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Social structure and drivers behind asynchronous burrow associations of the desert tortoise

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Abstract

For several species, refuges (such as burrows, dens, roosts, nests) are an essential resource for protection from predators and extreme environmental conditions. Refuges also serve as focal sites of social interactions including mating, courtship and aggression. Knowledge of refuge use patterns can therefore provide information about social structure as well mating and foraging success of wildlife populations, especially for

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species considered to be relatively solitary. In this study, we sought to (a) infer social associations of the desert tortoise through their asynchronous burrow associations, and (b) provide mechanisms behind individual-level variation in burrow use networks using a model of burrow switching (to describe variation in tortoise behavior) and burrow popularity (to describe variation from the perspective of burrows). Although considered to be relatively solitary, we found tortoise social networks to be significantly different than null networks of random associations, with geographical locations having a moderate influence on tortoises' associations. Seasonal variation and local tortoise/burrow density had a strong impact on individual's burrow switching behavior. Among the three population stressors included in the model (translocation, drought, disease), translocation had the largest effect on burrow switching. Analysis of variation in burrow popularity revealed older burrows and burrows at rough higher elevation sites are more popular than other burrows in desert tortoise habitat. Our study emphasizes the role of combining graph theoretic and statistical approaches to examine the social structure of (relatively) solitary species to design effective conservation and management strategies including control of future infection spread.

Key words: behavioral stress response, *bipartite networks*, *generalized linear mixed models*, *Mycoplasma agassizii*, *seasonality*, *URTD*.

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INTRODUCTION

Incorporating behavior into conservation and management of species has garnered increased interest over the past twenty years (Clemmons 1997; Swaisgood 2007; Festa-Bianchet and Apollonio 2013). Adaptive behavioral responses such as habitat selection, patch use, and foraging (Morris et al. 2009; Berger-Tal et al. 2011), can be efficient indicators of population disturbances because, unlike population dynamics, they can respond instantaneously to altered conditions. Refuge use can affect fitness as refuges, by providing shelter, protection from predators and sites for nesting, are central to survival and reproductive success. Altered patterns of refuge use may therefore indicate a disturbance or change in population fitness and provide an early warning to conservation biologists.

Quantifying patterns of refuge use is especially useful for relatively solitary species, as it can provide important information about their social structure. Social structure of wildlife populations is typically derived from observational studies on direct social interactions (e.g. in primates (Griffin and Nunn 2011; MacIntosh et al. 2012), dolphins (Lusseau et al. 2006), ungulates (Cross et al. 2004; Vander Wal et al. 2012) etc.). Direct interactions are less frequent and thus harder to quantify for relatively solitary species. For such species, social interactions may be limited to certain areas within their habitat, such as refuges (e.g., roost, den, burrow, nest) or watering holes that provide increased opportunities of direct contact between individuals. Monitoring these resources can therefore help establish relevant social patterns among individuals. In addition to establishing social structure, knowledge of refuge use patterns can serve as a key tool in efforts to control the spread of infection in solitary species. Transmission of pathogens occurs either through close contacts among hosts or through fomites. As refuges often serve as focal sites of host contacts in solitary species, patterns of refuge use can be used to establish relevant contact networks for prediction of infectious disease dynamics.

Here we investigate patterns of burrow use in the desert tortoise, *Gopherus agassizii*. The desert tortoise is a long-lived, terrestrial species in the Testudinidae family that oc-

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curs throughout the Mojave Desert north and west of the Colorado River. Desert tortoises use subterranean burrows (excavated by both adults and non-reproductives) as an essential adaptation to obtain protection from temperature extremes and predators. Constructing new burrows can be an energy-intensive process, and tortoises often use existing burrows when available (Duda and Krzysik 1998). Because tortoises utilize existing refuges and spend a majority of their time in or near burrows, most of their social interactions are associated with burrows (Bulova, 1994). Documenting asynchronous burrow use can therefore provide insights towards sociality in desert tortoises.

Social behavior in desert tortoises is not well understood, though evidence suggests some dominance hierarchies or structure may be present (Niblick et al. 1994; Bulova 1997) which can influence burrow choice in tortoises. In addition to social structure, environmental conditions and burrow attributes can likely influence burrow-use behavior. Multiple tortoises have been observed visiting a subset of burrows on the landscape (Woodbury and Hardy 1948), suggesting popularity of a burrow may increase the likelihood of social interaction (Bulova 1994). At an individual scale, previous research suggests factors such as sex (Harless et al. 2009), age (Wilson et al. 1999), season (Bulova 1994); and environmental conditions (Duda et al. 1999; Franks et al. 2011) may influence burrow use in desert tortoises. However, we currently lack a mechanistic understanding of heterogeneity in burrow use patterns, as the relative effect of various factors influencing burrow switching in desert tortoises and popularity of burrows is unknown.

If conspecific cues and environmental factors exhibit strong influence on burrow use, population stressors impacting these characteristics could alter typical burrow behavior. Desert tortoises are currently listed as a threatened species under the US Endangered Species Act (Department of the Interior: US Fish and Wildlife Service 2011). Three major threats to desert tortoise populations have been identified, the first being anthropogenic interference such as overgrazing, urban development, development of solar power plants, etc. (Boarman 2002). In the recovery plan for the species, the recovery guidelines recommend translocating

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animals in affected populations in response to these anthropogenic disturbances (Department of the Interior: US Fish and Wildlife Service 2011). Translocation in other reptilian species, however, has had limited success due to high rates of mortality (Dodd and Seigel 1991; Germano and Bishop 2009). The second threat is an infectious disease called upper respiratory tract disease caused by *Mycoplasma agassizii* and *Mycoplasma testudineum* (Brown et al. 1994; Sandmeier et al. 2009; Jacobson et al. 2014). The third threat to desert tortoise populations is extreme environmental conditions, particularly drought (Lovich et al. 2014). All three of these stressors: translocation, disease, and drought, have been linked to differences in tortoise behavior (Duda et al. 1999; Nussear et al. 2012; McGuire et al. 2014).

In this study we combined graph theoretic and statistical approaches to: 1) investigate social structure in desert tortoises populations as reflected by their asynchronous burrow use, and 2) analyze the relative contribution of tortoise attributes, burrow attributes, environment, density conditions as well as population stressors towards patterns of burrow use in desert tortoises. To achieve this goal we combined data-sets from nine study sites in desert tortoise habitat (Fig.1), spanning more than 15 years to derive burrow use patterns and tease apart the effect of various drivers and population stressors. We first constructed bipartite networks of burrow use in desert tortoise to infer social associations due to asynchronous burrow use. We then used generalized linear mixed models to examine the potential variables influencing burrow use patterns from the perspective of both individuals and burrows. Our analysis, unlike previous research, attempts to describe the population level consequences of asynchronous burrow use as well as tease apart the role of various drivers of burrow use while controlling for others. In addition, as desert tortoises are long lived species, quantifying demographic consequences of population stressors can be difficult. Our analysis instead focuses on behavioral consequences of population stressors that are linked to foraging and mating, and thereby survival success.

