, species with life history traits tied to low lifetime reproductive rates do appear to be at the greatest risk for road-related declines.

Studies on reptiles are too scarce to develop quantitative predictions of life history traits associated with sensitivity to roads (Rytwinski and Fahrig, 2012). Behavioral traits in reptiles, such as road-side basking for thermoregulation (Sullivan, 1981), and human behavior, such as intentionally crushing reptiles encountered on roads (Ashley, 2007), may increase risk in reptiles relative to mammals. Although there are limited studies, documented effects of roads on reptiles include high mortality, altered demographic structure near roads, and increased risk of local extirpation, particularly in long-lived species (Gibbs and Shriver, 2002; Gibbs and Steen, 2005; Row et al., 2007; Taylor and Goldingay, 2010). Most turtle species have life history characteristics consistent with the predictions of high risk in mammals: late sexual maturity, low reproductive rates, and long generation times. If population-level effects apply mostly to species with "slow" life histories, turtles and tortoises are expected to be particularly vulnerable to impacts from roads. Additionally, demographic studies in turtles indicate that small increases in annual mortality of as little as 1–3% can result in population declines (Congdon et al., 1993, 1994; Doroff and Keith, 1990; Heppell, 1998). Simulated movement patterns composed of short- and longer-distance movements for terrestrial turtles indicate that roads increase annual mortality rates to over 5% (Gibbs and Shriver, 2002), a rate higher than that estimated to result in population declines. Given that turtles and tortoises are among the most threatened taxonomic groups globally (Buhlmann et al., 2009), quantifying and reducing the effects of roads on their populations represents an important contribution to biodiversity preservation.

Road effects may be especially important in structuring populations of Agassiz's desert tortoise (*Gopherus agassizii*). Agassiz's desert tortoise is a federally- and state-protected species, native primarily to the Mojave Desert, USA, and roads have been identified as a major threat to their persistence (USFWS, 2008). Desert tortoises have large home ranges (Harless et al., 2009), can require up to 20 years to reach sexual maturity (Mueller et al., 1998), and only produce an average of 4.5 eggs per clutch (Turner et al., 1986). Their slow life histories suggest that desert tortoise populations should be sensitive to the negative impacts of roads, especially increased mortality. Previous studies indicate that tortoises in the Mojave and Sonoran Deserts are negatively affected by the presence of roads and occur at lower densities near heavily traveled roads (Boarman and Sazaki, 2006; Nicholson, 1979; Von Seckendorff and Marlow, 2002). However, tortoises are also noted to be attracted to gravel roads, as demonstrated by their increased presence near these roads (Grandmaison et al., 2010). The different effects of gravel roads versus highways may be a consequence of traffic volume. Here, we examine the impact of roads and traffic volume on desert tortoises with three main objectives. Our first objective was to determine whether traffic volume correlated with the abundance of tortoise sign (scat, pallets, burrows, tracks, and live or dead individuals) along roads. Next, to determine whether the road-effect zone extends farther from roads as a result of increased traffic volume, we examined roads that varied in vehicle use. Finally, we examined the demographic structure of tortoises found near roads of differing traffic volume. Collectively, we sought to demonstrate the comparative influence of traffic volume on nearby tortoise populations.

2. Methods

2.1. Relative abundance of tortoises and tortoise sign

We conducted our study in Mojave National Preserve, a 650,000 ha protected area managed by the National Park Service and located in the eastern Mojave Desert, California, USA (34°53′N, 115°43′W). We surveyed four areas of the preserve known to have tortoises: Ivanpah Valley, Fenner Valley, Clypepper Valley, and Kelso. We grouped roads into three general categories depending on the traffic volume: paved with high traffic volume (HIGH), paved with intermediate traffic volume (MED), and paved or dirt roads with low traffic volumes (LOWs) (see Table 1 for more information).

In 2012, we surveyed nine roads across the four locations in the preserve during 5–29 June. Three roads were assigned to each of three road categories (HIGH, MED, and LOW) based on traffic volume data (Table 1). We were limited by the number of suitable roads available. We measured road-effect zones along each of the roads by surveying for tortoise sign at various distances from the road. We selected the survey distances based on previous work by Boarman and Sazaki (2006) and based on preliminary data we collected during a pilot study in 2011. The work by Boarman and Sazaki (2006) in the western Mojave Desert indicated no change in the abundance of tortoise sign between 800 and 1600 m from the road but a significant increase in sign between 0 and 400 m. Thus, we did not include a 1600 m distance and instead increased the sampling resolution of our study closer to the road. Therefore, to measure the road-effect zone, we documented tortoise sign at distances of 0, 50, 100, 200, 400, and 800 m from the road. At each distance we walked two 1600 m transects parallel to the road, separated by 20 m. We recorded all tortoise sign (scat, tracks, pallets, burrows, and live and dead tortoises) visible in a 10 m wide transect (i.e., 5 m on either side of the observer) and surveyed only one transect site per road. A 5 m sight distance from the observer is a standard used during line distance sampling for desert tortoises (Boarman and Sazaki, 2006; Zylstra et al., 2010), which maximizes detection of the animals and minimizes variation among habitat types, vegetation, or observers. Distances greater than 5 m from the observer may lead to differential detection among areas, which we sought to avoid. The total tortoise sign recorded during the two parallel transects at each survey distance was summed. We corrected for bias by treating adjacent associated signs (e.g., a tortoise inside a burrow, tracks behind a tortoise) as a single encounter, as described by Boarman and Sazaki (2006).

