

Movement patterns and habitat use of soft-released translocated spur-thighed tortoises, *Testudo graeca*

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Abstract Wildlife translocations, the deliberate movement of animals from one part of their distribution to another, are increasingly used as a conservation method for the reestablishment of rare and endangered species. The objective of this study was to examine the movement patterns and macro- and microhabitat use of translocated and resident spur-thighed tortoises. This translocation was considered a soft-release as the tortoises were forced to be relatively inactive due to their being released at the beginning of the aestivation season. Our results suggest that forced aestivation soft-releases may succeed in reducing dispersal by forcing spur-thighed tortoises to spend time at the release site as the majority of translocated tortoises had similar activity range sizes and movement path tortuosity as resident tortoises. Spur-thighed tortoise conservation will require protecting habitat at multiple scales, with the remaining native forests in the country of Jordan being important to

the spur-thighed tortoise during the activity and aestivation/hibernation seasons, as this macrohabitat was used significantly more than the human-modified habitats. Microhabitat structures such as leaf litter and availability of large stones may also be especially important in human-modified landscapes, as these microhabitats may help reduce the effects of habitat degradation.

Keywords Aleppo pine · Fractal dimension · Forced aestivation · Leaf litter · Microhabitat · Repatriation · Translocation

Introduction

Wildlife translocations, the deliberate movement of wild individuals from one part of their distribution to another, are increasingly used as a conservation method for the reestablishment of rare and endangered species (Dodd and Seigel 1991; Kingsbury and Attum 2009). Excessive movement is among the main obstacles that can reduce the likelihood of translocation success. When animals are hard-released into an area without any prior experience or acclimation to their new environment, they often immediately undergo mostly linear, long-distance dispersal away from the release site that suggest that animals are unfamiliar with the release site or could be homing to their original activity range. In contrast, the movement of resident animals is more convoluted as animals visit numerous sites that are confined to a limited area (Fritts et al. 1984; Reinert and Rupert 1999; Oldham and Humphries 2000; Plummer and Mills 2000; Caldwell and Nams 2006; Hester et al 2008). The linear movements of translocated animals result in activity ranges that are much larger than resident animals (Reinert and Rupert 1999; Plummer and Mills 2000;

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Nowak et al. 2002; Moehrensclager and Macdonald 2003; Tuberville et al. 2005; Hester et al 2008; Kingsbury and Attum 2009). For example, hard-released translocated gopher tortoises (*Gopherus polyphemus*) had polygon activity ranges up to 80× larger than recorded for other resident populations (Tuberville et al. 2005).

One method that may reduce dispersal is a soft release, which consists of placing an individual in an outdoor enclosure at the release site (Lockwood et al. 2005; Tuberville et al. 2005) or releasing the animal just prior to or during the inactive season when hibernation occurs (Eastridge and Clark 2001; Clark et al. 2002). Generally, as time in a soft-release increases, dispersal distance from release site decreases, survivorship increases, and release site fidelity increases (Kingsbury and Attum 2009). Releasing animals just prior to their inactivity season may increase release site fidelity because some species show fidelity to hibernacula by utilizing the same site yearly (Macmillan 1995). Releasing animals late in the year, just prior to when animals normally enter hibernation, or even releasing animals in a hibernation state into hibernacula is a method that has been used to reduce dispersal from release sites and increase survival in translocations of large mammals (Eastridge and Clark 2001). The only known forced inactivity soft release study to occur in reptiles did show promise, as eastern massasauga rattlesnakes that were released in the autumn just prior to entering hibernation were found to have smaller activity ranges than hard animals released individuals in the spring (King et al. 2004).