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MATERIALS AND METHODS

Dataset

We combined datasets from nine study sites across desert tortoise habitat in the Mojave desert (Fig.1) of California, Nevada, and Utah. At each site, individuals were monitored at least weekly during their active season and at least monthly during winter months using radio telemetery. All tortoises were uniquely tagged, and during each tortoise encounter, data were collected to record the individual identifier of the animal, date, GPS location, microhabitat of the animal (e.g., vegetation, pallet, or a burrow), any visible signs of injury or upper respiratory tract disease, and environmental conditions. The unique burrow identification (id) was recorded for cases where an animal was located in a burrow. New burrow ids were assigned when an individual was encountered at a previously unmarked burrow. Each site was monitored over multiple but not simultaneous years (SI Table1).

Network analysis

We constructed burrow use networks of desert tortoises in five out of the nine sites (CS, HW, MC, PV, SL; where no translocations occurred) during active (March - October) and inactive season (November - February) of each surveyed year as a two-mode *bipartite network* that consisted of burrow and tortoise nodes (Fig.2). An edge connecting a tortoise node to a burrow node indicates usage of that burrow by the individual. Edges in a bipartite network always connect the two different node types, thus edges connecting two tortoise nodes or two burrow nodes are not permitted. Tortoise nodal degree in the bipartite network therefore denotes the number of unique burrows used by the individual and burrow nodal degree is the number of unique individuals visiting the burrow. The power of using bipartite networks of burrow use is to represent both animals and burrows as nodes, thus representing an interaction between individual tortoises and burrows. To reduce bias due to uneven sampling, we did not assign edge weights to the bipartite networks.

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We further examined the social structure of desert tortoises by converting the bipartite network into a single-mode projection of tortoise nodes (Tortoise social network, Fig.2). For these tortoise social networks, we calculated network density, degree centralization, modularity, clustering, and assortativity of individuals by degree and sex/age class. Network density is calculated as the fraction of observed edges to the total possible edges in a network. Degree centralization measures the variation in node degree across the network, such that high values indicate a higher heterogeneity in node degree and that a small number of nodes have a higher degree than the rest. Modularity measures the strength of the division of nodes into subgroups (Girvan and Newman 2002) and clustering measures the tendency of neighbours of a node to be connected (Bansal et al. 2009). The values of modularity and clustering can range from 0 to 1, and larger values indicate stronger modularity or clustering. We generated 1000 random network counterparts to each empirical network using the configuration model (Molloy and Reed 1995) to determine if the observed network metrics were significantly different from random expectation. The generated random networks had the same degree distribution, average network degree, and number of nodes as empirical networks, but were random with respect to other network properties.

We next examined the spatial dependence of asynchronous burrow associations by using coordinates of burrows visited by tortoises to calculate centroid location of each tortoise during a particular season of a year. Distances between each tortoise pair (i, j) were then calculated as $d_{ij} = d_{ji} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$ where (x, y) is the coordinate of tortoise centroid location. Pearson correlation coefficient was used to calculate the correlation between observed social associations and geographical distances between the tortoises. We compared the observed correlation to a null distribution of correlation values generated by randomly permuting spatial location of burrows 10,000 times and recalculating correlation between social associations and distance matrix for each permutation.

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Regression Analysis

We used generalized linear mixed regression models with Poisson distribution and log link function to assess burrow use patterns. To capture seasonal variation in burrow use, we aggregated the response counts over six periods (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec). Patterns of burrow use were analyzed in two ways. First, we investigated factors affecting burrow switching, which we define as the number of unique burrows used by a tortoise in a particular sampling period. Second, we investigated burrow popularity, defined as the number of unique individuals using a burrow in a particular sampling period. Model variables used for each analysis are summarized in Table 1. All continuous model variables were centered (by subtracting their averages) and scaled to unit variances (by dividing by their standard deviation). This standard approach in multivariate regression modeling assigns each continuous predictor with the same prior importance in the analysis (Schielzeth 2010). All analyses were performed in R (version 3.0.2; R Development Core Team 2013).

Investigating burrow switching of desert tortoises:

In this model, the response variable was burrow switching, defined as the total number of unique burrows used by desert tortoises during each sampling period. An individual was considered to be using a burrow if it was reported either inside a burrow or within 25 sqm grid around a burrow. The predictors included in the model are described in Table 1. In addition to the fixed effects, we considered three interactions in this model (i) sampling period $\times$ sex, (ii) sampling period $\times$ seasonal rainfall and (iii) local tortoise density $\times$ local burrow density. Tortoise identification and year $\times$ site were treated as random effects.

Investigating burrow popularity:

For this model, the response variable was burrow popularity defined as the total number of unique tortoises using a focal burrow in a sampling period. The predictors included in

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the model are also described in Table 1. In this model, we also tested for three interactions between predictors including (i) sampling period × seasonal rainfall, (ii) sampling period × local tortoise density, and (iii) local tortoise density × local burrow density. We treated burrow identification and year × site as random effects.

Population stressors:

Disease as a stressor: We considered field observations of tortoises exhibiting typical signs of URTD including nasal discharge, swollen (or irritated/ sunken) eyes and occluded nares to be indicative of an unhealthy animal. As diagnostic testing was not the focus of the studies collecting the data, we were unable to confirm the infection status of individuals. Knowledge of confirmed infection status of animals, however, was not central to our study as our aim was to measure behavioral response of symptomatic individuals only. We included health condition in the regression model as a categorical variable with two levels - healthy and unhealthy. An individual was considered to be unhealthy if it was reported to display clinical signs of URTD at least once during the sampling period.

Translocation as a stressor: Translocations were carried out at four (BSV, FI, LM, SG) out of nine sites in our dataset for purposes described in previous studies (Drake et al. 2012; Nussear et al. 2012). We categorized all animals native to the site prior to translocation as controls. Post translocation, all control animals at translocation sites were categorized as residents and introduced animals as translocated. Translocated and resident animals were labeled as ex-translocated and ex-residents, respectively, after a year of translocation to account for potential acclimatization of introduced animals (Nussear et al. 2012). We note that one of four translocation sites (SG) did not have native animals prior to translocation. No translocations were carried out at the rest of the five sites, so all animals surveyed at those sites were labeled as controls. We accounted for translocation in the regression model by giving each surveyed tortoise one of the following five residency status at each sampling period: Control (C), Resident (R), Translocated (T), Ex-Resident (ER) or Ex-Translocated

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(ET).