To determine whether road category differentially affected the relative abundance of tortoise sign between categories and at the various distances, the total tortoise signs were square root transformed and analyzed using R 2.11.1 (Institute for Statistics and Mathematics, Wien, Austria) at an $\alpha = 0.10$ level. We were willing to accept higher Type I error due to our low sample sizes ($n = 3$) and with the understanding that the cost of a false negative has greater negative implications for species management than the cost of a false positive. We used analysis of covariance (ANCOVA) with tortoise sign as the dependent variable and distance from the road as the independent predictor with road name and road category included as covariates. Post hoc pairwise comparisons among means were completed using Tukey's honestly significant difference (HSD) test to compare the total relative abundance of tortoise sign and road category. We used Holm's least significant difference (LSD) test to look at the effect of each distance from the road on relative tortoise sign abundance for each road category. Because burrows represent areas where tortoises have chosen to settle, they may be more indicative of suitable habitat than other types of tortoise sign. Thus, we separated burrows from other

Table 1
Roads in Mojave National Preserve along which transects were conducted in 2011 and 2012. Category is road category based on number of vehicles per day. Type refers to whether the road was paved with asphalt or a dirt road. Lane number refers to the road width (number of lanes) present.

Road name	Location	Transect coordinates (UTM, NAD83)	Category	Vehicles per day	Type	Lane number	Posted speed limit (mph)
Goffs Rd.	Fenner Valley	0675305, 3864702	HIGH	1089[a]	Paved	2	55
Cima Rd.	Kelso	0632705, 3886389	HIGH	346[a]	Paved	2	55
Morning Star Mine Rd.	Ivanpah Valley	0643597, 3913717	HIGH	325[a]	Paved	2	55
Essex Rd	Clypepper Valley	0646939, 3858103	MED	59[a]	Paved	2	55
Ivanpah Rd.	Ivanpah Valley	0650922, 3915068	MED	47[a]	Paved	2	55
Lanfair Rd.	Fenner Valley	0675683, 3867932	MED	35[a]	Paved	2	55
None	Kelso	0618555, 3861175	LOW	<1[b]	Dirt	2	Not posted
None	Ivanpah Valley	0645971, 3911505	LOW	<1[b]	Paved	1	Not posted
None	Fenner Valley	0672552, 3869031	LOW	<1[b]	Dirt	1	Not posted

[a] Data source: <http://www.sbcounty.gov/dpw/trafficadt/>, (accessed 10.03.12).
[b] Estimated from personal observations during data collection.

tortoise signs and repeated the analyses used for total tortoise sign to test for the effects of road category and distance on the relative abundance of burrows.

We compared the number of live to dead tortoises encountered during all road transects among road categories throughout Mojave National Preserve. In order to do so we used a Chi-square test of independence to test for departures from random between road category and the number of live and dead tortoises.

From 14–24 October 2011, we measured the distance (m) from the road to the first burrow located for three roads, one in each road category (HIGH, MED, and LOW), in Ivanpah Valley. We selected five haphazard points along each of the three roads and walked perpendicularly away from the road until we located a burrow. We analyzed the effect of road category on square root transformed distance to first observed burrow using a one-way analysis of variance with $\alpha = 0.10$.

2.2. Habitat

In Ivanpah Valley from 01 April – 20 July 2011, we collected habitat data on three roads, each of which was categorized into the HIGH, MED, and LOW road category. We collected habitat data at distances of 0, 200, 400, 600, 800, 1000, 1200, 1400, and 1600 m at five randomly selected points along each road. At each sampling point we measured perennial shrub volume (height and width [cm]), distance to nearest three shrubs (m), and number of small mammal burrows (important refugia for juvenile tortoises) under the nearest three shrubs. We used ANCOVAs to examine the effect of distance with road category included as a covariate on the dependent factors perennial shrub volume (cm^3), distance to the nearest three shrubs (m), or the number of small mammal burrows with $\alpha = 0.10$. Perennial shrub volume was log transformed prior to analyses.

2.3. Tortoise demography

To determine whether tortoises varied in size among road categories, we measured all tortoises that we encountered in Ivanpah Valley from 01 April – 25 October 2011. Our 2011 Ivanpah Valley road transects overlapped spatially with areas where we conducted additional field research. Thus, all tortoises encountered in Ivanpah Valley during road transects and any other ongoing field work in 2011 were included in demographic comparisons. We located tortoises visually and handled them when shaded air temperatures were below 35 °C. For each animal encountered, we recorded location (UTM, NAD83) using a Global Positioning Unit (Garmin eTrex Venture HC [±3 m]), mid-line carapace length (MCL; distance from the nuchal scute to the pygal scute [mm]), mass (g), and sex when possible based on external secondary sexual characteristics. Desert tortoises begin showing secondary sexual characteristics around 180–190 mm MCL (Turner et al., 1987). Individuals below 180 mm MCL were recorded as sexually immature juveniles. We permanently marked each individual by notching unique combinations of marginal scutes with a triangular file (Cagle, 1939). We released all tortoises at the point of capture immediately following handling, which was limited to 30 min. We also measured MCL and recorded sex for all intact shells of dead animals that were encountered. We marked each shell with a permanent marker to ensure they were not double counted. We followed procedures approved by the Institutional Animal Care and Use Committee through the University of California, Davis (IACUC # 15997) and the University of Georgia (A2010 04-059-Y3-A0) during our handling of all animals. Our study was done in accordance with permits provided by US Fish and Wildlife Service (Permit # TE-17838A), California Department of Fish and Game (Permit # SC-11072), and Mojave National Preserve (Permit # MOJA-2011-SCI-0023).

We assigned live and dead tortoises encountered during all field activities in Ivanpah Valley during 2011 to a road category using the location selection tool in ArcEditor 9.31.1 (ESRI, California, USA). We assigned individuals within a 500 m buffer of the HIGH road to HIGH; individuals within a 500 m buffer of the MED road to MED, excepting those already assigned to HIGH; and individuals within a 500 m buffer of the LOW road to LOW, excepting any previously assigned to either HIGH or MED. Individuals that were not encompassed by HIGH, MED, or LOW buffers were assigned to a new category of NONE. For tortoises that were encountered multiple times, we used only the first encounter for analysis. After category assignment, we tested MCL data for normality and found the assumptions of normality and homogeneity of variance were violated. Thus, we used the non-parametric Kruskal–Wallis rank sum test to examine the relationship between road category and tortoise MCL. To reduce potential bias from sex- or stage-specific (e.g. adult or juvenile) mortalities near a given road, we included sex and stage as covariates, as both can affect MCL. We completed a post hoc comparison test on the results from the Kruskal–Wallis rank sum test using the "pgirmess" package in R with $\alpha = 0.10$.