Studying the habitat use and movement patterns of repatriated animals can provide valuable insights into their responses in a new environment. Habitat use studies are one of the most effective ways to create management guidelines to assist in wildlife conservation as habitat has to be protected in order to ensure the long-term survival of the species. Habitat use takes place along multiple spatial scales (George and Zack 2001). For example, macrohabitat is often defined by the dominant vegetation cover or biome type within a landscape, while microhabitat is defined as the structural and vegetative composition of a particular site occupied by an individual within a broader landscape (Litvaitis et al. 1996). Identifying macrohabitat use allows the development of large-scale conservative initiatives to prevent habitat loss or fragmentation, whereas identifying important microhabitats determines the importance of particular habitat structures needed within a broader scale.

Repatriation and habitat use studies have the potential to assist in the conservation of the spur-thighed tortoise in Jordan. The spur-thighed tortoise, *Testudo graeca*, is a globally vulnerable species that occurs in Europe, Asia Minor, the Middle East, and North Africa (IUCN 2009). In the country of Jordan, the spur-thighed tortoise has a

limited distribution, occurring mostly in the northern and western parts of the country (Disi et al. 2001). The main threats facing the spur-thighed tortoise in Jordan are collection for the pet trade, habitat loss, overgrazing, and feral dogs (Disi et al. 2001).

The objective of this study was to examine the movement patterns and habitat use of translocated and resident spur-thighed tortoises. This translocation was considered a soft-release, as the tortoises were forced to be relatively inactive due to being released at the beginning of the aestivation season. To the best of our knowledge, this is the first published study that examines the effectiveness of a forced inactivity soft release on chelonians. We also examined spur-thighed tortoise macrohabitat and microhabitat use.

Methods

Study site

This study took place in Dibbeen forest reserve, Jordan (3573145N, 761438E northeast and 3567236N, 768674E southeast), which is 8.5 km² over mountainous terrain, 570–1,050 m. Annual rainfall is 400 mm, which mostly falls in the winter and spring, with a mean temperature of 8°C in January and 27°C in July, with a maximum of around 38°C. Dibbeen is dominated by two main forest types: Aleppo pine (*Pinus halepensis*) and deciduous oak (*Quercus calliprinos*). Dibbeen forest represents the southeastern-most and driest limits of the Aleppo pine forest and is one of the few remnants of pine-oak forest that once covered large areas of the Middle East. Other trees present include arbutus (*Arbutus andrachne*), pistachio (*Pistachia palaestina*), and wild olive (*Olea europaea*).

The seven translocated tortoises were confiscated on 25 May 2007 from individuals trying to sell the tortoises at a major shopping center in Amman, Jordan. Although the exact origin of the tortoises was unknown, the tortoises are believed to have originated from northern Jordan, within the vicinity of the study site, because northern Jordan contains the largest tortoise population, has the greatest amount of available habitat, and is the area where humans are most likely to encounter tortoises outside a protected area. The confiscated tortoises were temporarily housed in an outdoor enclosure away from the release site at Dibbeen and visually examined for any signs of ocular and nasal discharge and eyelid edema. All animals were found to be asymptomatic. The tortoises were intentionally released inside pine forest, as we predicted that pine forest would provide the appropriate aestivation and hibernation habitat due to canopy cover, refuge availability, and deeper leaf litter. Unfortunately, we were only able to capture two

resident tortoises, which were found opportunistically while tracking the translocated tortoises. The small sample size of resident tortoises did not allow for statistical comparison between translocated and resident individuals.

The transmitters, approximately 10 g, were glued onto the lateral-posterior end of the carapace with an epoxy glue and weighed less than the recommended 4–6% of body weight (Cochran 1980). All translocated tortoises were released at the same location, whereas the resident tortoises were released at their original capture location. All tortoises were tracked approximately three times a week. The variation in tracking history was due to transmitter failure. See Table 1 for a description of the tortoises and their tracking history.

For each tortoise location, the following information was recorded: date, time, ambient air temperature, and UTM coordinates via GPS hand-held unit. In addition, we recorded the following microhabitat attributes: distance to nearest tree that was taller than 1.5 m, distance to nearest stone (20 cm diameter or larger), leaf litter depth using a metric ruler, and canopy cover, which was measured by treating the tortoise location as the center point of 1-m² square, and visually (based on previous experience and calibration) estimating the proportion of cover within the square. We then recorded the above described microhabitat attributes for a randomly selected point 20 m from the tortoise's location.