Drought as a stressor: The desert tortoise habitat in Mojave desert typically receives most of the rainfall during the winter season. We therefore use winter rainfall to assess drought conditions in desert tortoise habitat. We defined winter rain during a year as average rainfall from November to February and used it as a proxy of drought condition for the following year. We note that summer rainfall in desert tortoise habitat varies from west to east, where summer rainfall becomes a larger component of the total annual precipitation in East Mojave desert (Henen et al. 1998). Therefore, although we used winter rainfall as a proxy of drought conditions, we considered the effects of summer precipitation implicitly by including seasonal rainfall as a separate predictor (see Table1).

Model selection and validation

Following Harrell (2002) we avoided model selection to remove non-significant predictors and instead present results of our full model. Using the full model with insignificant predictors allows model predictions conditional on the values of all the model predictors and results in more accurate confidence interval of effects of interest (Harrell 2002). The Bayesian information criterion (BIC) of model selection was used only to identify the best higher order interactions. A potential drawback of including all independent variables in the final model is multicollinearity. We therefore estimated Generalized Variance Inflation Factor (GVIF) values for each predictor. GVIF is a variant of traditional VIF used when any predictor in the model has more than 1 degree of freedom (Fox and Monette 1992). To make GVIF comparable across dimensions, Fox and Monette (1992) suggest using $\mathrm{GVIF}^{(1/(2.\mathrm{Df}))}$ which we refer to as adjusted GVIF. We sequentially removed predictors with high adjusted GVIFs, recalculated adjusted GVIF, and repeated the process until all adjusted GVIF values in the model were below 3 (Zuur et al. 2010).

We carried out graphical diagnostics by inspecting the Pearson residuals for the conditional distribution to check if the models fit our data in each case. We detected under-

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dispersion in both the regression models. Under-dispersed models yield consistent estimates, but as equi-dispersion assumption is not true, the maximum-likelihood variance matrix over-estimates the true variance matrix which leads to over-estimation of true standard errors (Winkelmann 2003). We therefore estimated 95% confidence intervals of fixed and random effects using bootstrapping procedures implemented in 'bootMER' function in package lme4.

We tested for the significance of fixed factors in both the models using likelihood ratio test (R function mixed from afex package Singmann (2013)). For significant categorical predictors, we used Tukeys HSD (R function glht from the multcomp package, (Hothorn et al. 2008)) as a post-hoc test of significant pair-wise differences among means. All reported p-values of post-hoc tests are adjusted for multiple comparisons using the single-step method (Hothorn et al. 2008).

RESULTS

Network analysis

We constructed bipartite networks of asynchronous burrow use in desert tortoises for active and inactive seasons of each year at five sites where no translocation were carried out. An example is shown in Fig.2. Tortoise nodal degree in the bipartite network represents the number of unique burrows used by the individual and burrow nodal degree is the number of unique tortoises visiting the burrow. Bipartite networks demonstrated considerable heterogeneity in tortoise degree and burrow degree (Fig.3). Tortoises visited more unique burrows on average (= 4.03 ± 3.43 SD) and had a greater range of burrows visited (1-9) in active seasons than in inactive seasons (average = 1.46±0.72 SD, range = 1-5). More than 60% of tortoises used a single burrow during Nov-Feb (inactive) months (Fig.3a). Most burrows in desert tortoise habitat were visited by a single tortoise during active and inactive season (Fig.3b). Heterogeneity in the number of animals visiting burrows, however, was slightly more during the months of March-November than November-February (active = 1.21±0.56

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SD, inactive = 1.08±0.35 SD).

Single mode projection of tortoise nodes from the bipartite network (henceforth called the tortoise social network) demonstrated moderate clustering (0.36 ± 0.21 SD) and modularity (0.53 ± 0.15 SD). Out of the total 24, 23 social networks had higher clustering and 18 social networks were more modular than random networks. Thirteen social networks out of the total 20 demonstrated significant degree homophily and 11 of those had positive associations (SI Table S3). Positive degree homophily (when nodes with similar degree tend to be connected) suggests that tortoises using many unique burrows often use the same set of burrows and are therefore connected in the social network. Tortoise social networks also had a moderate positive degree centralization which indicates a small subset of individuals used more burrows than the rest in the sampled population. Within sexes, positive degree centralization was observed both within males (0.20 ± 0.08 SD) and females (0.17 ± 0.06 SD). Homophilic association by sex ranged from -0.6 to 0.11 indicating a preference for one sex to associate with the opposite. These negative sexwise associations, however, were not different than those expected by chance.

The magnitude of correlation between geographical distances and social association in tortoise social network due to asynchronous burrow use ranged from -0.22 – -0.89 with an average value of -0.49 (Fig. 4). The p-value of the permutation test for all sites across active seasons of all surveyed years was less than 0.05, indicating a significant effect of geographical location on social associations. This result of spatial constraints driving social interactions is not surprising as geographical span of surveyed sites were much larger (>1500m) than the normal movement range of desert tortoises (Franks et al. 2011). However, the moderate value of correlations suggest other factors (such as environmental, social, density) could play an important role in desert tortoise's asynchronous burrow associations.

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Regression analysis

Based on the observed heterogeneity in bipartite networks, we next investigated the relative effect of natural variables and population stressors on burrow switching patterns of desert tortoises (*viz* degree of animal nodes in bipartite networks) and popularity of burrows in desert tortoise habitat (*viz* degree of burrow nodes in bipartite networks). SI Table4 presents the best models of BIC values for interactive predictors that explain burrow switching in desert tortoises and burrow popularity. The three interactions tested for burrow switching models were sampling period $\times$ sex, sampling period $\times$ seasonal rainfall and local tortoise density $\times$ local burrow density. We tested all possible combinations of the three interactions. The best model contained an interaction of sampling period $\times$ seasonal rainfall (SI Table4). The evidence ratio of this model was over 92 times higher than the second best model containing an additional interaction of local tortoise density $\times$ local burrow density.

For the burrow popularity model, we tested all possible combinations of the sampling period $\times$ seasonal rainfall, the sampling period $\times$ the local tortoise density and the local tortoise density $\times$ local burrow density interactions. The best model included the sampling period $\times$ the local tortoise density and the local tortoise density $\times$ the local burrow density interaction term. All three measures of temperature (average, max and min) had adjusted GVIF values of >3 and were therefore removed from the models. We also removed the sampling period $\times$ tortoise density interaction from the burrow popularity model as it inflated adj GVIF value of tortoise density to >3. σ^2 estimate of tortoise id and burrow id was negligible (tortoise id: $\sigma^2 = 0$, CI = 0-0.004, burrow id: $\sigma^2 = 0$, CI = 0-0.01). Both the random effects were therefore removed from the regression models.