We summed all tortoise encounters in Ivanpah Valley in 2011 by road category (i.e., HIGH, MED, LOW, or NONE) and used Chi-square tests to examine the ratio of adult to juvenile tortoises and male to female tortoises among the various road categories.

3. Results

3.1. Relative abundance of tortoises and tortoise sign

The average abundance of the total tortoise sign recorded for all six transect distances in 2012 was 253.7 ± 98.2 (mean ± 1 SE, $n = 3$) for LOW roads, 195.3 ± 80.8 ($n = 3$) for MED roads, and 149.0 ± 23.6 ($n = 3$) for HIGH roads. We found a significant effect of road

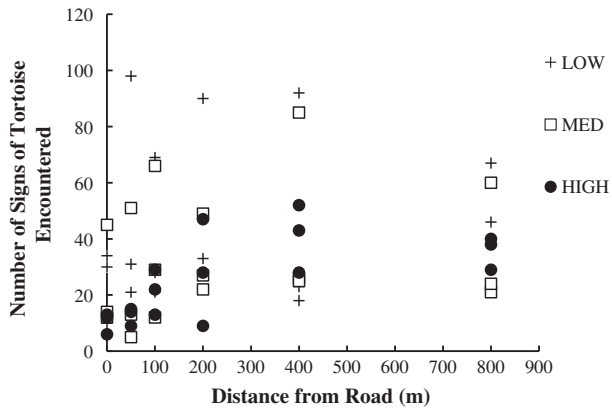

Fig. 1. Abundance of tortoise sign at each of distance sampled for the high traffic volume (HIGH), intermediate traffic volume (MED), and low traffic volume (LOW) road categories in Mojave National Preserve.

category on relative abundance of tortoise sign (road category effect: $F_{2,36} = 8.7$, $p < 0.001$) and of distance on the relative abundance of tortoise sign (distance effect: $F_{1,36} = 9.8$, $p = 0.003$; Fig. 1). We did not find any significant interaction between road category and distance (interaction effect: $F_{2,36} = 2.3$, $p = 0.11$). Tukey's HSD revealed LOW roads had significantly greater abundances of total tortoise sign than either MED ($p = 0.07$) or HIGH ($p < 0.001$) road categories. Holm's LSD tests showed no significant differences between relative abundance of tortoise sign at any of the distances for the LOW and MED roads. However, relative abundance of tortoise sign along HIGH roads increased significantly at 400 m ($p = 0.04$) and at 800 m ($p = 0.08$) compared with 0 m.

In 2012 we encountered a total of 56.0 ± 9.0 burrows on LOW traffic roads, 36.0 ± 5.5 on MED, and 32.3 ± 7.6 on HIGH roads. We found a significant effect of road category (road category effect: $F_{2,36} = 5.1$, $p = 0.01$), distance (distance effect: $F_{1,36} = 23.8$, $p < 0.001$), and an interaction of distance and road category (interaction effect: $F_{2,36} = 2.6$, $p = 0.09$) on the relative abundance of burrows (Fig. 2). Tukey's HSD revealed LOW roads had significantly greater abundances of burrows than either MED ($p = 0.06$) or HIGH ($p = 0.01$) road categories. Holm's LSD tests showed no significant differences between relative abundance of burrows at any of the distances for the LOW and MED roads. Relative abundance of burrows along HIGH roads increased significantly at 400 ($p = 0.01$) and 800 m ($p = 0.01$) compared with 0 m. Distance to the first burrow also correlated positively with traffic volume, such that the first burrow encountered was farthest from HIGH roads and closest to LOW roads ($F_{2,12} = 6.7$, $p = 0.01$; Fig. 3).

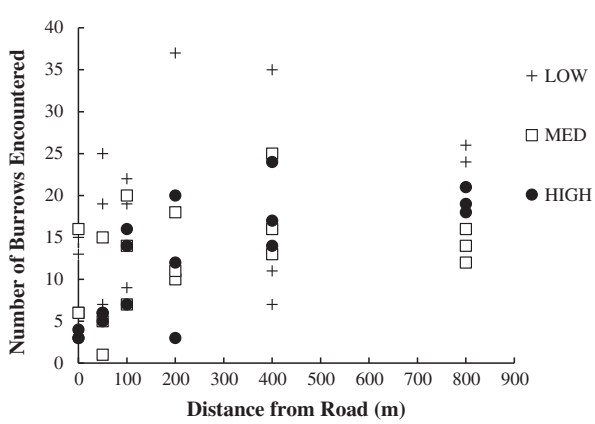

Fig. 2. Abundance of burrows at each distance sampled for the high traffic volume (HIGH), intermediate traffic volume (MED), and low traffic volume (LOW) road categories in Mojave National Preserve.

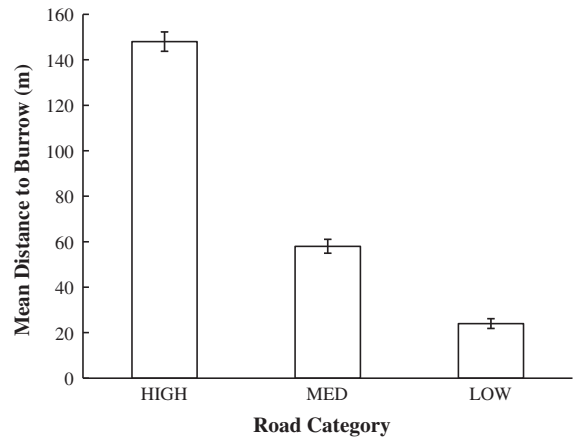

Fig. 3. Mean (±1 SE) distance to the first burrow encountered along high traffic volume (HIGH), intermediate traffic volume (MED), and low traffic volume (LOW) road categories. Road category had a significant effect on distance to the first burrow observed.