We defined five macrohabitat categories based on the dominant tree species or land use. Pine forests were dominated by the Aleppo pine (*P. halepensis*) and oak forests were dominated by the deciduous oak (*Q. calliprinos*). Tree-based farms were areas dominated by summer irrigated, tree-based agriculture such as olive, fig, or peach trees. Open habitats were classified as areas not containing trees or large shrubs and dominated by a bare, rocky landscape mixed with grasses. These areas are typically

grazed by livestock. Urban/agricultural macrohabitats were described as human dwellings that were associated with irrigated monoculture, agricultural areas. Macrohabitats used were determined by plotting the tortoise locations on a GIS-based vegetation map created by Dibeen forest reserve (Fig. 1).

We used the software Fractal V4.09 (Nams 1996) to determine the mean fractal dimension (FD), which consists of a value between 1 and 2 for each individual tortoise path. Fractal values near one indicate relatively straight paths, suggesting animals are unfamiliar with the environment. These animals do not exhibit release-site fidelity or attachment to an established activity range and move in a linear path. Fractal values closer to two indicate more tortuous paths, convoluted movements with greater turning rates (Nams 1996; Caldwell and Nams 2006). We suggest that these animals are familiar with their environment since they are staying within their activity range.

We studied habitat selection during the activity season through the use of compositional analysis, which compares macrohabitat availability to actual use to determine if habitats are used at random (Aebischer et al. 1993). The activity season was defined as the time period outside of the inactive season when tortoises were aestivating and hibernating. Compositional analysis first tests if overall habitat use is random. If habitat use is non-random, then habitats are ranked in order of importance (Aebischer et al. 1993).

Macrohabitat use was compared at two spatial levels, the population and activity range levels. The population level, sometimes referred to in the literature as the landscape or geographic level, compares the proportion of macrohabitat availability at the study site to use based on individual locations in each macrohabitat for members of a population. The study site (223.4 ha) was delineated by calculating a minimum convex polygon (MCP) around the combined

Table 1 Tracking history and activity range sizes (ha) of spur-thighed tortoises

	Tortoise ID	Sex	Carapace length (cm)	Tracking history	MCP (ha)	Fractal dimension
Translocated	28A	Female	15.5	6/24/07–5/7/08	5.5	1.18
	115A	Female	13.3	6/24/07–5/7/08	6.9	1.29
	135A	Female	13.1	6/24/07–5/7/08	2.4	1.35
	146A	Female	14.7	6/24/07–10/27/07	0.9	1.16
	186B	Female	12	7/14/07–3/7/08	97.6	1.11
	206B	Male	13.8	7/14/07–3/1/08	10.4	1.01
	245B	Male	10.8	7/14/07–5/7/08	6.1	1.11
					Mean=18.5±13.2 SE Mean=6.3±1.3 SE ^a	
Resident	146AA	Female	11.1	2/23/08–9/22/08	8.5	1.20
	325C	Female	18.4	10/18/07–4/12/08	2.6	1.30
					Mean=5.5±3.0 SE	

^a The mean activity range size when individuals 146A and 186B are removed from the analysis due to insufficient tracking history and outlier activity range size