Effect of animal attributes

Sex/age class had a significant effect on burrow switching (χ^2=16.75, P=0.0002). Overall, adults used more unique burrows than non-reproductives. Among adults, males used a slightly higher number of unique burrows than females (Fig. 5). There was no effect of body

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size on individuals' burrow switching behavior (χ^2= 0.2, P=0.65).

Effect of burrow attributes

Out of the six burrow attributes included in the model, burrow age and surface roughness around burrow had the highest impact on burrow popularity, i.e., number of unique individuals visiting the burrow (burrow age: χ^2= 46.07, P $<$ 0.0001, surface roughness: (χ^2= 14.37, P $<$0.0001). Burrow popularity was positively correlated with surface roughness indicating that burrows in flat sandy areas were visited by fewer unique tortoises than burrows in rough rocky areas. Older burrows were visited by more unique individuals, with burrow popularity increasing $e^{0.08}$ times with each increment of age (Fig. 5). Burrows in areas with higher topographical position as indicated by GIS raster images were also more popular (χ^2= 5.71, P= 0.02).

Effect of environmental conditions

Sampling period had a large effect on number of unique burrows used by desert tortoises (χ^2= 160.96, P $<$ 0.0001) as well as on burrow popularity (χ^2= 176.25, P $<$ 0.0001). Burrow switching of desert tortoises was highest during the months of May-June and September-October when they are typically more active, and lowest in winter months (Fig. 5). In the late summer (July-August), tortoises demonstrated slightly lower burrow switching than during the active season, but higher than the winter season. Within a particular year, the direction of the effect of seasonal rainfall varied across different sampling periods (sampling period $\times$ seasonal rain: χ^2= 107.46, P $<$ 0.0001). For example, high rainfall during the months of March-April reduced burrow switching in desert tortoises. On the other hand, individuals exhibited higher burrow switching with higher rain during the months of July-August (SI Fig. S3b).

In contrast to the large variation in individuals' burrow switching behavior between sampling periods, popularity of burrows did not vary during a large portion of the year (May

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- December). Total unique animals visiting burrows tended to be lower in the months of January-February and March-April, as compared to other months of the year (Fig. 5, S4c). Seasonal rainfall had a positive correlation with burrow popularity (χ^2= 6.02, P= 0.01).

Effect of density conditions

An increase in the number of active burrows around individuals promoted burrow switching, whereas an individual used fewer burrows when there were more tortoises in the vicinity (Fig. 5). In the burrow popularity model, higher tortoise density around burrows increased number of individuals visiting these burrows (Fig. 5). There was a significant interactive effect of the two density conditions on burrow popularity (χ^2= 177.37, P $<$ 0.0001) – increase in burrow popularity with higher tortoise density was lower when there were more burrows in the vicinity of the focal burrow (SI Fig. S4d).

Effect of population stressors

Population stressors of drought, health and translocation had variable influences on burrow switching of desert tortoises (Fig.5, S5). As compared to residents and controls, translocated animals demonstrated lower burrow switching during the year of translocation and also in the subsequent years. We did not find any differences between burrow switching levels of individuals exhibiting clinical signs of URTD and clinically healthy individuals (χ^2= 2.51, P = 0.11). Burrow switching levels of all surveyed animals (indicated by lower winter rainfall), however, was slightly lower in comparison to non-drought years (burrow switching: χ^2= 3.5, P = 0.06).

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DISCUSSION

Although previous studies have found evidence towards the existence of social hierarchies in desert tortoises (Niblick et al. 1994; Bulova 1997), there has been no attempt to quantify their social structure. Moreover, few studies have evaluated the social structure of wildlife species that do not display overt forms of gregariousness [but see (Corner et al. 2003; Leu et al. 2011; Hirsch et al. 2013)]. Even though direct social interactions among such species are relatively infrequent, individual preference for certain shared refuge and foraging spaces may lead to highly structured social system (Leu et al. 2011). Understanding social network of relatively solitary wildlife species through refuge/forage associations can provide insights towards vulnerability of populations towards future infections, identify key individuals that play a disproportionate role in disease spread, and provide early-warning signal for environmental (or anthropogenic) disturbances that may ultimately affect population fitness.

This study uses social network analysis tools to study social structure of desert tortoise population formed due to asynchronous burrow associations. We found social networks of desert tortoises to be significantly different than null networks of random associations. Desert tortoise asynchronous burrow associations were negatively density-dependent (as reflected in SI Table S3 and SI Fig S3c) and were subject to spatial constraints. In general, the social networks of desert tortoises had higher modularity (0.34 - 0.68) and clustering coefficient values (0.23-0.59) than random null networks. However, higher clustering coefficient values have been reported in other social species [e.g, 0.54-0.57 in bottlenose dolphins (Mann et al. 2012), 0.57-0.87 in guppies (Croft et al. 2004), 0.81 in squirrels (Manno 2008), 0.57-0.67 in primates (Pasquaretta et al. 2014)] and even in a few relatively solitary species that have been studied [e.g., 0.7 in raccoons (Hirsch et al. 2013), 0.59 in brushtail possum (Porphyre et al. 2011)]. The clustering coefficient metric indicates preference for an individual's neighbors to associate with each other, and low (but significant) clustering coefficient value in desert tortoises indicates that they do not form tight social bonds as compared to more social wildlife species. The spatial constraints to asynchronous burrow associations along with high

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modularity values can have important implications in infection spread through desert tortoise populations. For example, few connections between communities in a social network can effectively localize new infections to a few individuals. For chronic infections such as URTD, these pockets of infection, however, can serve as sources of re-infection to other uninfected communities, eventually leading to persistent infection across the entire population.

While describing network metrics is a standard approach to social network analysis, we sought to gain a mechanistic understanding behind individual heterogeneity in asynchronous burrow associations in desert tortoises. Degree of tortoises' nodes in bipartite networks has biological and ecological importance as it indicates a decision to switch burrows. Burrow switching in desert tortoises is associated with a tradeoff between the costs of increasing exposure to heat, predators, increased risk of infection and the benefits of finding food and mates. The outcome of burrow switching patterns observed in desert tortoise populations is important as theoretical models predict reduced survival of populations due to suboptimal refuge use decisions (Cooper Jr 2015). Modeling optimal burrow switching that maximizes fitness in desert tortoises is challenging as it is difficult to quantify fitness costs in a long-lived species. Our study instead provides a baseline of burrow use patterns in desert tortoises. Any large deviation to these baseline levels may lower the survival and thus burrow switching may serve as an immediate indicator of potential long-term fitness consequences.