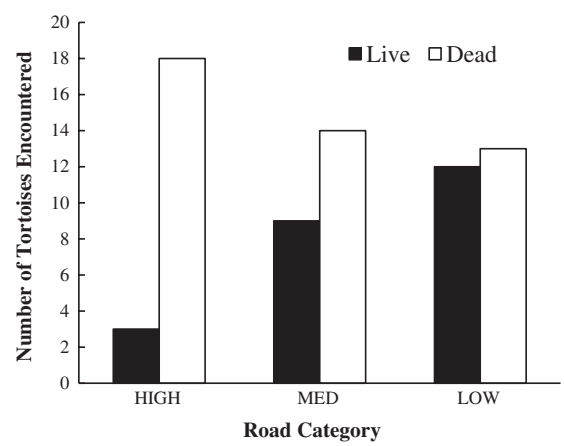

Fig. 4. Number of live and dead tortoises encountered during road transects in Mojave National Preserve. The distribution of observations differed significantly from random.

The number of live and dead tortoises encountered among the different road categories was nonrandomly distributed ($\chi^2 = 6.01$, $df = 2$, $n = 69$, $p = 0.05$; Fig. 4). The number of dead tortoises encountered increased with increasing traffic volume, whereas the number of live tortoises decreased.

3.2. Habitat

We found no effect of distance from road ($F_{1,122} = 1.2$, $p = 0.28$), road category ($F_{2,122} = 1.8$, $p = 0.17$), or their interaction ($F_{2,122} = 0.35$, $p = 0.70$) on perennial shrub volume. Similarly, we found no effect of distance from road ($F_{2,122} = 0.16$, $p = 0.69$), road category ($F_{2,122} = 1.1$, $p = 0.32$), or an interaction ($F_{2,122} = 1.6$, $p = 0.20$) on distance to the nearest three shrubs. Finally, we found no effect of distance from road ($F_{1,122} = 0.01$, $p = 0.93$), road category ($F_{2,122} = 2.1$, $p = 0.13$), or an interaction ($F_{2,122} = 0.13$, $p = 0.88$) on the number of small mammal burrows.

3.3. Tortoise demography

In 2011, we encountered 94 live tortoises (32 female, 47 male, 15 juvenile) and an additional 38 intact shells of dead animals (13 female, 13 male, 12 juvenile) in Ivanpah Valley during all field work. Search times in the different areas were not documented so

we cannot compare abundance of individuals by road category with this dataset. The mean MCLs for tortoises in each road category were 213 ± 7 mm for NONE, 235 ± 9 mm for LOW, 230 ± 10 mm for MED, and 146 ± 21 mm for HIGH. Mean MCL of tortoises differed significantly among road categories ($H = 22.2$, $df = 3$, $n = 132$, $p < 0.001$). Tortoises in the vicinity of HIGH roads were significantly smaller than tortoises near the LOW, MED, and NONE road categories, whereas tortoises near the LOW road were significantly larger than tortoises that were not associated with any road (Fig. 5).

The distribution of adults to juveniles differed significantly from random among road categories ($\chi^2 = 8.64$, $df = 3$, $n = 132$, $p = 0.05$). The proportion of juvenile tortoises found within 500 m from the HIGH road was greater than the proportion found near MED, LOW, or NONE road categories (Fig. 6). Males and females were encountered with similar frequency among all road categories ($\chi^2 = 0.37$, $df = 3$, $n = 105$, $p > 0.1$; Fig. 6).

4. Discussion

The results of our study demonstrate that roads of varying traffic volumes differentially affect the relative abundance of tortoise sign, the width of the road-effect zone, and the demography of desert tortoises in Mojave National Preserve. The relative abundance of tortoise sign and burrows were significantly greater along "low" traffic volume roads (<1 vehicle per day) than either "intermediate" (30–60 vehicles per day) or "high" (320–1100 vehicles per day) traffic volume roads. We also found significantly lower relative abundances of total tortoise sign and burrows at the 400 and 800 m distances for high traffic volume roads. The lack of significant differences in measured habitat characteristics with distance from the road or between road categories suggests that changes in the relative abundance of tortoise sign were related to direct effects of traffic volume, via mortality or avoidance by the animals, and not to indirect effects such as changes in habitat resulting from road presence. However, we did not measure annual vegetation abundance near roads, though roads may have a greater effect on annual vegetation than perennial vegetation. A previous study found that the abundance of tortoise sign was lower at least 400 m from the roadside along a single heavily used highway, although traffic data are not available for that site (Boarman and Sazaki, 2006). Thus, our observations are consistent with previous findings that the relative abundance of tortoise sign is lower near roads, but provide new information about the interaction between traffic volume, demography, and relative abundance.

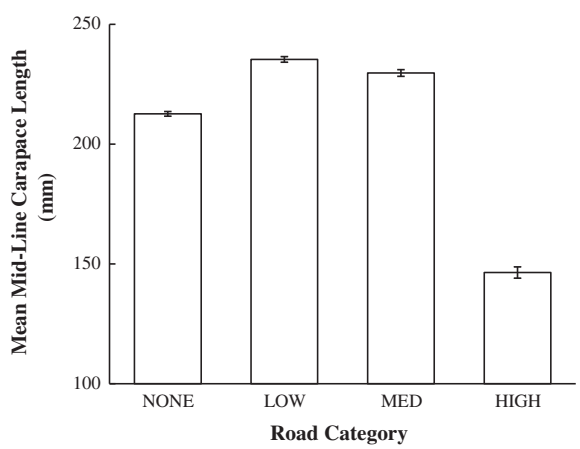

Fig. 5. Mean (±1 SE) mid-line carapace length (MCL) of tortoises encountered within 500 m of HIGH, MED, LOW, or NONE road categories for all tortoises encountered in Ivanpah Valley. Traffic volume correlated significantly with tortoise MCL.