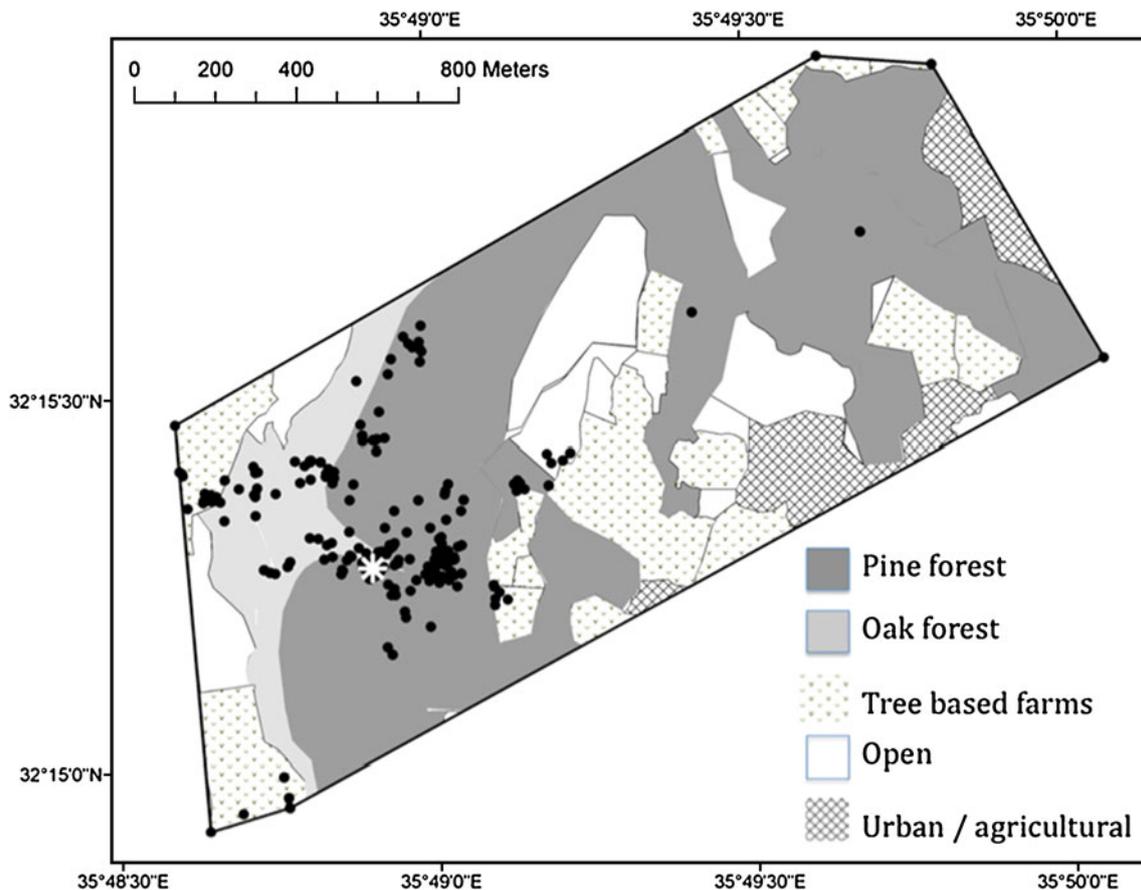


Fig. 1 Macrohabitat composition of the study site. The *black circles* represent spur-thighed tortoise locations and the *white asterisk* represents the release site

locations of all the tortoises (Beyer 2004). The activity range level, sometimes referred to in the literature as home range, signifies habitats that are available to the individual to choose from within their activity range (MCP) and was tested by comparing the proportion of macrohabitats available within the activity range to the proportion of animal locations within each macrohabitat. Tortoise movement patterns and activity range size were determined by using Hawth's analysis tools (Beyer 2004). Habitat selection was analyzed by compositional analysis (Aebischer et al. 1993), using the software Compos Analysis 6.2 (Smith Ecology) (Smith 2005). Habitats that were not used but available were replaced with the proportion 0.01% based on the recommendations of Aebischer et al. (1993).

We examined microhabitat use during the activity and inactive season separately by comparing the microhabitat characteristics at the tortoise location to the randomly selected point through the use of a series of logistic regressions that tested every possible combination of the characteristics (leaf litter depth, distance to tree, and distance to stone). Canopy cover was removed from all microhabitat analysis due to significant correlations with distance to tree ($p < 0.0001$) and leaf litter depth ($p <$

0.0001). There were no significant correlations among the remaining three variables ($p > 0.05$). The model with the lowest Akaike Information Criterion score (AIC) was chosen as the final model. We combined the data from the translocated and the resident tortoises in both the macro- and microhabitat analyses due to the low sample size of resident tortoises ($N=2$).