Our analysis revealed local burrow density and time of the year to have the largest influence on burrow switching behavior of desert tortoises. Low burrow switching during winter and summer months reflects reduced movement of desert tortoises to avoid severe weather conditions (Eubanks et al. 2003). High burrow switching in May-June and September-October coincides with high activity of nesting and mating in adults. Seasonal rainfall also influenced burrow switching in desert tortoises. Tortoises demonstrated low burrow switching during high rainfall conditions in the months of March-April, which possibly reflects their reduced activity due to the cold weather associated with spring storms. The infrequent summer rains, on the other hand, increase tortoise activity as individuals emerge from burrows

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to rehydrate (Nagy and Medica 1986; Peterson 1996). Our results of high burrow switching during summer (July-August) are consistent with these reports of increased activity. We note that previous studies report sex differences in activity across seasons, with adult female tortoises moving longer distances and having larger home ranges during nesting season, and males being more active during mating season (Bulova 1994). However, our models suggests seasonal differences in burrow use behavior between adult sexes to be minor compared to other drivers of burrow use.

Among individuals, the differences in burrow use behavior between adults and non-reproductives were much larger than differences among adult males and females. These differences may reflect the different costs and benefits of switching burrows for reproductive adults and non-reproductive individuals. Leaving a refuge may present a greater risk to non-reproductives that are more vulnerable to predation (Wilson 1991), are prone to thermal stress due to their smaller size (Mushinsky et al. 2003), and do not benefit from the mating opportunities gained by burrow switching. Our results of lower burrow switching in non-reproductives also corroborate previous studies that found juveniles forage closer to their burrows and minimize time spent out of burrows (Mcrae et al. 1981; Mushinsky et al. 2003; Halstead et al. 2007). Future studies and management plans may consider differences in burrow switching between different non-reproductive tortoises including neonates, juveniles and subadults in order to mitigate increased predation risk by pervasive predators such as ravens.

Earlier studies report only one-fourth of burrows in desert tortoise habitat to be popular, i.e., used by more than one animal in a year (Bulova 1994; Harless et al. 2009). We show variables such as topographical variables (of surface roughness and elevation), age of burrow and density of tortoises around the burrow affect burrow popularity, which may explain why only a small fraction of burrows are visited by multiple animals. Knowledge of active and popular burrows can have two important implications for management of the species. First, population density estimates usually rely on observations of animals located above ground.

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Desert tortoises, however, spend most of the time in a year in burrows (Bulova 1994), which may lead to underestimation of true population densities (Nussear and Tracy 2007; Inman et al. 2010). Survey of active popular burrows at high tortoise density areas can augment the current survey methods in order to get a more accurate estimate of population density of desert tortoises. Our results suggest that popular burrows can be identified using certain burrow characteristics such as surrounding topographical variables and age. We believe older burrows have a higher chance to be a known resource to individuals which increases their popularity compared to younger burrows. As true burrow age is often hard to determine, we demonstrate the use of historical survey data to estimate proxy age of burrows. Once identified, these popular burrows can be surveyed throughout the year as there is only a minor effect of sampling period and seasonal rainfall on burrow popularity. Secondly, declines of popular burrows in desert tortoise habitat can indicate reduced social interactions and mating opportunities for individuals. Reduced burrow popularity can also be indicative of higher mortality risk - Esque et al. (2010) found higher mortality in flat open areas where burrows, as our results indicate, are less popular compared to rough higher elevation sites. Active popular burrows can be therefore used (a) as sentinels of population health and (b) to identify critical core habitat of desert tortoises for conservation and adaptive management of the species.

We investigated the effect of three population stressors - drought, translocation and disease - associated with major threats to the conservation of this species. Of the three stressors our results suggests translocation to have the strongest impact on burrow switching behavior of desert tortoises. Although translocated animals are known to have high dispersal tendencies (Nussear et al. 2012; Hinderle et al. 2015) and hence are expected to encounter and use more burrows, we found translocated individuals use fewer unique burrows than residents. Our results are supported by evidence of translocated tortoises spending more time on the surface and taking shelter under vegetation rather than using burrows (Hinderle 2011). Surprisingly, even after one year of translocation, relocated animals continued use

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of fewer burrows than residents in the population. The use of fewer burrows coupled with high dispersal rates can increase exposure of translocated animals to thermal stress and dehydration, potentially increasing mortality. Therefore, to improve translocation success, a fruitful area of investigation for future research will be to determine potential causes of this change in burrow use behavior in translocated tortoises.

There was no major effect of drought or disease on burrow switching patterns of tortoises in our data-set. Severe clinical signs of URTD have been associated with changes in burrow use pattern in Gopher tortoises (McGuire et al. 2014). Our results do not indicate any effect of disease, possibly because we could not distinguish severe clinical signs with milder forms in the dataset. Although there was no evidence of disease influencing burrow use behavior in the present study, we note that it is likely for burrow use behavior (and in particular the burrows themselves) to drive infectious disease patterns in desert tortoises either directly, through cohabitation instances, or indirectly, by serving as focal sites of social interactions. We used winter rain as a proxy of drought conditions as the Western Mojave receives most of its annual rainfall during the months of November-February, and because winter rain is important for the availability of food for desert tortoises in the spring (Duda et al. 1999; Lovich et al. 2014). Our results show the average number of unique burrows visited by tortoises were slightly reduced during drought years. Reduced burrow switching may correspond to smaller homeranges of desert tortoises observed during drought years (Duda et al. 1999). Years of low winter rainfall have been known to cause increased predation of desert tortoises due to diminished prey resources (Peterson 1994; Esque et al. 2010). Lower burrow switching during drought years can also be a behavioral response to avoid predation or reduce energy expenditure and water loss in years of low resource availability (Nagy and Medica 1986).

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CONCLUSIONS

We examined the social structure of desert tortoises through their asynchronous burrow use. Although typically considered to be solitary, we found the social network of desert tortoises to be significantly different that null networks of random associations. In order to gain a mechanistic understanding of individual level heterogeneity in bipartite burrow use networks, we first consider animals as units of interest and model tortoise degree (or tortoises' burrow switching behavior). Using burrows as units we next model burrow degree (or burrow popularity) in desert tortoise habitat. We compare and identify various factors of tortoise attributes, burrow attributes, environment and population stressors that affect burrow use patterns in desert tortoises.

Burrows are essential for survival and are the focal points of social interactions of several wildlife species that are not overtly gregarious. For these species burrow switching may therefore correlate to reproductive and foraging success, and patterns of burrow use can be an important aspect to consider before implementing any management or conservation strategy. For example, popular burrows can be used to identify core habitat areas. In addition, sudden change in burrow switching behavior of individuals can be used as an early warning signal of disturbances that may ultimately affect population fitness. Burrows might also play an important role in spread of infectious diseases by either providing refuge for prolonged contact or facilitating indirect transmission. Understanding the drivers of burrow use patterns can therefore provide insights towards the social (contact) structure of species typically considered to be solitary and, in future, help design models of infectious disease spread such as URTD in desert tortoises.