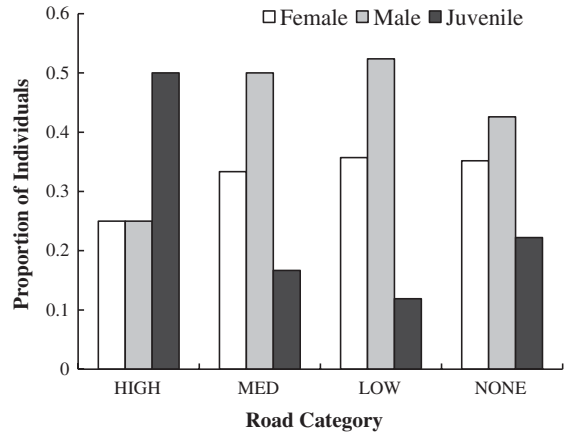

Fig. 6. Proportion of male, female, and juvenile tortoises ($n = 132$) found within 500 m of roads in the high traffic volume (HIGH, $n = 12$), intermediate traffic volume (MED, $n = 24$), low traffic volume (LOW, $n = 42$), and no road (NONE, $n = 54$) categories. Within a traffic volume category, proportions sum to 1. The proportion of adult to juvenile tortoises was significantly non-random across road observations, with the greatest proportion of juveniles observed near the HIGH category.

Abundance or density of tortoise sign may generally reflect the abundance of individuals or their relative use of habitats (McCoy and Mushinsky, 2007; Mushinsky et al., 1994). Density or abundance of individuals is thus often considered a reflective measure of habitat quality (Andersen et al., 2000; Van Horne, 1983). Our methods did not allow us to determine whether the lower relative abundance of tortoise sign was a result of increased mortality, behavioral avoidance of roadways, or both. However, both increased mortality in an area and behavioral avoidance of that area can result in reduced use by a population. Consequently, we suggest that lower relative abundances of tortoise sign and burrows near roads with traffic volumes as low as 300 vehicles per day result in decreased quality or loss of adjacent habitat for desert tortoises.

Demography of populations near roads may also be differentially affected depending on vehicle use (Taylor and Goldingay, 2010). In Ivanpah Valley, using additional data that we had available, we found that the demographic structure of tortoises along the high traffic road differed from those found along the low, intermediate, and no road categories. Although male desert tortoises, being more vagile and having larger home ranges (Franks et al., 2011; Harless et al., 2009), might be more susceptible to roads than females, we did not find that sex ratios differed among the road categories. However, we encountered only three male and three female tortoises near our high traffic volume road, so we caution against forming strong inferences on effects on the sexes from this dataset. Larger sample sizes may yield more information about the relative effects of traffic volume on male versus female tortoises. We did find that tortoises located near the high traffic road were at least 30% smaller on average than tortoises associated with lower traffic volumes or no roads. These results are similar to a study of road impacts on freshwater turtles, which found that areas with lower densities of roads had turtles of larger sizes (Patrick and Gibbs, 2010). In our study, the mean size of tortoises associated with the highest traffic volume road was below the typical size of sexual maturity in either sex.

A reduction in the average size of individuals along the high traffic road may result in lower population growth rates, even if individuals do reach sizes great enough for sexual maturity. Body size in many reptiles, including the desert tortoise, correlates strongly with fecundity (Congdon et al., 1987; Ford and Seigel, 1989; Mueller et al., 1998; Winck and Rocha, 2012). Though tortoises in different populations or geographic regions may exhibit

responses that vary from those we observed, our results are similar to previous predictions for terrestrial turtles. Gibbs and Shriver (2002) found that traffic volumes of >100 vehicles per lane per day would be sufficient to result in population declines of terrestrial turtle species. In this paper, we provide evidence that traffic volumes of 300 vehicles per day may be sufficient to decrease the abundance of individuals through direct mortality and indirectly by removing larger reproductive animals that contribute more to population growth (Doak et al., 1994).

In addition to the smaller sizes of adult tortoises, there were also a greater proportion of juvenile tortoises near the high traffic volume road than along any of the other road categories. There are several potential explanations as to why we observed more juveniles in this area. One likely explanation is that juveniles are less susceptible to road mortality due to smaller home ranges and lower rates of movement than adults (Harless et al., 2009; Hazard and Morafka, 2002; O'Connor et al., 1994). Thus, juveniles may less frequently encounter and be killed on roads. Alternatively, areas near high traffic volume roads may be attractive to dispersing juveniles due to lower densities of tortoises and associated reductions in intraspecific resource competition. Additionally, tortoises near the high traffic volume road may have shorter lives from greater mortality. The lower number of live animal encounters and the greater number of observed mortalities near our highest traffic road category provides supporting evidence. The longer an individual lives near a road or the greater the frequency of passing vehicles, the more likely an individual is over time to encounter a vehicle with potentially fatal consequences, thus leading to the loss of older animals. The presence of greater relative proportions of juveniles in habitat near high traffic volume roads may ultimately indicate that habitat near roads used by as few as 300 vehicles per day represents sink habitat for desert tortoises.

We found no significant negative effects on tortoises near low and intermediate traffic volume roads as measured by road-effect zone, tortoise size, or demographic composition. However, we did observe that intermediate traffic volume roads had lower over-all relative abundance of tortoise sign compared to low traffic volume roads. We further observed that our intermediate traffic volume roads had mean values that were frequently between the low and high traffic volume roads, suggesting that even our intermediate traffic roads may affect nearby tortoises. Availability of roads within the preserve limited our sample size, which may have limited our ability to statistically detect the more subtle effects of intermediate traffic volume roads. Interestingly, tortoises encountered along the low traffic volume road were larger on average than were tortoises found >500 m from any road. One possible explanation is that roads may physically alter habitat in a way that can be beneficial when mortality is not also increased from tortoise-vehicle collisions. For example, roads increase water runoff, a factor that may increase drinking opportunities in water-limited deserts and which may also increase biomass of road-side annual vegetation. Increased water and forage availability can increase growth rates and survival of the desert tortoise (Nagy and Medica, 1986; Peterson, 1996). However, even if roads have the potential to positively alter habitat characteristics, the increased disturbance or mortality that occurs with as few as 300 vehicles per day likely negates such changes. Furthermore, the interaction between low traffic roads and local habitat quality is likely species-specific. For instance, previous studies have indicated that black bears avoid gravel roads (Reynolds-Hogland and Mitchell, 2007). Although none of the metrics we measured had statistically observable negative associations between our lowest traffic road and desert tortoises, other metrics, tortoise populations, or species may demonstrate negative responses.