Results

Shortly after the release, the translocated tortoises underwent reduced activity and began aestivating in August and September. There was a short period of activity in October, with hibernation generally taking place from November until February. The highest activity took place between March and April (Fig. 2). All the translocated tortoises had activity ranges of 10 ha or less, except for one individual that had an activity range of 97.6 ha (Table 1). However, after the outlier 97.6 ha activity range and the activity range of an individual were removed from consideration due to insufficient tracking, the mean activity ranges of the translocated and resident tortoises were similar (Table 1). Both resident tortoises had relatively convoluted paths,

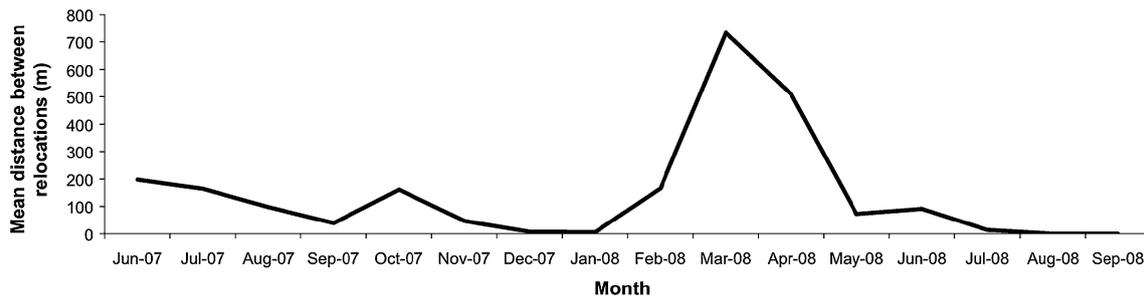


Fig. 2 The relationship of activity, measured as the mean distance between relocations, and month. The highest activity took place between March and April. *N* = number of individuals: June 2007 *N*=4; July, August, and September 2007 *N*=7; October 2007 *N*=8;

November and December 2007, and January 2008 *N*=7; February 2008 *N*=8; March 2008 *N*=7; April 2008 *N*=6; May 2008 *N*=5; June, July, August, and September 2008 *N*=1

whereas there was greater variation among the translocated tortoises, with three individuals having relatively convoluted paths (FD=1.18–1.35) and the remaining four having relatively unidirectional paths (FD=1.01–1.16).

At the population level, spur-thighed tortoises differentially used and selected macrohabitats during the activity season ($\chi^2=55.13$, *df*=4, *p*=0.0090). The most- to least-used macrohabitats were pine forest > oak forest > tree-based agriculture > open > urban/agriculture (Fig. 3). Pine forest was used significantly more than tree-based agriculture (*t*=2.54, *df*=8, *p*=0.046), open (*t*=7.65, *df*=8, *p*=0.0090), and urban/agriculture (*t*=29.05, *df*=8, *p*=0.0050), but not oak forest (*t*=1.16, *df*=8, *p*=0.25). Oak forest was used significantly more than open (*t*=2.81, *df*=8, *p*=0.043) and urban/agriculture (*t*=4.83, *df*=8, *p*=0.0050), but not tree-based agriculture (*t*=1.46, *df*=8, *p*=0.17). There was no significant difference in habitat use between tree-based agricultural and open (*t*=1.80, *df*=8, *p*=0.14), urban/agriculture areas (*t*=2.30, *df*=8, *p*=0.063) or between open and urban/agricultural areas (*t*=0.64, *df*=8, *p*=1.00).

At the activity range level, it was not possible to test differences in habitat use of tree-based agriculture, open, and urban/agriculture, as those habitats were not available

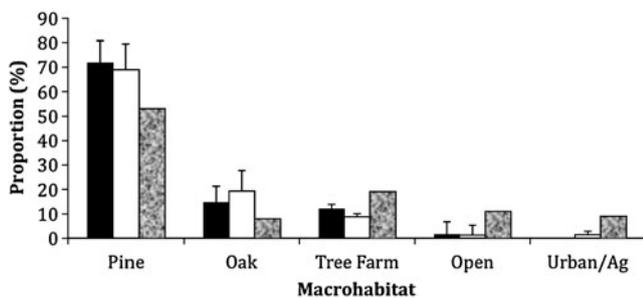


Fig. 3 Mean ± SE proportion of macrohabitat use during the activity season at the population and activity range level. The black bars represent the mean proportion of habitat use by the tortoise. The white bars represent the mean proportion of habitat available within the activity range as estimated through the use of a MCP. The speckled gray bars represent the proportion of habitat available within the study site

in the activity ranges of four of the nine tortoises. Therefore, we only tested selection between pine and oak forest habitats, which revealed no significant difference in use ($\chi^2=1.81$, *df*=1, *p*=0.18; Fig. 3).

The final model that predicted microhabitat use within pine forests contained only the predictor, leaf litter depth (AIC=62.2), and correctly predicted 59.2% of tortoise locations ($\chi^2=28.9$, *df*=1, *p*<0.0001). Tortoises were significantly more likely to occur in areas with deeper leaf litter (Wald=24.52, *B*=0.25±SE 0.05, *df*=1, *p*<0.0001; Fig. 3). The final model that predicted microhabitat use within oak forests only contained the predictor distance from tree (AIC=55.8), which correctly predicted 79.8% of tortoise locations ($\chi^2=5.89$, *df*=1, *p*=0.015). Tortoises were significantly more likely to be found farther away from trees (Wald=5.41, *B*=0.50±SE 0.22, *df*=1, *p*=0.020; Fig. 2). The three macrohabitats, tree-based farms, open, and urban/agriculture were combined into one category: human-modified landscapes due to the small sample size from the low use. Within human-modified landscapes, the final model with the lowest AIC score (6.00) contained the predictors, distance from stone, and leaf litter depth. However, the log-likelihood values were approaching zero and the slopes were infinite as a result of a complete separation in the data from the model perfectly predicting the response variable, tortoise vs random location (Webb et al. 2004). Due to the impossibility of determining which variables to remove from the model as a result of simultaneous effects (Webb et al. 2004), we therefore report the results of the regression models for each variable separately. The model with only distance to stone (AIC=8.28) correctly predicted 97.7% of tortoise locations ($\chi^2=116.3$, *df*=1, *p*<0.0001), as tortoises were significantly more likely to be found closer to stones (Wald=4.72, *B*=-1.60±SE 0.74, *df*=1, *p*=0.032; Fig. 3). The model with only leaf litter depth (AIC=32.09) correctly predicted 60.2% of tortoise locations ($\chi^2=6.40$, *df*=1, *p*=0.01), as tortoises were significantly more likely to be found in deeper leaf litter (Wald=5.31, *B*=0.26±SE 0.11, *df*=1, *p*=0.021; Fig. 3).

During the inactive season, six tortoises aestivated in pine and two inside oak forest and five hibernated in pine, two in oak forest, and one in a tree-based farm. None of the tortoises aestivated or hibernated in animal burrows. Data from the aestivation and hibernation season were combined due to small sample sizes into an aestivation/hibernation category. The final microhabitat model contained the predictor leaf litter depth (AIC=24.99), which correctly predicted 78.1% of tortoise locations ($\chi^2=9.85$, $df=1$, $p=0.002$), as tortoises were significantly more likely to aestivate/hibernate in areas with deeper leaf litter (Wald=6.75, $B=0.52\pm SE\ 0.20$, $df=1$, $p=0.009$; Fig. 4).

Discussion

Conservation efforts should test a variety of methods to increase release site fidelity, as this is among the main factors that limit repatriation success (Kingsbury and Attum 2009). A forced inactivity soft release has two major potential conservation benefits when translocating chelonians. First, our results suggest that forced aestivation soft-releases, releasing tortoises in the summer just prior to aestivation, may have succeeded in reducing dispersal by forcing the majority spur-thighed tortoises to spend time at the release site and becoming familiar with the area. This ensured that the animals had limited time to disperse since selecting an aestivation site may have overridden the tendency to disperse upon release. Our inference that forced aestivation soft-release may have succeeded in reducing dispersal is based on our finding that the majority of translocated tortoises had activity ranges that were similar in size to resident individuals. However, the fractal values suggest that, despite translocated and resident tortoises having similar activity ranges, the slight majority of translocated tortoises needed additional exploration to establish an activity range. Soft-releases are not able to reduce excess movement for all translocated individuals, as one tortoise had an activity range of 97.6 ha, which, to the best of our knowledge, is the largest known home range size for a *Testudo* species (Longepierre et al. 2001; Lagarde et al. 2003; Anadon et al. 2006; Attum et al. 2007, 2008).

A forced inactivity soft release may also be advantageous when it is financially prohibitive or logistically not possible to build a large outdoor enclosure at the release site. Forced inactivity soft releases may be beneficial to chelonians and other reptiles because it may be logistically simpler to house them in captivity. Unlike large mammals that are difficult and expensive to house in captivity, most chelonians have smaller body sizes, require smaller temporary housing, and are less expensive to feed and transport to and from an offsite facility. The main challenge keeping chelonians at a temporary offsite facility will be to

ensure appropriate captive conditions and addressing issues with keeping animals in captivity, such as disease transmission (Kingsbury and Attum 2009).

Spur-thighed tortoise conservation will require protecting habitat at multiple scales. Spur-thighed tortoises used a mosaic of macrohabitats at the population level, with microhabitats being differentially used according to macrohabitat. Jordan's remaining native forests, which are rapidly disappearing, must be protected. Our study has shown these habitats to be important to the spur-thighed tortoise during the activity and aestivation/hibernation seasons (Khresat et al. 2008). At the

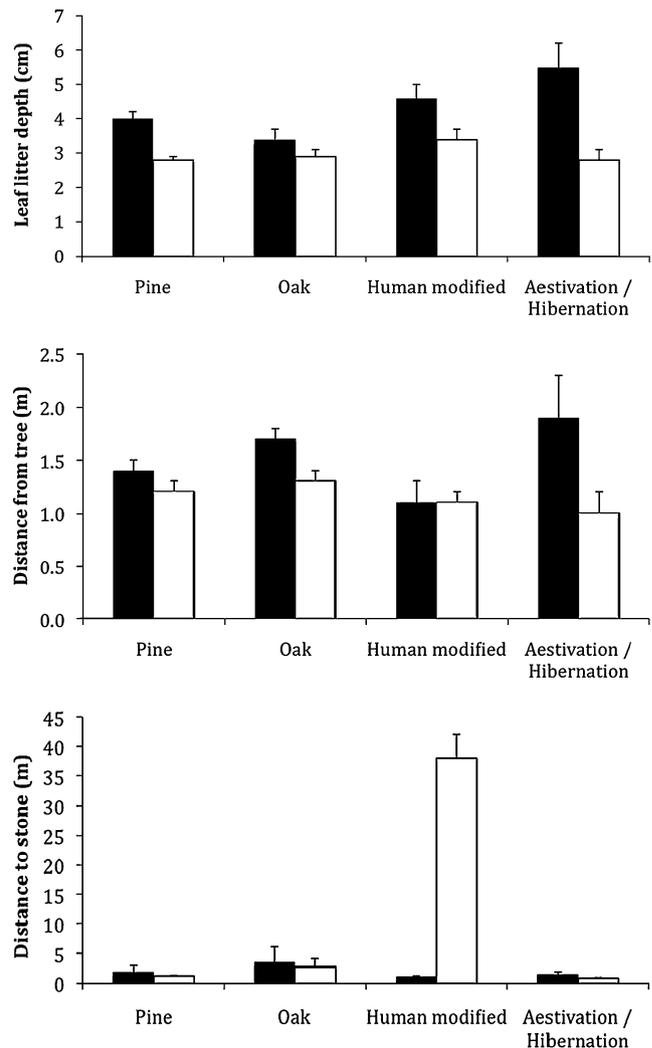


Fig. 4 Microhabitat use within macrohabitats by the spur-thighed tortoise. See methods for description of macrohabitats. *Aestivation/Hibernation* represents microhabitat use during the combined aestivating/hibernating season. Values reported are mean \pm SE. The *black bars* represent the microhabitat at the tortoise location. The *white bars* represent the microhabitat at the randomly selected location. Sample sizes: pine (tortoise location $N=213$, random $N=211$); oak (tortoise location $N=53$, random $N=53$); human-modified landscape (tortoise location $N=44$, random $N=44$); aestivation/hibernation (tortoise location $N=16$, random $N=16$)

population level, tortoises avoided the structurally simpler human-modified macrohabitats and preferred the structurally more complex forest habitats during both the activity and aestivation/hibernation seasons. Jordan is approximately 8.9 million hectares in size but only contains less than 3,600 ha (0.0004%) of native forest. These forests also represent a genetic resource of Aleppo pines that have adapted to the exceptionally dry climatic conditions at the outer limits of their distribution (RSCN, unpublished data). Protecting spur-thighed tortoise habitat in Jordan will prevent soil erosion, maintain soil fertility, and benefit other rare and endangered species such as the stone martin (*Martes foina*) (Khresat et al. 2008).

Microhabitat use confirms that tortoises are selecting for the structural components within macrohabitats. The tortoises utilized areas covered by deep leaf litter in areas with canopy openings, as suggested by the intuitive relationship of canopy cover decreasing as the distance from tree increases (Fig. 4). However, the tortoises were always within the general vicinity of trees since trees are the source of leaf litter. Preference for open canopy within the forest and deeper leaf litter is probably related to thermoregulatory needs of ectotherms (Anadon et al. 2006; Cunnington et al. 2008). Leaf litter depth acts as a complex microhabitat that is utilized by many herpetofauna, as deeper leaf litter generally serves as a refuge from predation, has stable temperature profiles, and has higher humidity than other structures, such as downed trees or rock crevices (Seebacher and Alford 2002; Jennings 2007; Rittenhouse and Semlitsch 2007; Steen et al. 2007; Cunnington et al. 2008). Deeper leaf litter allows animals to thermoregulate by moving vertically through the leaf litter while remaining cryptic vs having to move conspicuously between basking and shaded sites (Rittenhouse and Semlitsch 2007). Deeper leaf litter would also provide stable temperature and moisture profiles for aestivating and hibernating tortoises, while the more open tree canopy exposes the tortoises to higher temperatures in the brief fall activity season after aestivation and in the spring after hibernation emergence (Steen et al. 2007).

Habitat structure was also an important predictor of microhabitat when using human-modified landscapes, as tortoises were significantly more likely to occur in areas covered with deeper leaf litter and closer to stones (Fig. 3). Microhabitat structure may be especially important to tortoises when using degraded or human-modified habitats, as microhabitat structure may provide a localized buffer and compensate for more extreme environmental conditions that occur in structurally simpler degraded habitats (Cunnington et al. 2008; Hillers et al. 2008).

This study shows that more research is needed to test the effectiveness of translocating chelonians just prior to the hibernation/aestivation season to induce inactivity at the

release site as a conservation tool to reduce post-release dispersal and that habitat conservation for the spur-thighed tortoise will need to occur at multiple scales.

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