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Invasion and Infection: Translocation and Transmission: An Experimental Study with Mycoplasma in Desert Tortoises.

DATA ACCESSIBILITY

The data used for burrow switching and burrow popularity model can be accessed at http://dx.doi.org/10.79

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TABLE CAPTIONS

Table 1. Potential variables considered to characterize burrow use patterns in the desert tortoise, *Gopherus agassizii*.

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FIGURE LEGENDS

Figure 1. Critical habitat range of the desert tortoise within the Mojave desert, USA as determined by the US Fish and Wildlife Services in 2010(http://www.fws.gov/carlsbad/GIS/CFWOGIS.html) Critical habitat is defined as those geographical areas that contain physical or biological features essential to the conservation and management of the species (US Fish & Wildlife Service 1973). Points represent centroids of survey sites where tortoises were monitored using radio-telemetry. Point size is proportional to the number of animals monitored at the site. Site abbreviations: BSV - Bird Spring Valley, CS - Coyote Springs, FI - Fort Irwin, HW - Halfway, LM - Lake Meade, MC - McCullough Pass, PV - Piute Valley, SG - St. George, SL - Stateline Pass.

Figure 2. (a) Bipartite network of burrow use patterns at MC site during the year 2012. Node type indicated by color (Blue = adult males and red = adult females). Node positions were fixed using Yifan Hu's multilevel layout in Gephi. In this paper, we quantify burrow switching and burrow popularity as degree of tortoise nodes and burrow nodes, respectively, in the bipartite network. For example, burrow switching of the female tortoise X is five and burrow popularity of burrow Y is one. (b) Single-mode projection of the bipartite network into tortoise social network.

Figure 3. Frequency distribution of (a) Tortoise degree i.e., unique burrows used by desert tortoises and (b) Burrow degree i.e., unique tortoises visiting burrows during active (Mar-Oct) and inactive (Nov-Feb) seasons. Values are averaged over each surveyed year and study site. y-axis represents normalized frequency counts of tortoises/burrows.

Figure 4. Spatial constraints on asynchronous burrow associations during active seasons at study sites with control animals. Correlation between geographical distance and edge occurrence in tortoise social network. Correlation values are averaged over each surveyed year and error vars are standard errors. P-value associated with each correlation measure was < 0.05.

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Table 1:

Variables	Variable type	Description
Tortoise attributes (Burrow switching model only)		
Sex/age class	Categorical	Three levels - adult males, adult females and non-reproductive individuals
Size	Continuous	Midline carapace length averaged over the year for each individual
Burrow attributes (Burrow popularity model only)		
Burrow azimuth	Categorical	Direction in which burrow entrance faces forward. We converted the 1 to 360° range of possible azimuth values to eight categorical azimuth directions: Q1 (1-45), Q2 (46-90), Q3 (91-135), Q4 (136-180), Q5 (181-225), Q6 (226-270), Q7 (271-315) and Q8 (316-360)
Burrow surveyed age	Continuous	Number of years between the first report of burrow and current observation
Soil condition	Categorical	The soil conditions at the nine sites varied from sandy to mostly rocky. We therefore categorized burrow soil into four categories - mostly sandy, sand and rocky, mostly rocky and caliche and rocky
Percentage wash	Continuous	Percentage area covered by dry bed stream within 250 sqm area around burrow
Surface roughness	Continuous	See (Inman et al. 2014)
Topographic position	Continuous	See (Inman et al. 2014)
Environmental characteristics		
Sampling period	Categorical	The period of observation as described before. We divided a year into six periods of two months each
Seasonal rainfall*	Continuous	Total rainfall recorded at weather station nearest to the study site (in inches) during a particular sampling period
Temperature*	Continuous	Average, maximum and minimum temperature recorded at the weather station nearest to the study site and calculated over each sampling period in our model
Population stressors**		
Tortoise health	Categorical	Burrow switching model only. Two categories - healthy and unhealthy
Residency status	Categorical	Burrow switching model only. Each individual was assigned one the five residency status for each sampling period - Control (C), Resident (R), Translocated (T), Ex-Resident (ER) or Ex-Translocated (ET)
Drought condition	Continuous	Average rainfall from November to February used as a proxy of drought condition for the following year
Density condition		
Local tortoise density	Continuous	For burrow switching model: the average number of individuals found within 10,000 sqm grid around the focal tortoise each day of sampling period when the animal was surveyed. For burrow popularity model: number of individuals found in 10,000 sqm grid around the focal burrow averaged each surveyed day of the sampling period
Local burrow density	Continuous	For burrow switching model: the average number of active burrows in 10,000 sqm grid around the focal tortoise each day of the sampling period when the animal was reported. For burrow popularity model: the number of active burrows in 10,000 sqm grid around the focal burrow. A burrow was considered to be active if it was reported to be occupied at least once during the current or any previous sampling period
Survey condition		
Sampling days	Continuous	Total survey days during the sampling period
Individual level bias	Continuous	Burrow switching model: Total number of days when the focal tortoise was reported using any burrow to account for any survey biases between individuals. Burrow popularity model: Total tortoises surveyed during the sampling period

* Rainfall and temperature data was obtained from the nearest weather station to the study site using database available at National Oceanic & Atmospheric Administration website (http://www.ncdc.noaa.gov).
** See text for details.

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Figure 5. The effect of various predictors on the two models of burrow use patterns in desert tortoises. Error bars indicate 95% confidence intervals around the estimated coefficient value. For continuous predictors, the vertical dashed line indicates no effect - positive coefficients indicate increase in burrow popularity/switching with increase in predictor value; negative coefficients indicate decrease in burrow popularity/switching with higher values of predictors. For each categorical predictor, the base factor straddles the vertical line at 0 and appears without a 95% CI. Positive and negative coefficients for categorical predictors denote increase and decrease,respectively, in burrow popularity/switching relative to the base factor.