Our results should be considered a conservative estimate of road-effect zones for desert tortoises. The traffic volumes studied in this paper, even for our "high" traffic volume roads, were relatively low (Grilo et al., 2009). Our highest traffic volume roads were lower than volumes on highways and interstates, which are frequently targeted or recommended for wildlife mitigation measures (Bissonette and Rosa, 2012; Ford et al., 2011; Gonzalez-Gallina et al., 2013). Within Mojave National Preserve, a designated wilderness area, the traffic volumes are lower than those in much of the desert tortoise's range due to the absence of commercial traffic, highways, and interstates. Thus, our results likely under-represent road-effect zones that tortoises experience in many other parts of their distribution, as well as emphasize the importance of studying the effects of even relatively low traffic roads. The noticeable effects of the relatively low traffic volumes studied here highlight the need to estimate the species' capacity to absorb additive mortality associated with a variety of traffic volumes and the contribution of these differing roads to population declines.

Though the installation of barrier fencing is a prescribed mitigation tool for reducing road mortality and road impacts on many species including the desert tortoise (USFWS, 2008), the use of fencing is not without controversy. Fences can transform a semi-permeable barrier into an impermeable one. As a result, fencing may fragment populations, prevent recolonization of depauperate but otherwise suitable habitat, and subsequently increase extinction risk. Given the prospect of changing climate in coming decades, fencing as a mitigation tool may drastically reduce likelihood of species persistence by limiting the ability of populations to follow shifts in the location of suitable habitat. Consequently, fences are predicted to have greater negative effects on population persistence than roads when road mortality is sufficiently low (Jaeger and Fahrig, 2004). The development of predictive measures to estimate traffic threat to nearby populations, population responses to fencing, fencing effectiveness, and willingness of individuals to use culverts are thus important future topics. Our results suggest that additional preventative measures (such as fencing) against road mortality along roads with as few as 300 vehicles per day may be beneficial at least in preventing initial population declines.

Our findings provide additional evidence that a vagile reptile species with a large home range and long generation time (Franks et al., 2011; Mueller et al., 1998) is susceptible to road presence, and that increasing traffic volume exacerbates associated effects. For many species, particularly those already experiencing declines, the impact of roads is of growing concern. Protected areas, such as national parks, can represent important buffers against extinction. Often, protected areas are treated as secure from anthropogenic impacts and human encroachment. However, even in otherwise protected areas, roads can present important threats to wildlife populations (Roger et al., 2012). Furthermore, over half of surveyed US national parks have expressed concern for the adverse effects of roads on endangered species within their borders, but most parks have little data documenting road effects on wildlife (Ament et al., 2008). In spite of this, many agencies responsible for managing wildlife populations may lack the resources necessary to acquire these data for the majority of species encompassed. Thus, knowledge of life history characteristics or patterns that correlate with sensitivity to road presence is an increasingly valuable conservation tool. Such a tool would allow wildlife or land managers to easily identify species or habitat zones that require road mitigation efforts to prevent declines in local abundances of target species, even in protected areas where they are often presumed secure.

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References

Ament, R., Clevenger, A.P., Yu, O., Hardy, A., 2008. An assessment of road impacts on wildlife populations in US National Parks. Environ. Manage. 42, 480–496.

Andersen, M.C., Watts, J.M., Freilich, J.E., Yool, S.R., Wakefield, G.I., McCauley, J.F., Fahnestock, P.B., 2000. Regression-tree modeling of desert tortoise habitat in the central Mojave Desert. Ecol. Appl. 10, 890–900.

Andrews, K.M., Gibbons, J.W., Reeder, T.W., 2005. How do highways influence snake movement? Behavioral responses to roads and vehicles. Copeia 2005, 772–782.

Ashley, E.P., Kosloski, A., Petrie, S.A., 2007. Incidence of intentional vehicle-reptile collisions. Hum. Dimens. Wildl. 12, 137–143.

Benitez-Lopez, A., Alkemade, R., Verweij, P.A., 2010. The impacts of roads and other infrastructure on mammal and bird populations: a meta-analysis. Biol. Cons. 143, 1307–1316.

Bissonette, J.A., Rosa, S., 2012. An evaluation of a mitigation strategy for deer-vehicle collisions. Wildlife Biol. 18, 414–423.

Boarman, W.I., Sazaki, M., 2006. A highway's road-effect zone for desert tortoises (*Gopherus agassizii*). J. Arid Environ. 65, 94–101.

Buhlmann, K.A., Akre, T.S.B., Iverson, J.B., Karapatakis, D., Mittermeier, R.A., Georges, A., Rhodin, A.G.J., van Dijik, P.P., Gibbons, J.W., 2009. A global analysis of tortoise and freshwater turtle distributions with identification of priority conservation areas. Chelonian Conserv. Bi. 8, 116–149.

Cagle, F.R., 1939. A system for marking turtles for future identification. Copeia 1939, 170–173.

Carr, L.W., Fahrig, L., 2001. Effect of road traffic on two amphibian species of different vagility. Conserv. Biol. 15, 1071–1078.

Congdon, J.D., Breitenbach, G.L., van Loben Sels, R.C., Tinkle, D.W., 1987. Reproduction and nesting ecology of snapping turtles (*Chelydra serpentina*) in southeastern Michigan. Herpetologica 43, 39–54.

Congdon, J.D., Dunham, A.E., Van Loben Sels, R.C., 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation management of long-lived organisms. Conserv. Biol. 7, 826–833.

Congdon, J.D., Dunham, A.E., van Loben Sels, R.C., 1994. Demographics of common snapping turtles (*Chelydra serpentina*): implications for conservation and management of long-lived organisms. Amer. Zool. 34, 397–408.

Doak, D., Kareiva, P., Kleptetka, B., 1994. Modeling population viability for the desert tortoise in the western Mojave desert. Ecol. Appl. 4, 446–460.

Doroff, A.M., Keith, L.B., 1990. Demography and ecology of an ornate box turtle (*Terrapene ornata*) population in south-central Wisconsin. Copeia, 387–399.

Eigenbrod, F., Hecnar, S.J., Fahrig, L., 2008. The relative effects of road traffic and forest cover on anuran populations. Biol. Cons. 141, 35–46.

Fahrig, L., Rytwinski, T., 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecol. Soc. 14, 21.

Ford, N.B., Seigel, R.A., 1989. Relationship among body size, clutch size, and egg size in three species of oviparous snakes. Herpetologica 45, 75–83.

Ford, A.T., Clevenger, A.P., Huijser, M.P., Dibb, A., 2011. Planning and prioritization strategies for phased highway mitigation using wildlife-vehicle collision data. Wildlife Biol. 17, 253–265.

Forman, R.T.T., 2000. Estimate of the area affected ecologically by the road system in the United States. Conserv. Biol. 14, 31–35.

Franks, B.R., Avery, H.W., Spotila, J.R., 2011. Home range and movement of desert tortoises *Gopherus agassizii* in the Mojave Desert of California. USA ESR 13, 191–201.

Gibbs, J.P., Shriver, W.G., 2002. Estimating the effects of road mortality on turtle populations. Conserv. Biol. 16, 1647–1652.

Gibbs, J.P., Steen, D.A., 2005. Trends in sex ratios of turtles in the United States: implications of road mortality. Conserv. Biol. 19, 552–556.

Gonzalez-Gallina, A., Benitez-Badillo, G., Rojas-Soto, O.R., Hidalgo-Mihart, M.G., 2013. The small, the forgotten and the dead: highway impact on vertebrates and its implications for mitigation strategies. Biodivers. Conserv. 22, 325–342.

Grandmaison, D.D., Ingraldi, M.F., Peck, F.R., 2010. Desert tortoise microhabitat selection on the florence military reservation, south-central Arizona. J. Herpetol. 44, 581–590.

Grilo, C., Bissonette, J.A., Santos-Reis, M., 2009. Spatial-temporal patterns in Mediterranean carnivore road casualties: consequences for mitigation. Biol. Conserv. 142, 301–313.

Harless, M.L., Walde, A.D., Delaney, D.K., Pater, L.L., Hayes, W.K., 2009. Home range, spatial overlap, and burrow use of the desert tortoise in the west Mojave Desert. Copeia, 378–389.

Hazard, L.C., Morafka, D.J., 2002. Comparative dispersion of neonate and headstarted juvenile desert tortoises (*Gopherus agassizii*): a preliminary assessment of age effects. Chelonian Conserv. Bi. 4, 406–409.

Heppell, S.S., 1998. Application of life-history theory and population model analysis to turtle conservation. Copeia 2, 367–375.

Jacobs, L., Houlahan, J.E., 2012. Adjacent land-use affects amphibian community composition and species richness in managed forests in New Brunswick, Canada. Can. J. Forest Res. 41, 1687–1697.

Jaeger, J.A.G., Fahrig, L., 2004. Effects of road fencing on population persistence. Conserv. Biol. 18, 1651–1657.

Karraker, N.E., Gibbs, J.P., 2011. Contrasting road effect signals in reproduction of long- versus short-lived amphibians. Hydrobiologia 664, 213–218.

Marsh, D., Page, R., Hanlon, T., Corritone, R., Little, E., Seifert, D., Cabe, P., 2008. Effects of roads on patterns of genetic differentiation in red-backed salamanders, (*Plethodon cinereus*). Conserv. Genet. 9, 603–613.

McCoy, E.D., Mushinsky, H.R., 2007. Estimates of minimum patch size depend on the method of estimation and the condition of the habitat. Ecology 88, 1401–1407.

Mueller, J.M., Sharp, K.R., Zander, K.K., Rakestraw, D.L., Rautenstrauch, K.R., Lederle, P.E., 1998. Size-specific fecundity of the desert tortoise (*Gopherus agassizii*). J. Herpetol. 32, 313–319.

Mushinsky, H.R., Wilson, D.S., McCoy, E.D., 1994. Growth and sexual dimorphism of *Gopherus polyphemus* in central Florida. Herpetologica 50, 119–128.

Nagy, K.A., Medica, P.A., 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica 42, 73–92.

Nicholson, L., 1979. The effects of roads on desert tortoise populations. In: Proceedings of the Desert Tortoise Council Symposium 1978, pp. 127–129.

O'Connor, M.P., Zimmerman, L.C., Ruby, D.E., Bulova, S.J., Spotila, J.R., 1994. Home range size and movements by desert tortoises, *Gopherus agassizii*, in the eastern Mojave Desert. Herpetol. Monogr. 8, 60–71.

Patrick, D.A., Gibbs, J.P., 2010. Population structure and movements of freshwater turtles across a road-density gradient. Landscape Ecol. 25, 791–801.

Peterson, C.C., 1996. Ecological energetics of the desert tortoise (*Gopherus agassizii*): effects of rainfall and drought. Ecology 77, 1831–1844.

Reynolds-Hogland, M.J., Mitchell, M.S., 2007. Effects of roads on habitat quality for bears in the Southern Appalachians: a long-term study. J. Mammal. 88, 1050–1061.

Roger, E., Bino, G., Ramp, D., 2012. Linking habitat suitability and road mortalities across geographic ranges. Landscape Ecol. 27, 1167–1181.

Row, J.R., Blouin-Demers, G., Weatherhead, P.J., 2007. Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). Biol. Conserv. 137, 117–124.

Rytwinski, T., Fahrig, L., 2011. Reproductive rate and body size predict road impacts on mammal abundance. Ecol. Appl. 21, 589–600.

Rytwinski, T., Fahrig, L., 2012. Do species life history traits explain population responses to roads? A meta-analysis. Biol. Conserv. 147, 87–98.

Sullivan, B.K., 1981. Distribution and relative abundance of snakes along a transect in California. J. Herpetol. 15, 247–248.

Taylor, B.D., Goldingay, R.L., 2010. Roads and wildlife: impacts, mitigation and implications for wildlife management in Australia. Wildlife Res. 37, 320–331.

Trombulak, S.C., Frissell, C.A., 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conserv. Biol. 14, 18–30.

Turner, F.B., Hayden, P., Burge, B.L., Roberson, J.B., 1986. Egg production by the desert tortoise (*Gopherus agassizii*) in California. Herpetologica 42, 93–104.

Turner, F.B., Medica, P.A., Bury, R.B., 1987. Age-size relationships of desert tortoises (*Gopherus agassizii*) in southern Nevada. Copeia 1987, 974–979.

USFWS, 2008. Draft revised recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*). In: U.S. Fish and Wildlife Service, California and Nevada Region U.S.F.W.S., (Ed.), U.S. Fish and Wildlife Service, Sacramento, CA, pp. 209.

Van Horne, B., 1983. Density as a misleading indicator of habitat quality. J. Wildl. Manage. 47, 893–901.

Von Seckendorff, H.K., Marlow, R.W., 2002. Impacts of vehicle road traffic on desert tortoise populations with consideration of conservation of tortoise habitat in southern Nevada. Chelonian Conserv. Bi. 4, 449–456.

Winck, G.R., Rocha, C.F.D., 2012. Reproductive trends of Brazilian lizards (Reptilia, Squamata): the relationship between clutch size and body size in females. N. West. J. Zool. 8, 57–62.

Zylstra, E.R., Steidl, R.J., Swann, D.E., 2010. Evaluating survey methods for monitoring a rare vertebrate, the Sonoran desert tortoise. J. Wildl. Manage. 74, 1311–1318.

occupancy surveys for Sonoran desert tortoise on the Arizona Buckeye Army National Guard Training Area. Surveys were conducted on 120, randomly selected, 3 hectare plots. Each plot was surveyed five times from mid-July to mid-October. A total of four individuals were detected on eight occasions, suggesting that the site hosts a relatively low density population of tortoises. In 2014, we again conducted occupancy surveys at the site, but chose a cluster method for plot selection. We generated 71 new plots using observations of scat, carcasses, and live SDTs from 2013. As in the previous year, we surveyed each plot five times from mid-July to Mid- Oct. In 2014, however, both the number of individuals detected and total detections increased by 50%. Because occupancy surveys can be costly both monetarily and temporally, we feel that focusing effort around known occupied plots in areas of low density are better able to inform management decisions.

Whole Genome Resequencing Provides Novel Landscape Genomic Insights for Desert Tortoise Conservation

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The field of landscape genetics provides a powerful toolkit for understanding how organisms move across complex environments, and the discipline has been applied with great success to desert tortoise populations. However, inferences from previous work have been limited by the number of available genetic markers and the number and extent of landscape features available for analysis. To address these issues, we consolidated tortoise samples from across their range within California and southern Nevada, generated a DNA dataset consisting of full genomes of 270 tortoises, assembled a set of 83 high-resolution geospatial layers, and analyzed the way in which the geography and environment of the desert tortoise have determined modern patterns of relatedness and genetic diversity across the landscape. Rangewide, Principal Component Analysis (PCA) revealed two major clusters of tortoises split by PC1 that lie north and south of the mountains defining the Ivanpah valley, while PC2 further subdivided the southern group on either side of the low-lying Cadiz valley. These first two axes primarily recapitulated geographic patterns, and explained 8.3% of the variation in the data. Subtle patterns of movement and admixture between these regions were also evident in the data. Twenty of a set of 24 landscape rasters that are maximally uncorrelated are statistically associated with genetic divergence among tortoises after geographic distance is taken into account, suggesting that they explain a great deal of the remaining genetic variation in the data set. Although this work is still underway, preliminary analyses indicate that precipitation and temperature variables are the most

highly correlated with genetic divergence, with smaller, but still significant effects of vegetation, and slope. Our next goals are to build genomically explicit models with different hypothetical placements of alternative energy installations to quantify the future effects of such placements on population viability models over the next century.

Landscape Genomics Aid Restoration of Desert Tortoise Habitat: A Case Study with *Sphaeralcea ambigua* (Malvaceae) in the Mojave Desert

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Local adaptation is widespread across plant taxa and may influence the responses of species to climate change and the effectiveness of their use in ecological restoration. Natural populations are characterized by fine-scale physiological or 41olyphemus41l adaptations that drive intraspecific variability in demographic responses to altered environmental conditions. Restoration techniques that expose plant materials to novel environments (e.g., broadcast seeding) should account for adaptive genetic variability, which may influence seedling establishment and longer-term fitness. Landscape genomic approaches aim to identify environmental drivers of adaptive genetic variability and map spatial patterns of gene / environment associations — information that can guide habitat restoration (e.g., seed sourcing) and improve predictions of species' responses to climate change. There is a clear need for landscape genomic studies of Mojave Desert plant taxa that comprise habitat for the desert tortoise (*Gopherus agassizii*) because both climate change and novel disturbance regimes are expected to impact vegetation throughout this region. We conducted a landscape genomic analysis for an important restoration and forage plant species, *Sphaeralcea ambigua*, by analyzing the variation at 153 amplified fragment length polymorphism (AFLP) loci from 47 Mojave Desert populations. Adaptive allele frequencies were most strongly correlated with summer maximum temperature and indices for vegetation water stress and surface temperature. Variation in adaptive allele frequencies along precipitation and temperature gradients was non-linear, potentially indicating physiological thresholds separating ecotypes. However, a linear relationship between adaptive alleles and temperature seasonality may reflect continuous differences in 41olyphemus41l optima. Two approaches for mapping the predicted patterns of turnover in adaptive allele frequencies are compared. Techniques developed in this study offer a robust approach for deriving spatially-explicit models of adaptive genetic variability in non-model species and providing restoration specialists the guidance needed for collection and use of Mojave Desert plant materials.