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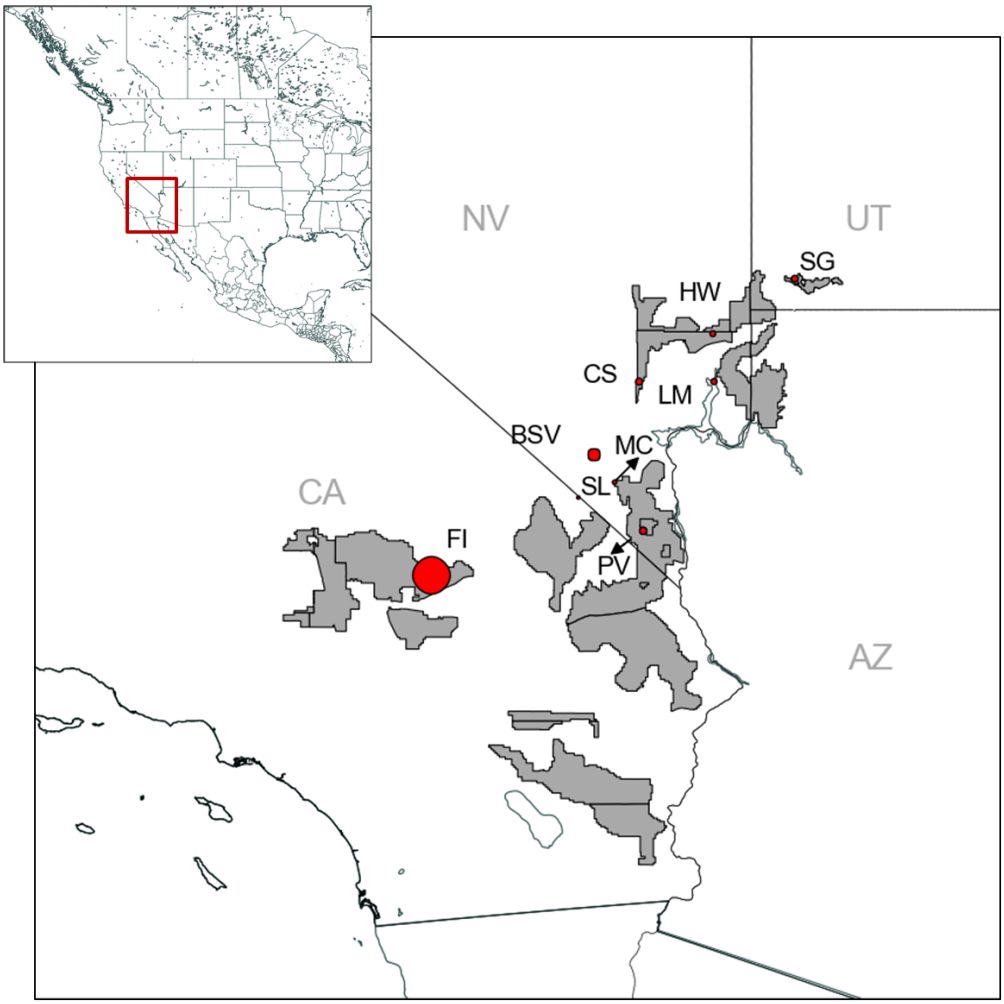

Figure 1:

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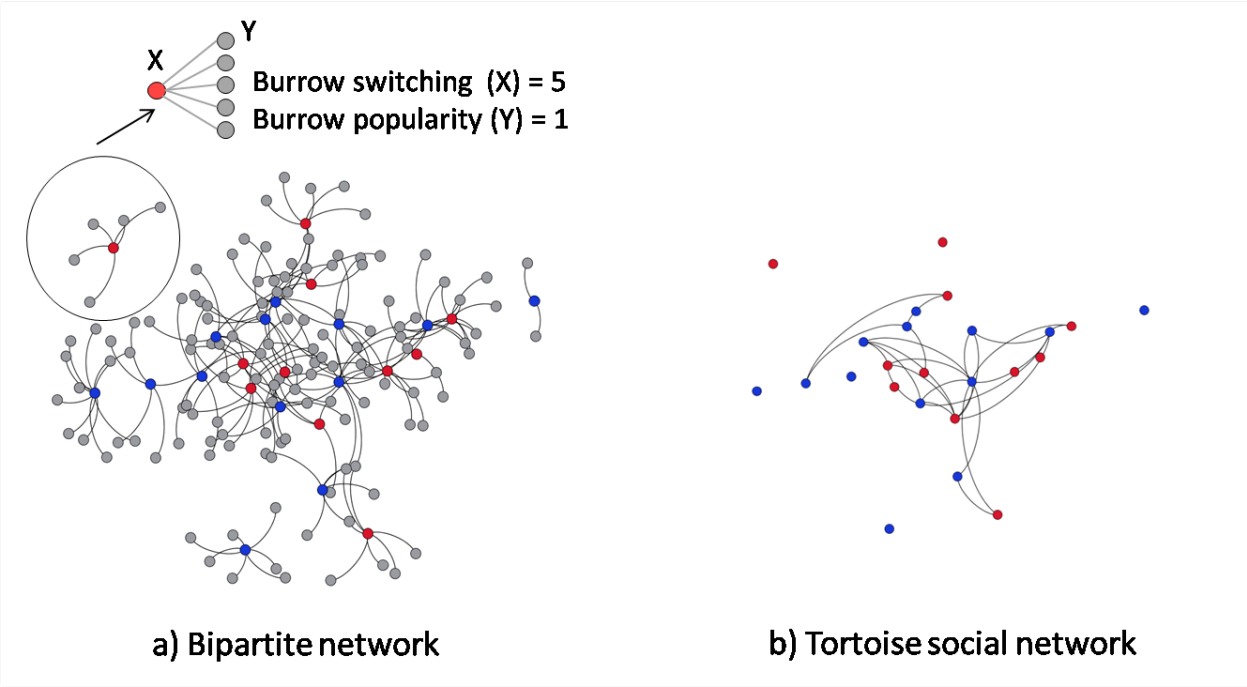

Figure 2:

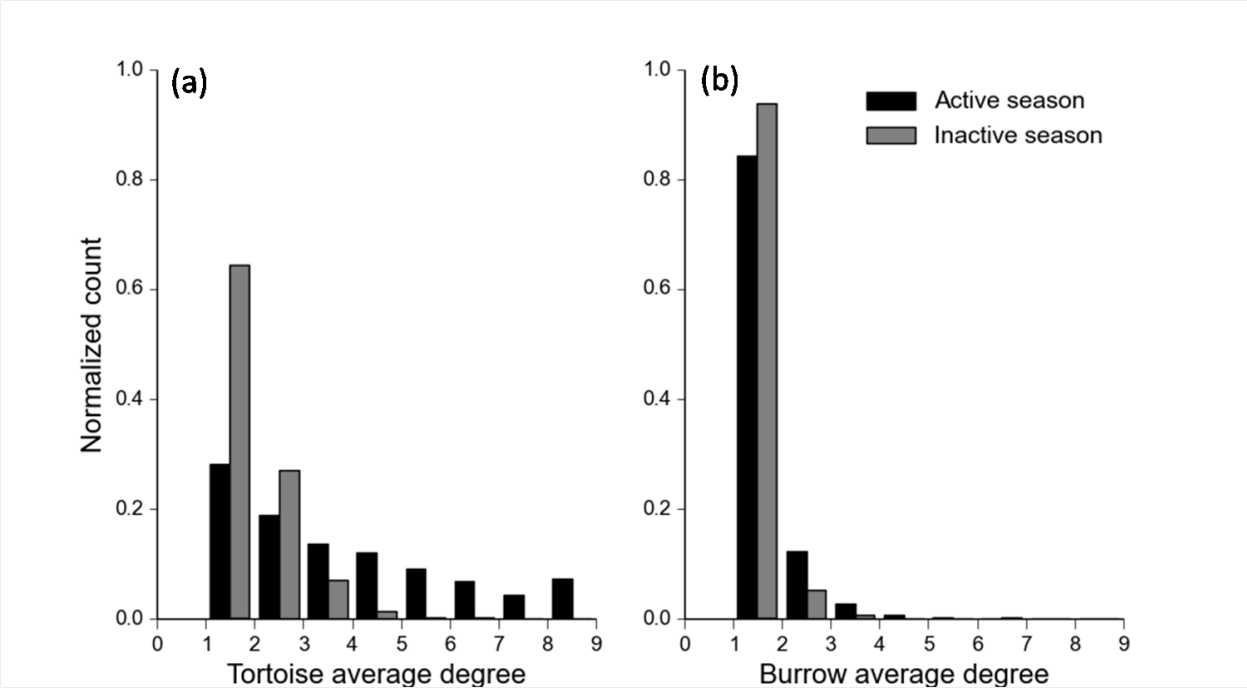

Figure 3:

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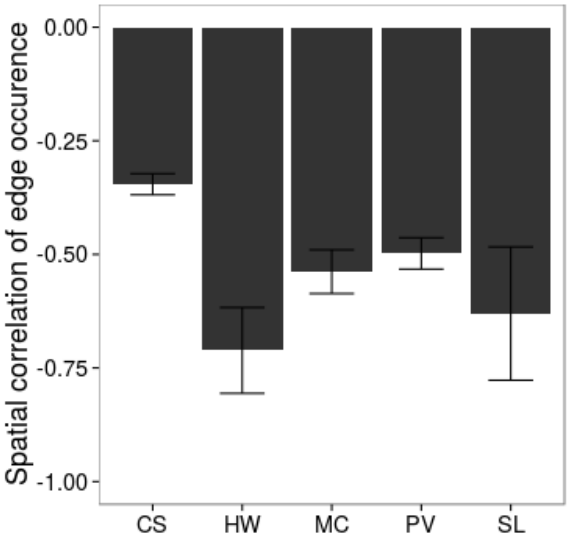

Figure 4:

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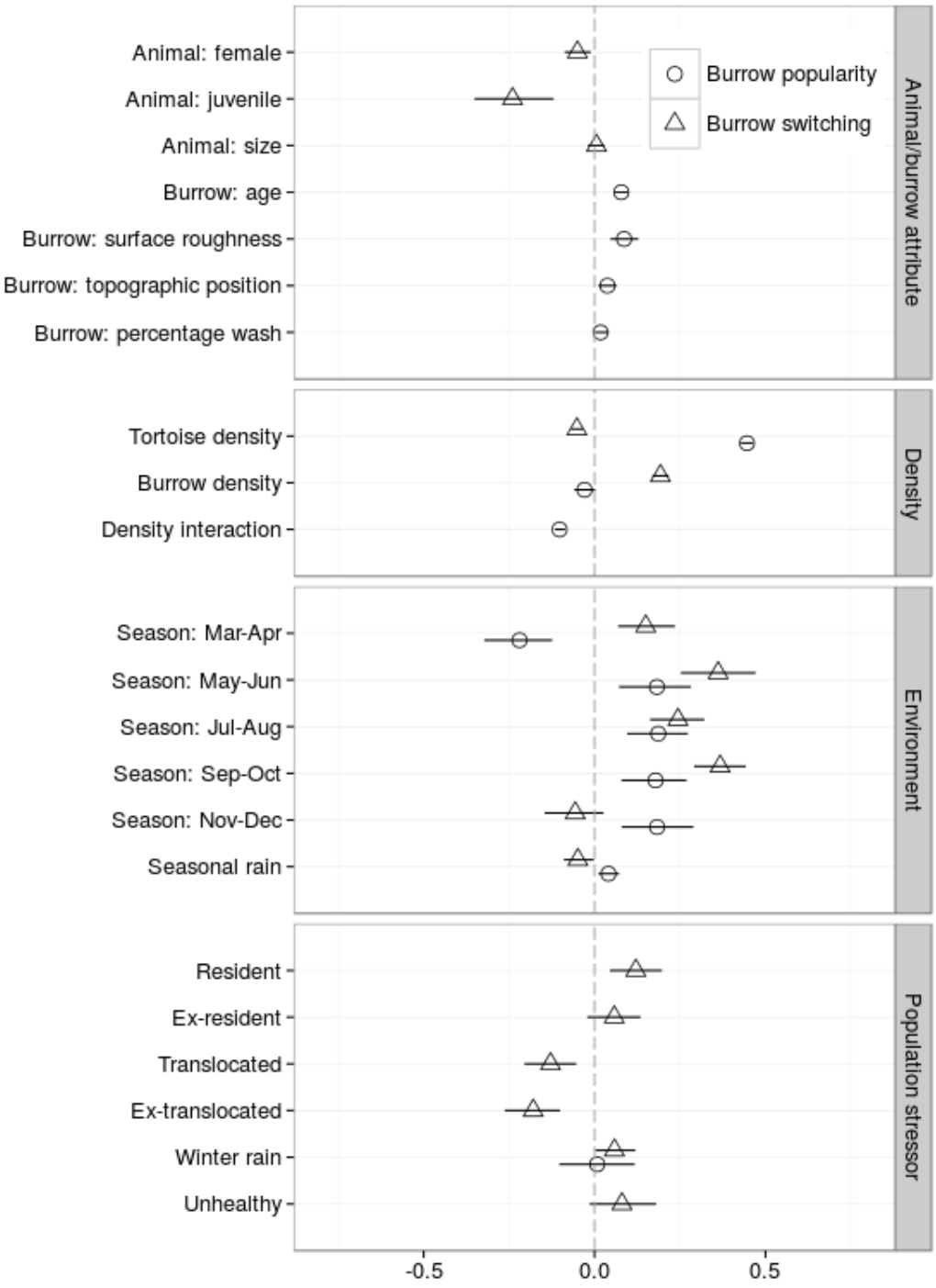

Figure 5: