"Circle of life" of the species *Testudo graeca* in Israel: Effect of temperatures on sex determination, and yearly activity patterns, habitats preference and home range utilization

Mai Bernheim

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

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University of Haifa

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ABSTRACT

Reptiles are one of the more susceptible groups to suffer from biodiversity loss as a result of human-induced changes in the environment, such as global climate change. There are solid evidences for the influence of global warming on species biology such as range-boundary changes and phenological shifts. Among reptiles there is a particular interest in the effects of climate change on species with temperature-dependent sex determination (TSD), where sex ratio is influenced by the temperature experienced by the eggs during the middle third of the incubation period. Therefore finding reptiles' habitat preferences and how temperature affect hatchlings' sex may provide vital information for managing their populations under climate change scenarios. Land tortoises are likely to be the most vulnerable to temperature changes, given their low dispersal capabilities, their occurrence in fragmented habitats and that most species are already threatened. The spur-thighed tortoise, *Testudo graeca,* is a terrestrial TSD tortoise with a circum-Mediterranean distribution. Courtship and mating season begins in March-April, but nesting occurs later between April and June, followed by 3 month incubation period. There is no data from natural environments in Israel on females nest habitats. Moreover, little data exists on adults' seasonal activity patterns, home range and their micro-habitat preferences. The objectives of this study were to identify tortoises' yearly activity pattern, home ranges, preferred micro-habitats and biotic conditions affecting the distribution of tortoises in Ramat Hanadiv; learn the effect of incubation temperature on sex determination of *T. graeca* in Israel, and the effect of nest location and temperature regimes on hatching success; and finally, to identify behavioral patterns of males and females in relation to righting behavior as a tool for sex identification. The research was conducted in two locations: Oranim academic college in Kiryat Tivon, where *T. graeca* eggs incubation experiments and hatchlings' behavioral experiment were performed; Ramat Hanadiv Park, where I surveyed and marked 181

adults' individuals and radio tagged 18 tortoises. MaxEnt models were performed for predicting distribution of individuals of *T. graeca* in the study area. The results show that between January and April both sexes exhibit maximum activity including courtship and copulations. Males precede females in decline of activity starting in May and lasting until October, while females decrease activity a month later. This study describes for the first time a long term summer-autumn activity hiatus of *T. graeca* in Israel. Models for predicting their distribution revealed that they prefer heterogeneous habitats that enable access to open and closed patches. Both sexes share same size of home ranges. There is an overlap among home ranges, and the tortoises do not show territoriality. Tortoises had a non-random occurrence on dark rendzina soil, where they were mostly found in shrub margins showing thermoregulatory activity, sleeping, resting, and mating. This particular micro-habitat probably provides them with relatively stable micro-climatic conditions. Sex determination of *T. graeca* in Israel follows the TSD mechanism, where incubation under constant temperatures above the pivotal point (a 1:1 sex ratio) during the thermo-sensitive period leads predominantly to females' development and below this temperature predominantly to males. In natural nests temperatures fluctuate and can rise significantly above the pivotal temperature without affecting the embryos' survival. It appears that females select carefully the specific nest location to ensure adequate incubation conditions. The effect of temperature fluctuations on sex determination in *T. graeca* is not known yet, mainly due to a lack of a reliable sex determination method to study hatchlings in the field. However, this study revealed the potential of using a behavioral method to distinguish between the sexes. While this study did not directly deal with the hazards of global warming on *T. graeca* in Israel, it shows the sensitivity of the sex determining mechanism to incubation temperatures, which should raise concern if global temperatures continue to rise.

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Introduction

Global species threat

The Survival and reproduction of organisms rely on their ability to cope with a combination of threats. However, while some threats are universal, others will be more important for specific taxa. Global biodiversity is changing at an unprecedented rate as a complex response to several human-induced changes in the global environment (Sala et al., 2000). Reptiles are one of the more susceptible groups to suffer from biodiversity loss. There are six factors known or suspected to be associated with reptile declines (Gibbon et al., 2000): 1) Habitat loss and degradation. For example, studies on the chicken turtle (*Deirochelys reticularia*) in Virginia indicated that the population is small, with possibly fewer than ten adults remaining. Although the population exists within a state park and natural area, only one-fifth of the original habitat is protected, whereas the remaining portion was destroyed by development outside the park boundaries (Buhlmann, 1995); 2) Introduced invasive species. For example: both the desert tortoise (*Gopherus agassizii*) and the gopher tortoise (*Gopherus polyphemus*) are threatened by the introduction of non-native plant species that alter habitat structure, native plant community composition, and even fire frequency (Gibbon et al., 2000); 3) Environmental pollution. For example: slider turtles (*Trachemys scripta*) inhabiting radioactive seepage basins. Stained DNA assays of spleen cells demonstrated significantly greater variation in DNA content for the basin turtles than for control animals from nearby, uncontaminated sites (Lamb et al., 1995); 4) Disease and parasitism. For example: an upper respiratory tract disease has been seen in populations of desert tortoise, *G. agassizi*, in the Mojave Desert, USA, and certain populations of the gopher tortoise, *G. polyphemus*, in Florida, USA (Jacobson, 1994); 5) Unsustainable use. For example: overexploitation of turtle populations in Asia results primarily from an increasing demand in China where a long tradition of using turtles for food and medicine arises from the belief that turtles have a significant role in improving human health (Le, 2007); 6) Global climate change.

Global climate change

The globally averaged combined land and ocean surface temperatures had shown a warming of 0.85°C over the period 1880 to 2012 (IPCC, 2013). There is sufficient evidence that these recent climatic changes have affected a broad range of organisms with diverse geographical distributions, often through species-specific physiological thresholds of temperature and

precipitation tolerance (Walther et al., 2002). On a small time scale, most of the short-term local changes are probably not caused solely by climate change but by land-use change and by natural fluctuations in the abundance and distribution of species (Parmesan and Yohe, 2003). Yet, there are solid evidences for the impact of global warming on species biology (for example: range-boundary changes and phenological shifts) in the long term. Parmesan and Yohe (2003) reported that range limits of species have moved on average 6.1±2.4 km per decade northward or 6.1 meter per decade upward, in the direction predicted by climate change. This shift in range limits poses a great challenge for species. As a result of the widespread habitats loss and fragmentation, many areas which may become climatically suitable are remote from current distributions, and beyond the dispersal capacity of many species (Walther et al., 2002). Numerous species show trends of phenological shifts in the direction expected from climate change towards spring advancement, such as earlier arrival of migrant birds. Therefore, climate change can reinforce the damaging effects of human exploitation and mismanagement, and push species and ecosystem tolerances over their limits.

Apart from shifting their range, species can cope with global climate change in two principal manners: through phenotypic plasticity and microevolution. Phenotypic plasticity, when present, could immediately compensate for a moderate change in environmental conditions. In contrast, as microevolutionary responses depend on heritable genetic variation, it is unknown whether, and how fast, such changes will occur in the future (Hulin et al., 2009).

Global climate change and reptiles

Climate change may have more direct impacts on ectothermic organisms where it directly affects their performance, survival and reproduction (Hawkes et al., 2007; Walther et al., 2002). Among reptiles there is a particular interest in the effects of climate change on species with temperature-dependent sex determination (TSD). Many reptile species exhibit temperature-dependent sexual determination, where primary sex ratio is influenced by the temperature experienced by the eggs during the middle third of incubation (Bull, 1980; Wibbels et al., 1994; Ciofi and Swingland, 1997; Valenzuela, 2004). The sex ratio of offspring in these taxa may be completely altered by as little as a 1°C shift in incubation temperature (Janzen, 1994a).

TSD mechanism

While genotypic sex determination (GSD) depends on genetic factors alone (sex chromosomes), environmental sex determination (ESD) depends on post-fertilization environmental factors. Among the potential environmental factors, eggs' incubation temperature appears to be the most critical factor affecting gonadal differentiation in those reptiles (Pieau, 1996; Ciofi and Swingland, 1997). TSD has been recorded in chelonians (turtles and tortoises), lizards, crocodilians (alligators and crocodiles) and recently, tuataras, but not in snakes.

Three TSD patterns have been generally recognized from artificial incubations of eggs at a constant temperature: Pattern la: low temperatures yield predominantly males, while high temperatures yield predominantly females; Pattern lb: low temperatures yield predominantly females, while high temperatures yield predominantly males; Pattern II: low and high temperatures yield predominantly females, with intermediate temperatures yielding various ratios of males (Pieau, 1996; Ciofi and Swingland, 1997). The pivotal temperature has been defined, under constant temperature conditions, as the incubation temperature which results in a 50% of individuals of each sexual phenotype. The thermosensitive periods for gonadal differentiation have been defined as the window of sensitivity to incubation temperature (Mrosovsky and Pieau, 1991; Delmas et al., 2008), mostly in the middle third of the incubation time. It is important to note that in some TSD species the range of temperatures between all-male and all-female clutches is less than 1°C (for example: *Emys orbicularis*, Pieau, 1982). In this regard, the ecological and evolutionary impact of temperature change by a local temperature shift and global warming is immediately apparent (Matsumoto and Crews, 2012).

Elevated expression of the aromatase gene, which is the enzyme that irreversibly catalyzes androgens into estrogens, is steadily observed in differentiating ovaries among taxa, while its expression is usually suppressed during development of testes (Matsumoto et al., 2013). It has been demonstrated that the increase in aromatase expression in embryonic gonads coincides with the thermosensitive period of development where embryonic gonads are sensitive to surrounding temperatures (Ramsey et al., 2007). Matsumoto and coauthors (2013) found in the red eared slider turtle, *Trachemys scripta*, temperature-specific pattern

of DNA methylation on the aromatase gene promoter region during gonad development.

The mechanism of TSD and its impacts on hatchlings' survival and sex ratio has been generally conducted under constant temperature experiments. However, it is of outmost importance to understand the effect of temperatures regimes under fluctuating temperatures, in natural nests. In nature, incubating reptilian eggs are subjected to daily thermal fluctuations, and females may preferentially select nest sites that maximize the female's fitness, based on the effects of the magnitude of thermal fluctuations on the offspring's phenotype (Bowden et al., 2014). The microhabitat surrounding the nest site has been shown to influence the thermal environment experienced by developing embryos of reptiles with temperature-dependent sex determination, and therefore the resulting sex ratio of the offspring (Wilson, 1998). Studies that have utilized fluctuating regimes have demonstrated that: 1) fluctuating temperatures allow eggs to survive short bouts of incubation at temperatures that would be lethal under constant conditions (below 22°C or above 35°C); and 2) fluctuating temperatures above and below the pivotal point produce a greater proportion of females compared with constant temperature incubations in TSD Ia species (Díaz-Paniagua et al., 2006; Les et al., 2007).

The spur thighed tortoise (*Testudo graeca*)

Among reptiles, land tortoises (family Testudinidae) are likely to be the most vulnerable to temperature changes, given their low dispersal capabilities, their occurrence in fragmented habitats and that most species are already threatened (Fernández-Chacón et al., 2011). These concerns for the family are particularly important in arid and semiarid ecosystems, such as the Mediterranean region, where the consequences of climate change will be more pronounced, and forecasting dramatic losses in biodiversity (Sala et al., 2000).

The spur-thighed tortoise, *Testudo graeca,* is a terrestrial tortoise with a circum-Mediterranean distribution whose populations are severely threatened by habitat loss and fragmentation (Anadón et al., 2006a). The status of this species according to the IUCN Red List of Threatened Species is Vulnerable A1cd (IUCN). The information about the nesting period of this species in Spain reveals that most females reproduce every year, laying one to four clutches from early April to the end of June. Nests were frequently located close to or under the cover of shrubs, which partially shaded them. Clutch size varied from 1 to7 eggs

and averaged 3.5 eggs (Díaz-Paniagua et al., 1996). *T. graeca* exhibited a bimodal annual activity pattern, with well-defined aestivation and hibernation periods. There are temporal differences between male and female activity patterns, consisting of an earlier emergence of males from hibernation and a delay in entering aestivation of females (Díaz-Paniagua et al., 1995).

Studies on egg incubation of this species under laboratory conditions have demonstrated that sex differentiation is temperature dependent, with a pivotal temperature of 30.5±0.5ºC (Pieau, 1975), were females dominantly formed above this threshold and males below. The eggs of *T. graeca* are assumed to be mostly influenced by incubation temperature and not moister because rigid egg shells prevent the loss of water (Díaz-Paniagua et al., 2006).

In Israel there are three sub-species: *T. graeca floweri* (south Israel), *T.g. terrestris* (central and north Israel) and *T.g. ibera* (Ramat Golan). It is common in the Mediterranean region and prefers rocky habitats. It feeds mostly on vegetation but sometimes add invertebrates to its diet. Courtship and mating season begins at the end of the winter (March-April) (Arbel, 1984). The female nests between April and June, and the duration of incubation is about 3 month. There is no data from natural environment, especially in Israel, on where females nest, and whether they nest within their home boundaries or migrate to preferable nesting sites. Moreover, little data exists on their seasonal activity patterns and their home range in Israel.

Testudo graeca's home range
Home range is the area traversed by the individual in its normal activities of food gathering, mating, and caring for young, if there any. The size of the home range may vary with sex, age, and season (Burt, 1943; White and Garrott, 1990). Population density also may influence the size of the home range. Home ranges of different individuals may, and do, overlap. This area of overlap is neutral range and does not constitute part of the more restricted territory of animals possessing this attribute (Burt, 1943). The mean home range size of the population of *T. graeca* in Sierra de la Carrasquilla-Murcia, Spain is 1.71 ± 1.61 ha for all individuals (Anadón et al., 2006b). There is no information about the size of home range for the populations in Israel and if there is territoriality among individuals.

Sex differentiation
Understanding the utilization of the home range and micro habitat preferences of *T. graeca*

under climate change, could contribute to proper management of populations that under threat of extinction. The morphological differences between the sexes start to appear at about the age of 5 years, when they are sexually mature. The current methods to identify the sex in this species before sexual maturity is only by invasive methods that rely on analyzing sex hormones from blood samples (Xia et al., 2011), histology of the gonads or by endoscopy (Rostal et al., 1994). All of these methods require laboratory facilities, and can not be practiced in the field. It is important to develop new un-invasive methods for sex identification that will be carried out to the field, for example: behavioral based sex discrimination tests.

Free-ranging tortoises may be flipped on their back accidentally during displacements by predators, rivals or uncooperative females during sexual interactions (Bonnet et al., 2001). Moreover, it is adaptive for hatchlings to have the ability to right themselves, to avoid exposure to predation and thermal stress (Freedberg et al., 2004). Righting performances have been examined in juvenile freshwater turtles but rarely in terrestrial species or adults. Golubović and coauthors (2013) found in *Testudo hermanni* strong differences in righting success between adults male and female, but no difference in other parameters of righting behavior, like net time to right. This suggests that individuals of both sexes exhibited similar 'motivation' to right themselves, but males were more agile to achieve this. Whether *T. graeca* demonstrate sex-dependent variability in their righting behavior at different ages has not been studied yet.

The current increasing global temperatures can lead to a skewed sex ratio in species with a TSD mechanism, that can result in a decreased effective genetic size (*Ne*), followed by genetic drift in small populations. Therefore, for managing land tortoises in Israel it is crucial to understand their current ecological behavior such as spatial and temporal micro-habitat preference, and what are the necessary micro-climatic conditions for successful nesting to provide balanced sex ratio. This study is a first step towards this direction.

Research objectives

1. Identification of tortoises' yearly activity pattern.

2. Determining the tortoises' home ranges.

3. Identification of tortoises' preferred micro-habitats and biotic conditions affecting the distribution of tortoises in the study area.

4. Learning the effect of incubation temperature on sex determination of *Testudo graeca* in Israel.

5. Learning the effect of nest location and temperature regimes on hatching success.

6. Identification of behavioral patterns of males and females in relation to righting behavior.

Methods

Study area

The research was conducted in two locations: 1) Oranim academic college in Kiryat Tivon, where *T. graeca* eggs incubation experiments and hatchlings' behavioral experiment were performed; 2) Ramat Hanadiv, a privately owned nature park, operated for the benefit of the general public by the Rothschild Foundation (Levin et al., 2013), where adults individuals of *T. graeca* were captured, identified and marked, of which 18 were mounted with radio-transmitters and were followed thereafter for one year.

The Ramat HaNadiv Park is located on the southern part of Mount Carmel, Israel (32°32`N 34°56`E). The climate in this region is Mediterranean with relatively cool, wet winters (average monthly temperature in January is 11.9 °C. Rainfall averages 574 mm annually, and it occurs mainly from November to March) and hot and dry summers (average monthly temperature in August 24.9 °C) (Geffen et al., 1999; Hadar et al., 1999).

The vegetation types in Ramat HaNadiv Park are batha (dwarf shrubs of up to 0.5 m and herbs), garrigue (a higher stage of shrubs, 1-2 m high, and small trees), scrub forest (`maquis', of medium-sized trees up to 12m) and planted coniferous stands of trees (Bar and Kaplan, 2005; Geffen et al., 1999). The most common shrubs in the park are *Phillyrea latifolia* (בר זית בינוני), *Pistacia lentiscus* (אלת המסטיק) *Calycotome villosa* (קידה שעירה) and the dwarf shrub *Sarcopoterium spinosum* (סירה קוצנית). There are also planted conifer groves, mostly the species *Pinus brutia* (אורן ברוטייה), *Pinus pinea* (אורן הצנובר) and *Cupressus sempervirens* (ברוש מצוי) and also natural trees such as *Ceratonia silique* (חרוב) and *Quercus calliprinos* (אלון מצוי) (Geffen et al., 1999; Hadar et al., 1999; Levin et al., 2013).

Yearly activity patterns

The research period lasted from February 2013 to August 2014. The size of the study area at Ramat Hanadiv was 133 ha (Fig.1). It was divided to five sub areas. Each sub area was scanned on foot once a week for tortoise sightings. Every tortoise that was found was marked with a numbered aluminum tag (1X1 Cm) that was attached with Epoxy glue to the right anterior carapace (Fig.2). The geographic coordinates of each sighting was taken using a GPS device (MobileMapperTMCX, Spectra Precision) or to a Collector application on smartphone. Additional measures were: date and time, sex, length (anterior plastron to posterior carapace) and width (widest part of the plastron) using a graph paper (Fig.2), as

well as dorsal, ventral, and lateral (with the aluminum tag) photos (Appendix 1). I took records of the activity status (for example: sleep; active; awake but static; aestivate; basking; mating; eating etc.), and the nearest plant' species.
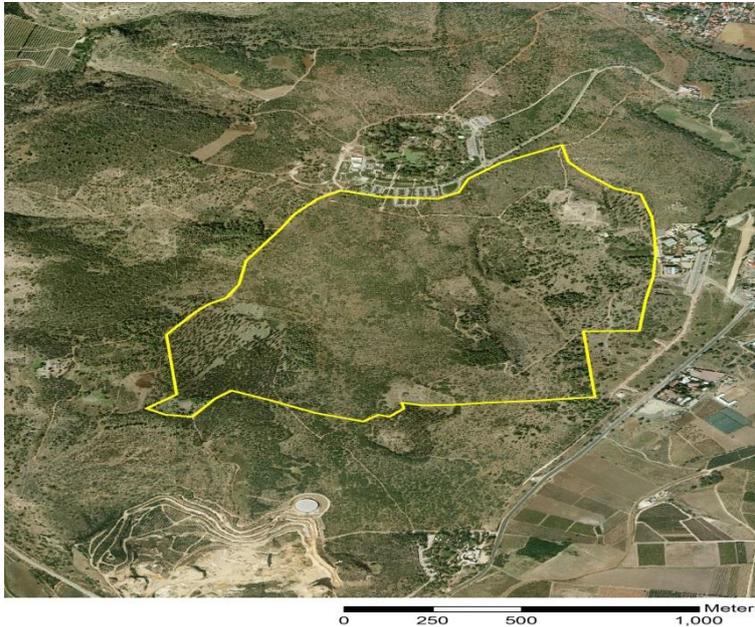
Fig.1. An aerial photo of Ramat Hanadiv Park. The border of the study area is in marked with a yellow line. At the bottom of the photo one can see the quarry bordering the southern border of the park and on the right upper side houses in Zichron Yaakov, bordering the northern part of the park. Road 652 on the bottom right borders the park on the east from the agricultural lands.

During October 2013 eighteen tortoises (10 males and 8 females) were equipped with radio telemetric transmitters (Glue-On Transmitter R1920, ATS Company), each weighing 14 g. Tracking the radio-transmitted tortoises was performed using an antenna (RA-23K VHF Antenna, Telonics, Inc.) and a telemetric receiver (R1000, COMMUNICATIONS SPECIALISTS, INC). From each radio-transmitted individual the same data mentioned above was collected and those parameters that do not require handling the tortoise were updated on every new observation. Continuous tracking took place four times a week until August 2014. Observation was conducted from morning to midday, or afternoon until evening. About three times a month searches and observations were performed at sunset and during the following sunrise to check if the last locations of the tortoises changed, for assessing nocturnal activity. The data from all observations was uploaded to a Collector for ARCgis application (Esri Company) on a smartphone, synchronized online to http://ramathanadiv.maps.arcgis.com/.

Fig.2. Male number 79 with aluminum tag and a radio-transmitter on a graph paper.

Selecting favorite micro-habitats

In all observations, descriptions of the tortoises' micro and macro locations were taken. Micro-location is synonymous with micro-habitat. This includes for example: an open area; under or within vegetation (shrub, scrub, herbaceous). The macro location is the habitat, for example: a spar stand of pine trees; garrigue; batha. A one sample Z test for proportion on the observed average amount of radio-transmitted tortoises under or near a shrub was performed, and compared to the null hypothesis value of the woody vegetation proportion in the study area (40%). The proportion of woody vegetation in the study area was estimate by selecting one-hundred random locations in the study area and determines their type: open area or woody area.

Biotic conditions affecting the distribution of tortoises in Ramat Hanadiv

Species distribution models (SDMs) are empirical models relating field observations (species occurrence or abundance) to environmental predictor variables. They are used to gain ecological and evolutionary insights and to predict distributions across landscapes (Guisan and Thuiller, 2005; Elith and Leathwick, 2009). Species data can be presence only observations, presence–absence, or abundance observations, which based on random or stratified field sampling, or observations obtained opportunistically, such as those in natural history collections (Guisan and Thuiller, 2005). I used MaxEnt as a tool for predicting species distribution of individuals of the species *T. graeca* in the study area at Ramat Hanadiv. MaxEnt is a machine learning method with a simple and precise mathematical formulation that estimates the probability distribution for a species occurrence based on environmental constraints, and is designed to make predictions from presence-only data using background environmental data of the study area (Phillips et al., 2006; Chetan et al., 2014). Presence only data in some sense releases us from the problems of unreliable absence records (Elith

et al., 2011).

10 MaxEnt models were performed, five for radio-transmitted males and five for radio-transmitted females (only individuals with over 50 observations were used in the analysis). The maps generated from the models were averaged and displayed on a single representative map. The data of tortoises' locations in the field were stored in http://ramathanadiv.maps.arcgis.com/. The environment data were collected from LiDAR technology made by Ofek Aerial Photography on the 10/5/12, 3200 feet above ground, with Optech ALTM 3100. Ofek preprocessed the data and created two products: a DTM (digital terrain model) - 3D mapping of the terrain at a precision of at least 25cm, and a DSM (digital surface model) - 3D mapping of the ground surface at a precision of at least 25cm. Then all the raw data (points-cloud) was analyzed in ENVI (ENvironment for Visualizing Images) software for creating seven variables that describe the surface. Such variables (Table 1) are commonly used in ecological studies to quantify habitat structure and heterogeneity (Bar Massada et al., 2008; Vierling et al., 2008), and may provide insight into habitat selection by tortoises.

Table 1- The variable names, description and their ecological meaning that were used in the MaxEnt models.

Variable name	Description	Ecological meaning
cv20	The coefficient of variation (ratio of the standard deviation to the mean highest of LiDAR returns within a 20 m radius around every pixel (1X1 m) in a map of the study area)	High cv20 is an indication for heterogeneous vegetation cover in a 20 m radius around every pixel (1X1 m) in the study area. Low cv20 is an indication for a homogeneous area.
h_cv	Coefficient of variation of LiDAR returns within each pixel in the study area map	High h_cv is an indication for heterogeneous area in a single pixel.
height	The value of the highest LiDAR return in every pixel.	Indication for the highest vegetation type in the pixel which means it either a tree, a shrub, a bush or an open spot
p0_1	The proportion of LiDAR returns below 1 m in each pixel.	Indicates for the proportion of bushes or open area in a pixel.
p1_2.5	The proportion of LiDAR returns between 1 and 2.5 m in each pixel.	Indicates for the proportion of shrubs in a pixel.
p_ground5	Proportion of LiDAR returns lower than 30 cm in a 5 m radius around each pixel.	High proportion indicates for an open area in a 5 m radius around each pixel.
p_veg5	Proportion of LiDAR returns higher than 30 cm in a 5 m radius around each pixel.	High proportion indicates for area with bushes, shrubs or trees in a 5 m radius around each pixel.

MaxEnt represents p, the distribution of *T.graeca* locations for a set n of (number of) sites in the study area at Ramat Hanadiv. Each site is assigned to a nonnegative value p(x), and these values across all sites sum to one. MaxEnt then generates distribution model based on environmental constraints obtained from the occurrence data, where each constraint is defined as a function of an environmental variable (Bar Massada et al., 2013). MaxEnt selects the probability distributions (among many) with the Maximum Entropy as the best representative of the data, and assigns a probability of occurrence to each location. Variable importance is quantified based on the increase of the standardized training gain through the repetitions of model development, where training gain denotes the increase in the probability of *T.graeca* in the training locations (Bar Massada et al., 2013). The predictive capability of the model is denoted by the area under the curve (AUC) of the receiver operator characteristic (ROC).The AUC is a common value for characterizing threshold-independent prediction accuracy applied for model of species presence (Franklin, 2010). AUC ranges from 0.5 to 1, where 0.5 is analogous to a completely random prediction and 1 implies perfect prediction. The common rule of thumb is: AUC values between 0.5 and 0.7 denote poor, between 0.7 and 0.9 denote moderate, and larger than 0.9 denote high model performance (Bar Massada et al., 2013).

In addition to generating distribution models, I examined whether the observed frequency of *T. graeca* locations (independent observations) on different soil types is consistent with their expected frequency, according to the occurrence distribution of the different soil types in the study area. I performed a Pearson's chi-squared test where H_0 is: The distribution of *T. graeca* observations on different soil types is equivalent to the areal distribution of soil types, and H_1: The distribution of *T. graeca* observations on different soil type is different from the areal distribution of soil types.

Home range
The home range area was measured for all radio-transmitted tortoises in Ramat Hanadiv. The area was calculated in R software according to the α- hull method (Edelsbrunner et al., 1983) that is based on four steps: 1) creating a Delaunay triangulation from all the samples (waypoints). The triangulation is created by drawing lines joining the points, constrained so that no lines intersect between points. 2) Measuring the lengths of all of the lines, and calculating the average line length. 3) Deleting all lines that are longer than a multiple (α) of

the average line length, in this case α = 50 (based on preliminary experiments in which I compared the sizes of polygons that were created, using α = 50, 75 and 100 and selected the most precise α that describes the individual polygons). 4) Calculating habitat area by summing the areas of all remaining triangles. The advantage of the α-hull approach is that it provides a more detailed description of the habitat's shape, as it is capable of breaking the hull into several discrete hulls when it spans an uninhabitable region (Burgman and Fox, 2003). For each radio-transmitted individual, about 50 observations (waypoints) were used.

Effect of incubation temperature on sex determination

Two custom made incubators were placed inside a caravan with an air conditioner working constantly on 26°C. The incubators temperatures were 31.8±0.1°C ('high temperature incubator') and 29.1±0.1°C ('low temperature incubator'). The size of each incubator was 50X50X100 cm, having three shelves. At the bottom of the incubator I placed a tray with a sponge soaked with water to produce a relative humidity of 80%. Once a week the humidity was checked and water was added if necessary. Twenty *T. graeca*'s clutches were collected with approval from Israel Nature and Parks Authority, from two farms: Balfuria and Hamat-Gader. The eggs were placed inside six plastic boxes, one on each shelf, with Vermiculite as bedding. Each clutch was equally divided between the two incubators (34 eggs in the 'low temperature incubator', and 32 eggs in 'high temperature incubator'). Eggs from a single clutch were placed in the same location in the plastic box and shelf in the respective incubators (Fig.3). Dates of egg laying and hatching were recorded. All hatchlings were weighted immediately after they hatched, and marked on the carapace with Latin letter painted with acrylic color. The egg shells and the amniotic fluid were collected from each hatchling, and frozen in -80°C for possible use in the future. The hatchlings were transferred into a big cage (4.16 m^2) and separated into two groups, one from each incubator. The cage was filled with soil (about 6 cm), dry leaves and clay fragments for shelter (Fig.4). The hatchlings were fed daily with lettuce and herbs.

Fig.3. An Incubator with *T. graeca* eggs inside plastic boxes with Vermiculite bedding.

Fig.4. The cage housing the hatchlings.

There is no known morphological difference between males and females hatchlings until they start to develop secondary sexual marks at around the age of 5 years. Several methods for sex identification, that do not require killing, were tested. MRI scan for identifying the gonads did not succeed. Another method: ELISA, which is an analytic biochemistry assay that uses a solid-phase enzyme immunoassay to detect the presence of a substance, in this case sex hormones, was not performed because it was impossible to take the required 500µl blood sample needed for the analysis from hatchlings without causing their death. The most effective method for sex identification was laparoscopy. The laparoscopy procedures were

carried out when the hatchlings were aged six to twelve months, between May and August 2014, at the veterinary clinic of Dr. Adi Ganz, a specialist in tropical animals and in the use of endoscopy. Laparoscopy was performed for thirty nine hatchlings (18 from 'high temperature' incubator and 21 from 'low temperature' incubator) at the age of eight month. The procedure included: 1) Anesthesia by an injection of 5 mg\kg Propophol to the venous sinus (sinus subcarapacial) using a 1 ml syringe with a 27G needle; 2) Additional local anesthesia performed at penetration (hind limb) by a subcutaneous injection of 2 mg\kg Lidocaine using a syringe and needle as above; 3) Celioscopy in caudal-lateral approach, between the hind limbs and the tortoise's shell. This was accomplished after making a skin incision of about 3 mm, followed by inserting a rigid endoscope (Storz 2.7mm 30deg, logged to Video) into the coelom space; 4) Locating and identifying the gonad, followed by withdrawal of the endoscope; 5) Skin incision stitched with a soluble string (6-0 PDS) (Fig.5).
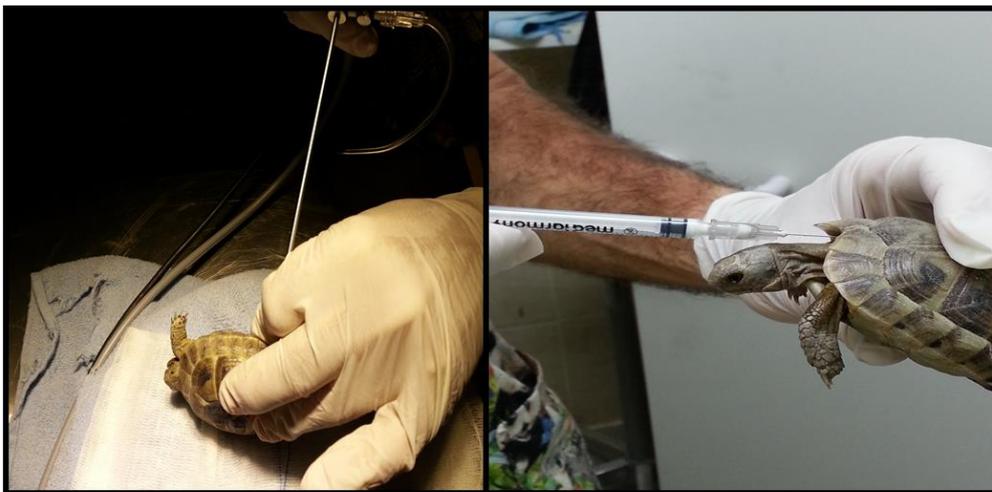
Fig.5. Laparoscopy procedure. On the left, an endoscope is inserted into a coelom space, and on the right is the injection of 5 mg\kg Propophol to the venous sinus.

Fisher's exact test was performed to test the relation between temperature of incubation and the sex of hatchlings. The H_0 was that there is no dependency between low incubation temperature and male development, and high incubation temperature and female development; H_1: there is dependency. The data included only individuals whose sex was identified in the laparoscopy.

Nest location preference and natural temperature regimes on hatchlings survival

Five females and four males were introduced on March 2014, to an outside meshed enclosure sized 12.88 m^2 located in the menagerie of Oranim College. The enclosure's (dark) ground was covered with a 2 cm (light colored) sand layer to enable location of nest

excavation by females as they throw the deep dark ground to the upper layer. Four dark meshed open boxes (58X58X35 cm) were placed in the enclosure for providing the tortoises shelters, and in particular shade for nesting (Fig.6).

Fig.6. The enclosure covered with white sand and shelter boxes.

After a nest was formed, the eggs were carefully removed in order to count them and to measure the depth and width of the nest. A temperature sensor (DT029 Fourier Systems Ltd) was placed in the middle of the nest and the eggs were placed back surrounding it and in their same original location. Another sensor was placed 15 cm nearby at the same depth for comparing the temperatures inside the nest to the same depth without the eggs (control). The sensors were connected to a Data logger (MultiLogPRO, Fourier Systems Ltd) that recorded the temperatures every 30 minute throughout the incubation period. I calibrated the sensors in iced water, as suggested by the manufacture, to activate the system. From each Data logger the data of temperatures was downloaded. The: mean, maximum and minimum temperatures were calculated (from every temperature sensor) for the all incubation period and for the twenty-four hours after the sensor started to collected data, immediately after the female excavated the nest. The purpose of this section was to learn the temperatures regime inside semi-natural nests and to see its effect on hatching success. In addition, comparing temperatures regime from the nests with the temperatures of the controls. This could reveal new information on females' nesting behavior, whether they nest in random areas or in specific areas that evade the extreme temperatures.

All hatchlings were weighted immediately after the hatch, and marked on the carapace with a number painted with acrylic color. The hatchlings were transferred into a big cage (see above and Fig.4). The hatchlings were fed daily with lettuce and herbaceous. The sex of the hatchlings could not be identified, because they are too small for laparoscopy at the time of writing this thesis.

Behavioral patterns among males and females before and after maturation

A method that has not been tried before to discriminate the sex of the hatchlings was based on their behavior. From my observations in the field I noticed that adult males are much more frenetic than adult females when handled, especially in the breeding season. I wanted to examine whether righting behavior (movement components and not the success to turn back on their limbs) is influenced by sex, no matter what the incubation temperature were. Two behavioral experiments were performed in May 2014, one with adult radio-transmitted tortoises in Ramat Hanadiv and the second with hatchlings in Oranim. In the adults experiments in Ramat Hanadiv 17 radio-transmitted tortoises and one additional non-radio transmitted female were examined. Each tortoise was placed on its carapace above Palziv surface (25X25 cm with a hole to fit for the transmitter) in a shadowed area. I filmed the tortoises with a video camera for 5 minutes starting when the tortoises' head emerged from the carapace. The experiment took placed between the hours 10:00 – 13:00. Males and females were filmed alternately. Discriminant analysis was preformed to distinguish between males and females based on: number of movements of lower limbs (for all 5 minutes), number of movements of upper limbs, number of movements of the head, number of head circles and number of "madness" behavior (intensive and synchronized movement of all limbs and head). Additionally, correlation tests were conducted between the individuals' body weight to summation of all movements. The movement variables were counted for every minute separately and then summed for the whole period.

Similar experiments were performed on the hatchlings from the incubation experiment. However this experiment lasted only 3 minutes, and was conducted indoors. Two cameras were used simultaneously to film two hatchlings, each from a different enclosure (different incubation temperature). The discriminant analysis to distinguish between males and females was carried out for the "correct" sex of the hatchlings (after the laparoscopy diagnosis), so only 25 individuals were included. The impact of genetic relation was not taken into account in the analysis because of lack of information about the genetic relationship between individuals.

Results

Yearly activity patterns

Seasonal activity

The study in Ramat Hanadiv took place from March 2013 to August 2014. One hundred and one adult females and 80 adult males were marked during the research. The observations of individual tortoises that were marked with aluminum tag through the research period showed a number of trends. The most prominent observation for both sexes was the lack of their detection during the summer period. The females were present between March and June 2013. From July 2013 there were no observations at all for three months, until the end of October of the same year. The females started to be detected again mostly in the middle of January 2014 parallel to the peak in the male observations. There were additional peaks in March, April and May 2014 and afterwards a decline from June 2014 and no observations until the end of the research, August 2014, (Fig.7). The males were observed between March and June 2013. From June to the middle of October 2013 there were no male observations. In the middle of October 2013, the males began to appear more frequently than the females. There was a peak in male abundance in January and then their number decreased until May 2014, and thereafter they were not observed until the end of the research.
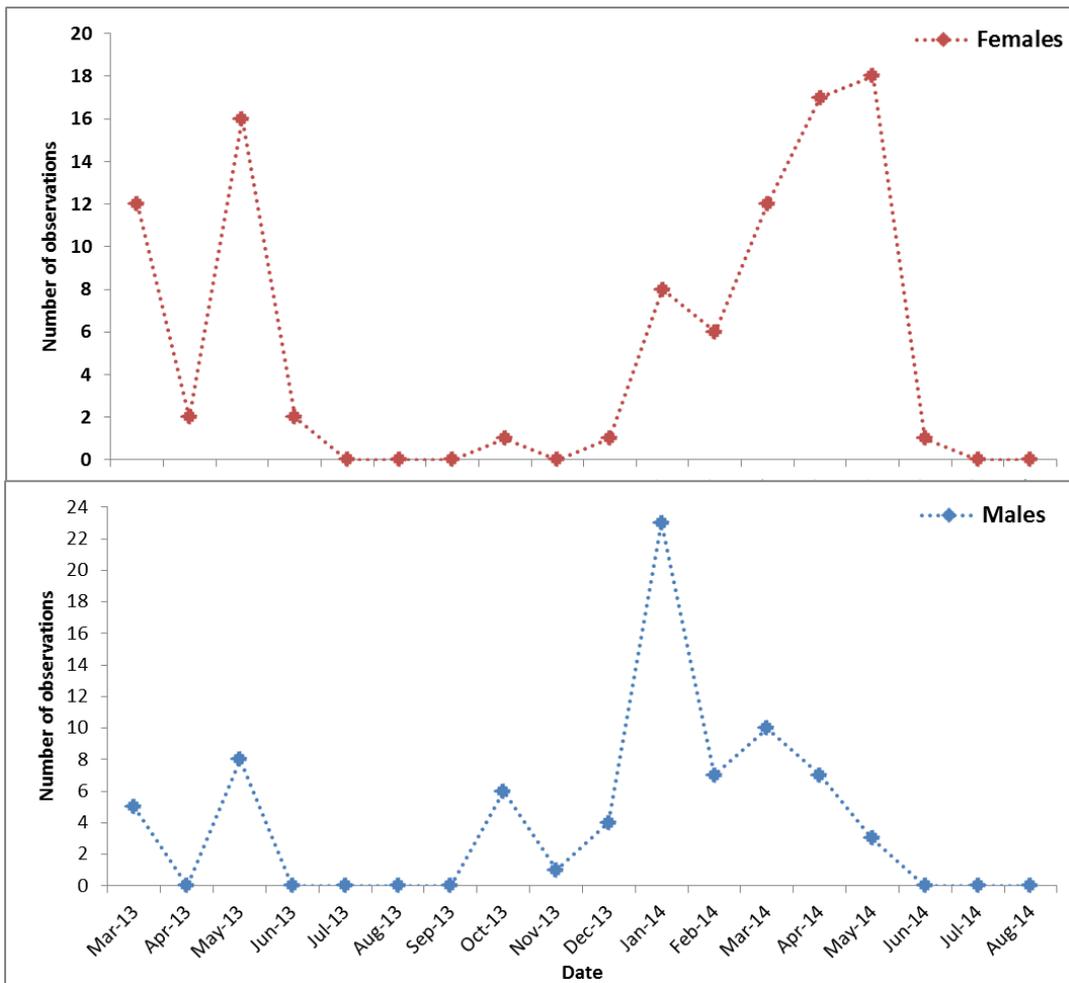
Fig.7. Observations number of different individuals, males and females, throughout the study period.

Radio-Transmitted tortoises

On October 2013, after a long period of zero observations, I located new individuals, and marked them with aluminum tags and attached radio-transmitters to their carapace (the first detection of every new radio-transmitted tortoise is included in the total observed tortoises, Fig.7). From October 2013 until the beginning of January 2014 the number of radio-transmitted tortoises was relatively low, with a majority of males (Fig.8). This tendency is compatible with the general tortoise observations in this season (Fig.7). At the end of January 2014 there was an increase in the number of tortoises observed and therefore an increase in the number of newly radio-transmitted tortoises. Radio-transmitters attachment was completed by February 2014 for ten males and by March 2014 for 8 females (Fig.8).
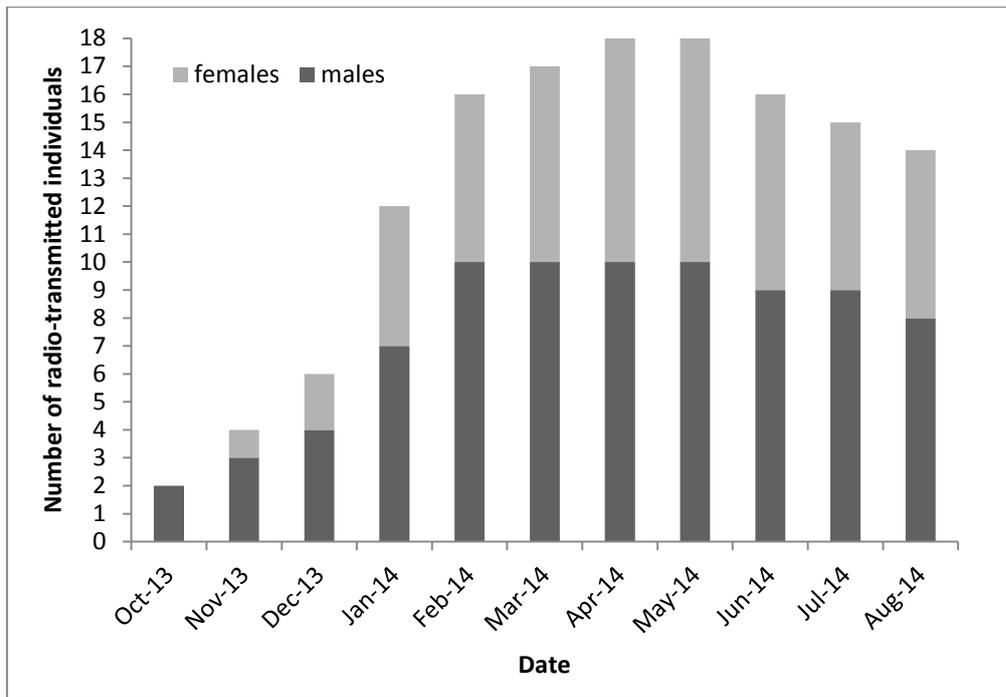
Fig.8. Number of tortoises with attached radio-transmitters during the research period. The radio transmission detection began in November 2013. At the end of the study some of the transmitter batteries were expired or fell off the tortoises.

The radio-transmitted tortoises provided a detailed data on the activity and inactivity pattern during the year. At the beginning of the rainy season, especially in November and late December, the small number of radio-transmitted tortoises showed low activity (Fig.9). Both sexes showed high activity level between January and May. The continuous tracking after the radio-transmitted tortoises revealed more than a few periods of inactivation during the research. The highest average days of inactivity were between July and August for both sexes. Males preceded females by one month (May 2014) in reducing activity (Fig.9). For example: male number 78 was inactive under a shrub of *Pistacia lentiscus* for 21 days during May. The duration of inactivity lasted from one week to three months depending on the individual. For example: male number 107 did not move from the same spot under a rock beneath a shrub of *Pistacia lentiscus* from 15/6/14 until 24/8/14 (70 days). Female number 74 was in the same place from 17/6/14 until 26/6/14 (9 days).
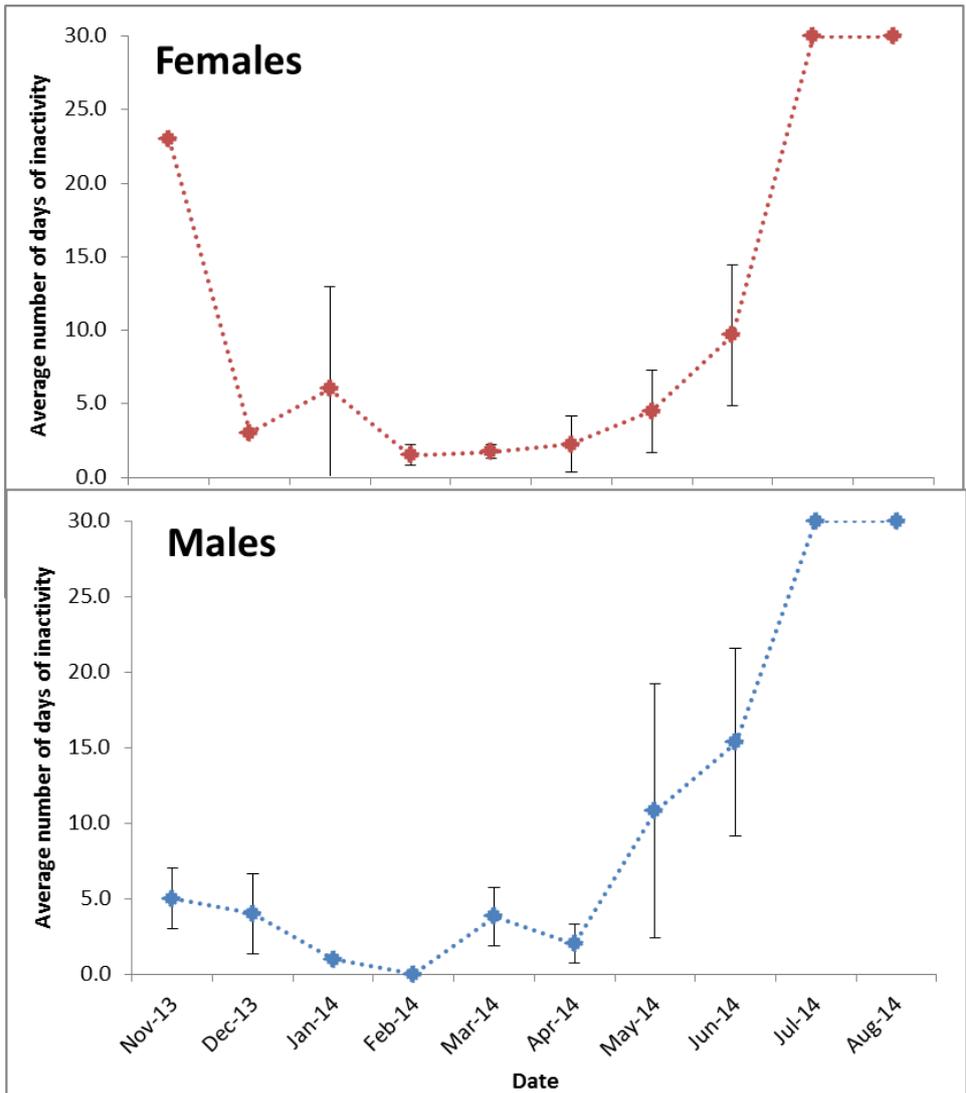
Fig.9. Days of inactivity (average and standard deviation) for the radio-transmitted tortoises during the transmission period.

Based on the radio-transmitted individuals' positions in the evening and the morning after, there was no indication for tortoise nocturnal activity in Ramat Hanadiv during the research period.

Courtship

During the research I observed ten courtship events. Most of the observations were in the second year while I was tracking the radio-transmitted tortoises. Courtship took place mostly in the winter and in the early spring (Fig.10).
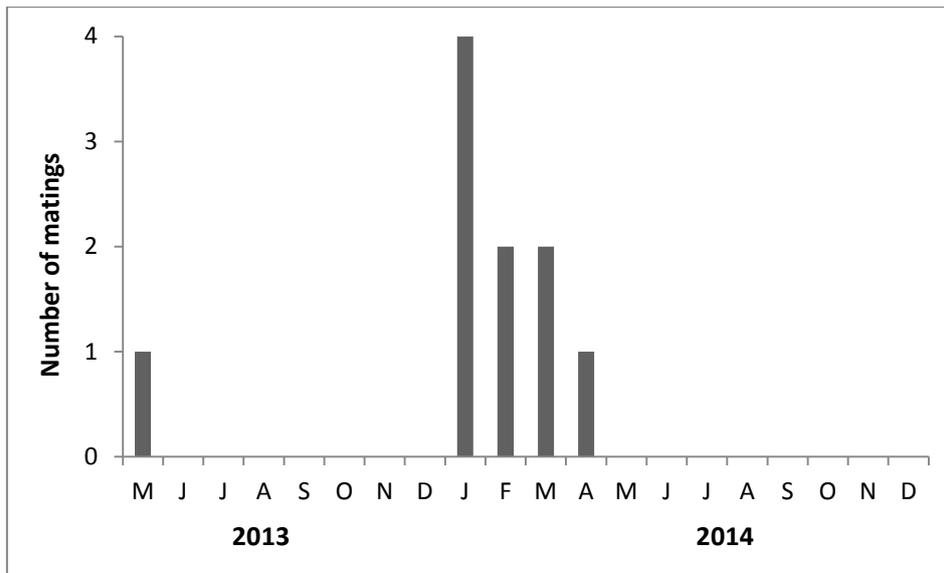
Fig.10. Courtship including copulation events during the research period.

Selecting favorites micro-habitats

When analyzing the micro-habitat data that was provided by observations of the radio-transmitted tortoises it stood out that the common place to find a tortoise was in the shrub margin or under it (Fig.11), where they could be found either basking during the hot or cold time of the day, digging in the soil for a hiding place, or spending long times of inactivity during the summer (putatively aestivating). The observed proportion of woody vegetation micro habitat for tortoises (76.34%) was significantly higher from the observed proportion of this specific habitat in the study area (40%) (Z= 3.15, p= 0.001, N= 18). The common shrubs that the tortoises occupied were: *Pistacia lentiscus*, *Phillyrea latifolia*, *Calycotome villosa* and *Sarcopoterium spinosum* (Table 2. This data is descriptive only as there is no data on relative cover for the study area of each of the shrub species). Male number 78 and female number 74 were exception to this general pattern and appeared more in the open areas than around shrubs (Fig.11).

An interesting phenomenon that I noticed during the study at Ramat Hanadiv was the absence of observations in dense stands of trees (black circles in Appendix 3) and in rocky areas with *Coridothymus capitatus* (Israeli Thyme) (orange rectangle in Appendix 4).
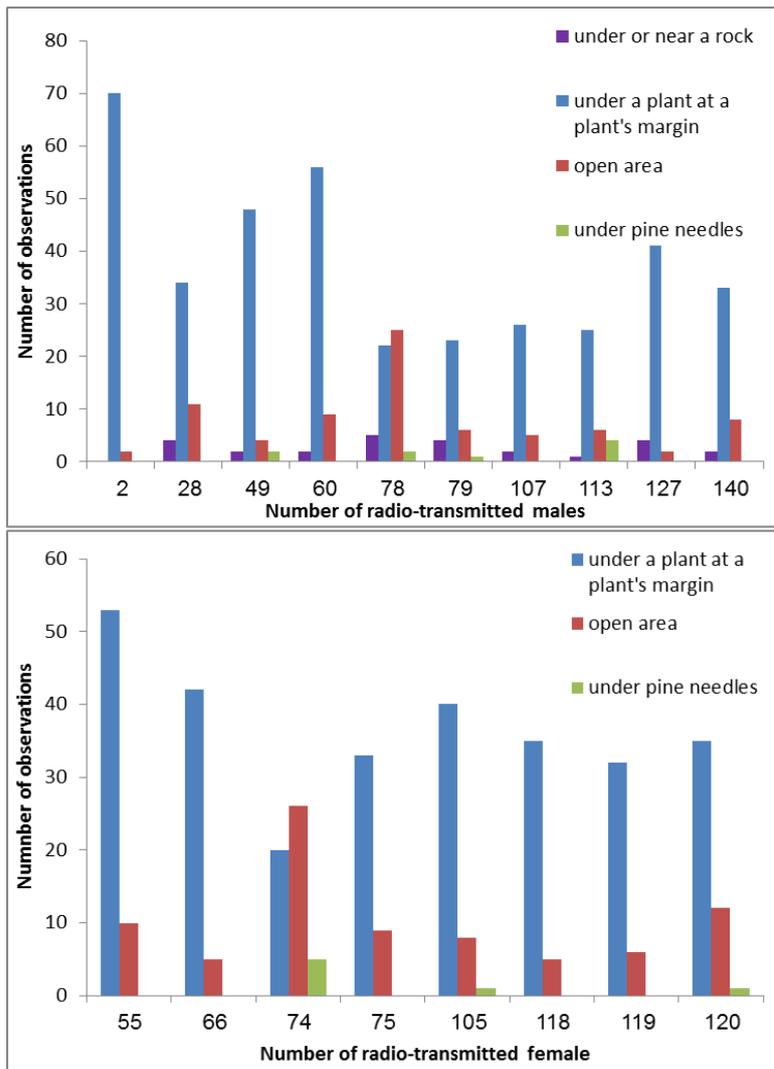
Fig.11. The micro-habitat where radio-transmitted males (upper) and females (lower) were located throughout the study.

Table 2- Observation number of radio-transmitted tortoises under, or on the margin of different shrubs species.

	Sarcopoterium spinosum	*Calicotome villosa*	*Pistacia lentiscus*	*Phillyrea latifolia*	*Rhamnus lycioides*	*Asparagus*	Other
Males	85	25	228	47	14	13	26
Females	85	12	139	13	18	15	16

Biotic conditions affecting the distribution of tortoises in Ramat Hanadiv

When taking into consideration all the results from the 10 different MaxEnt models (each for different radio-transmitted individual) three variables contributed most strongly to the tortoises' distribution in the study area: cv20, h_cv and p_ground5 (for descriptions, Table 1)

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(Fig.12). The average AUC of the 10 models is 0.75± 0.09, and it ranges from 0.601 to 0.848 (Table 3). Based on a visual interpretation of the habitat suitability map that was generated with MaxEnt (Fig. 13) tortoises tend to avoid areas with dense vegetation such as dense stands of conifers and large patches of shrubs (blue areas on the left map, Fig.13). Most of the tortoises preferred habitats with high cv20, which mean that heterogeneous vegetation cover serves as a suitable habitat (but some individuals preferred areas with lower heterogeneity). This was followed by half of the tortoises selecting also areas with high h_cv, which suggests that they tend to be found in small areas (1X1 m) that contain vegetation in different heights. However the other half tended to be found in more homogeneous locations in term of vegetation height. Tortoises also seem to favor high values of p_ground5, suggesting that they prefer sub-habitats that comprise a high proportion of open area.

Table 3- Number of observations used for training and testing, and the AUC values for the 10 models.

ID	Records used for training	Records for testing	Training data AUC	Test data AUC
2	65	17	0.823	0.848
28	51	13	0.833	0.759
49	50	14	0.744	0.601
55	62	16	0.855	0.881
60	64	16	0.851	0.82
74	45	12	0.806	0.803
75	40	10	0.672	0.639
78	57	14	0.713	0.799
105	45	11	0.677	0.705
120	38	12	0.766	0.628
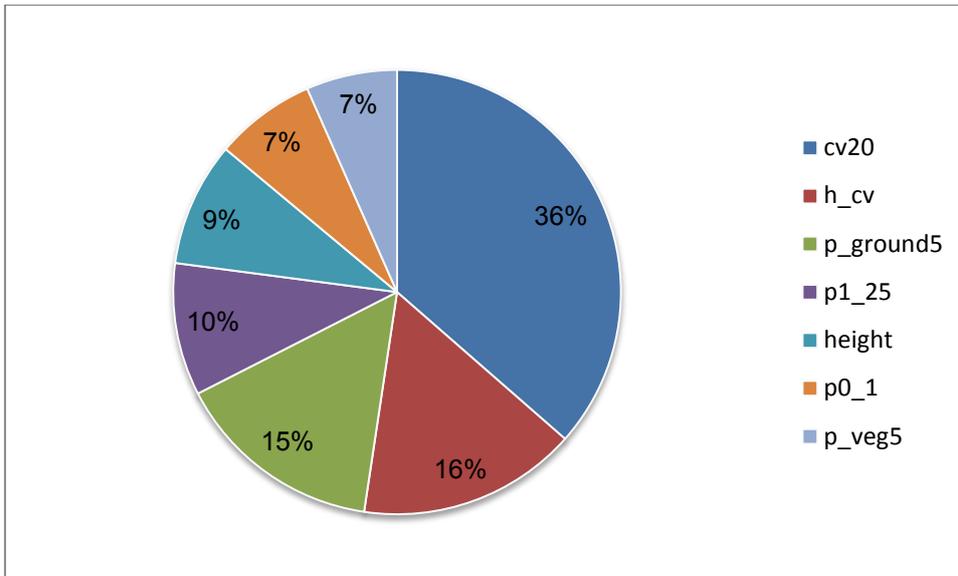
Fig.12. The average value for all 10 models of the variables that affect tortoises' habitat preference. The data based on Percent Contribution from Analysis of variable contributions.
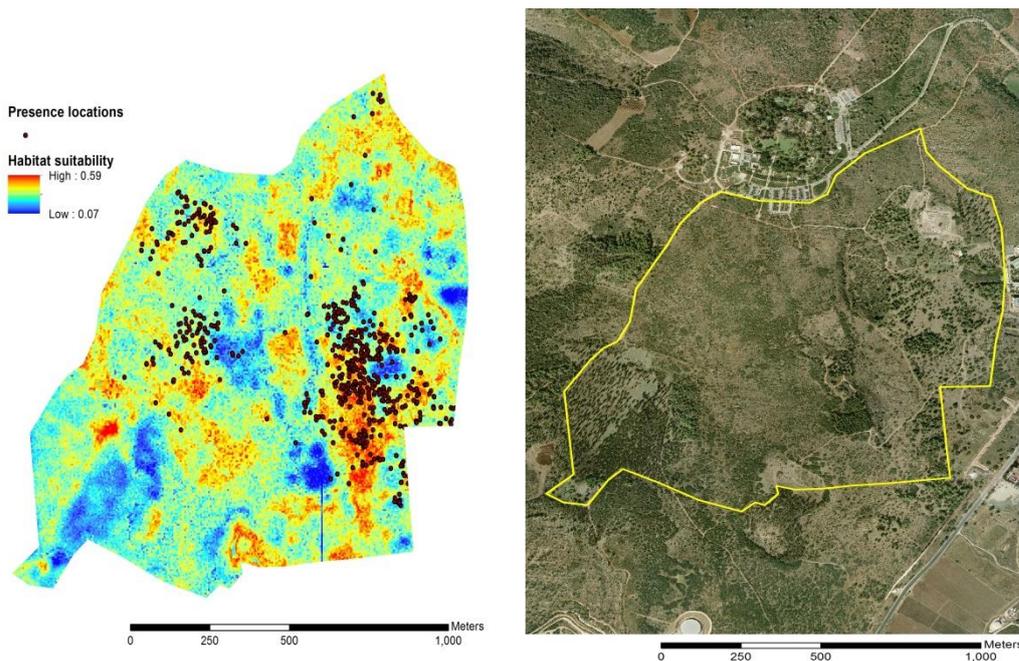
Fig.13. A map that averages and weighs all variables that explain the habitat preference of 10 radio-transmitted tortoises in the study area at Ramat Hanadiv (left). An aerial photo of the study area for comparison (right).

The tortoises' observed distribution on different soils in the study area was significantly different from the relative distribution of soil types at the same area (N=175; chi square= 70.82; df= 4; p< 0.001, Fig.14). Tortoises' observations on dark rendzina contributed above the expected distribution (chi square= 49.53) and observations on grumosol contributed below expected distribution (chi square= 14.90).
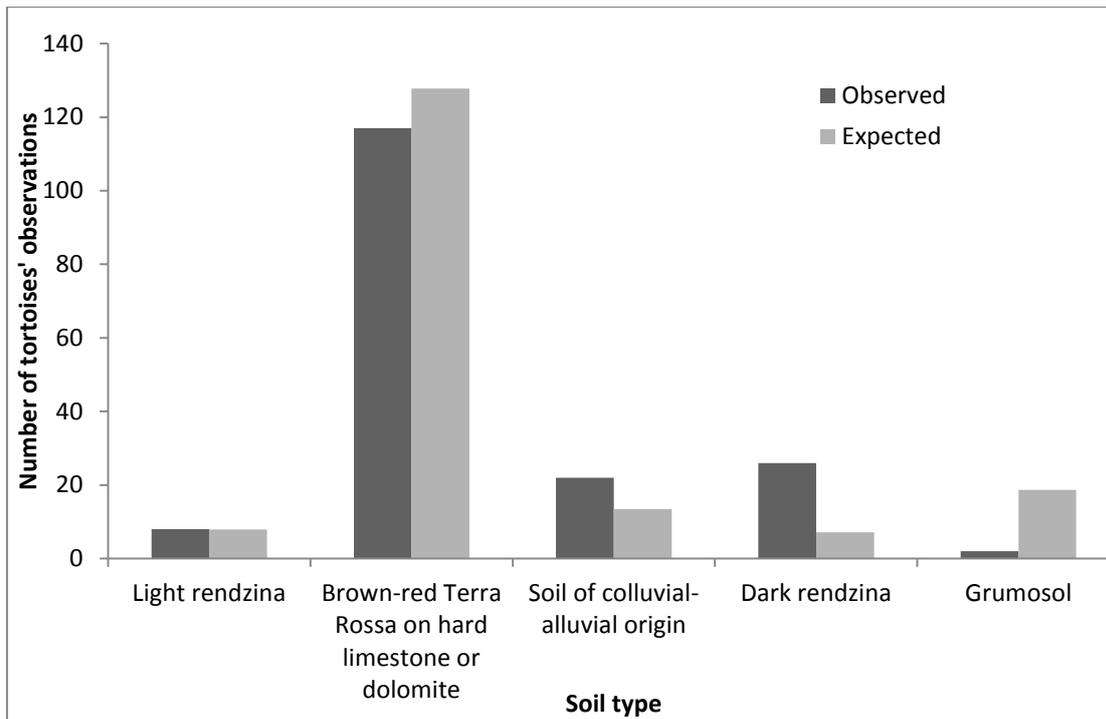
Fig.14. Observed and expected tortoises' distributions on different soils in the study area.

Tortoises' home ranges in Ramat Hanadiv

The average area of an individual's home range was 10383 ± 1517.38 m^2 (1.0383 ± 0.15 Ha) and 14897.31 ± 2998.05 m^2 (1.489 ± 0.29 Ha) for radio-transmitted females and males respectively (for all ranges see Appendix 5). There is no difference between the habitat sizes of the sexes (t_{16} =-1.243; p=0.232, Fig.15), and there are overlaps among individuals' home ranges (Fig.16, Fig.17, Appendix 3). For example: the range of male number 2 with the range of male number 127 (Fig.16a). Most of the home range areas are located on brown-red terra rossa soils and some of them are also found on colluvial-alluvial soil and dark rendzina (Fig.17). On April 2014 cattle was introduced into some parts of the study area. I assume that this shifted the home range of three males: 28, 49 and 79, because after the cattle were removed from the area, those three individuals moved back to the area that they inhabited before.
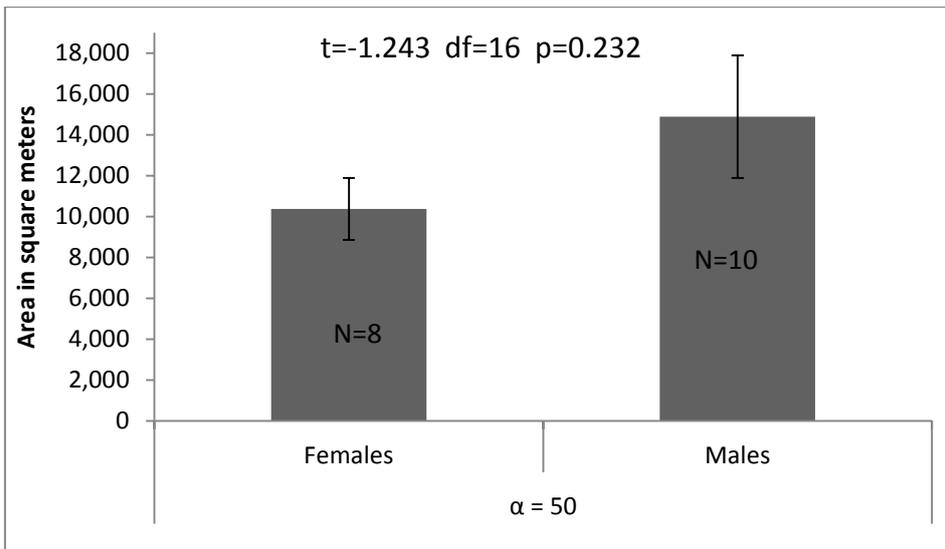
Fig.15. Home ranges for radio-transmitted males and females (mean ± standard error).
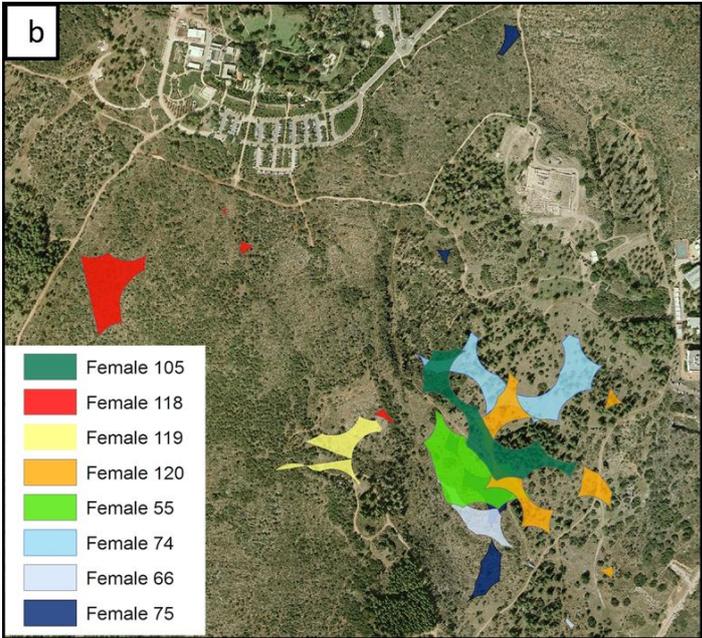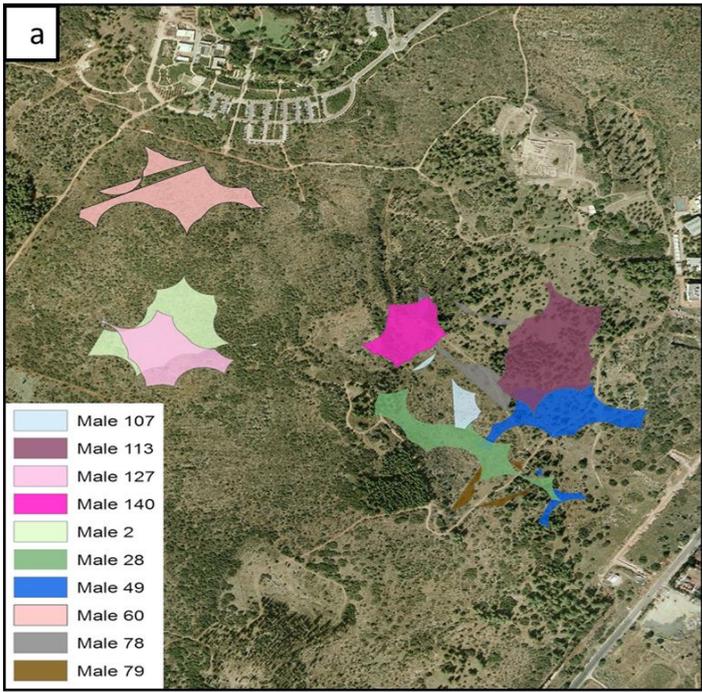
Fig.16. Radio-transmitted tortoises home range polygons of males (a) and females (b).
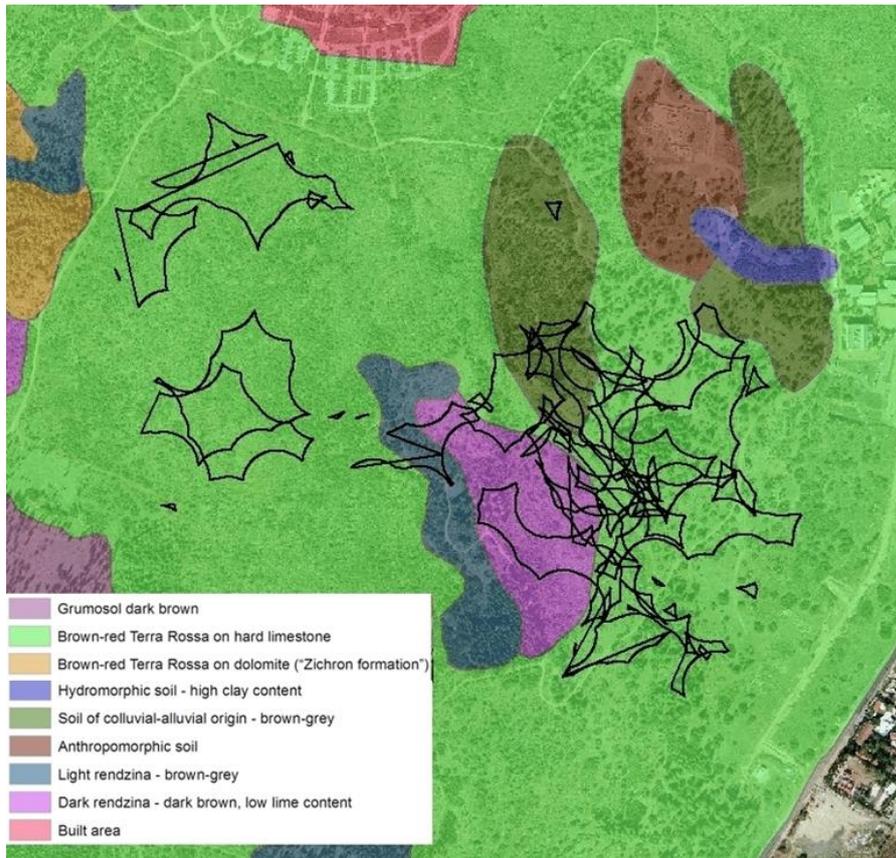
Fig.17. Home ranges polygons of radio-transmitted males and females on the different soils.

Effect of incubation temperature on sex determination

Twenty one of thirty two incubated eggs hatched from the 'high temperature' incubator (31.8°C). Three of them did not survive after the hatching, resulting in 18 hatchlings. From the 'low temperature' incubator (29.0°C) twenty one out of thirty four eggs hatched. There was no difference between the two incubators in hatchlings mass (average hatchlings' mass from low temperature was 13.85 gr, and average hatchlings' mass from high temperature was 12.80 gr; t= 1.59, df= 36, p= 0.12) and incubation length (average 84.8 days for 'high temperature', and 87.5 days for 'low temperature'; U= 129.5, Z= -1.48, p=0.139). The hatchlings from the two incubators were housed in separated enclosures (see Materials and Methods)

The gonads (Fig.18) of twenty six of thirty nine hatchlings were successfully identified in the laparoscopy procedure (ten individuals from the 'high temperature', and sixteen from the 'low temperature' incubators). There is a dependency between low temperature and males' development, and between high temperature and females' development (Fig.19). Nine individuals died as a result of the laparoscopy. Gonads histology was performed on three of them. All three samples were testes (Fig.20).
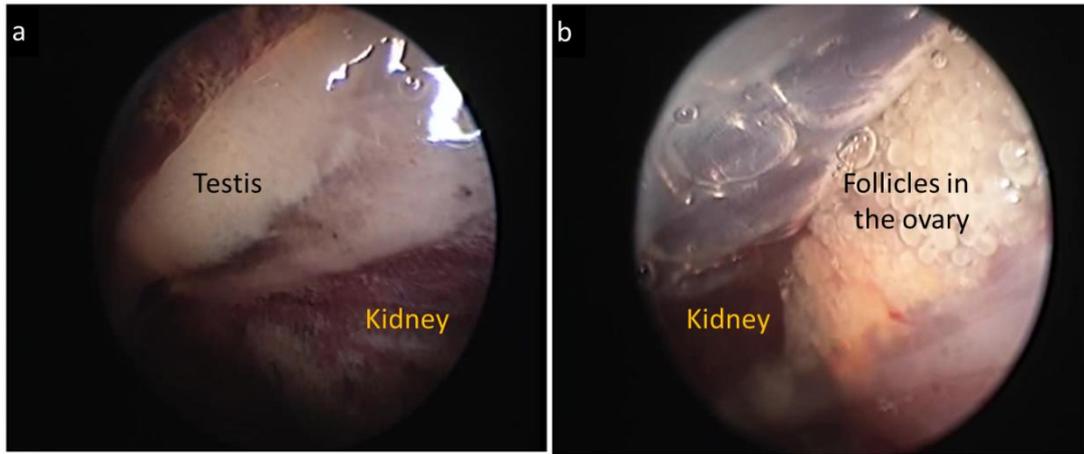
Fig.18. Hatchlings' gonads exposed in a laparoscopy procedure. a) Testis. b) Ovary.
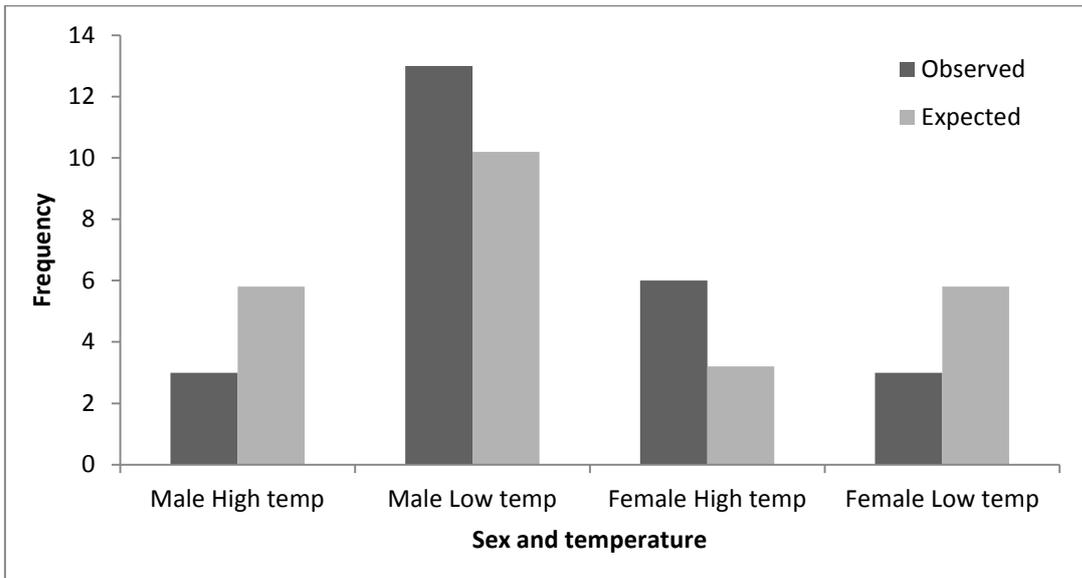
Fig.19. Observed and expected frequency of males and females developed under high or low incubation temperatures (p= 0.025, N= 25).
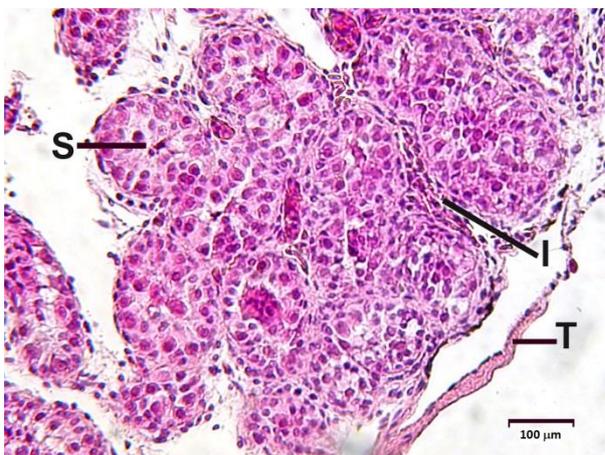
Fig.20. A microscopic section of a testis. S- Seminiferous tubule; T- Tunica albuginea and I- Interstitium.

Effect of nesting location and temperature regimes in the nest, on eggs' survival

Five nests were constructed between April and June 2014. All nests sites were under partial or full shade. The average nest depth was 9.86 cm. Nest numbers 1, 3 and 4 were constructed under a common shelter; nest number 2 was under complete shadow below a rock; and nest number 5 was in partial shade under a rock (Fig.21). All nests provided hatchlings, with different levels of success. The highest hatching successes were in nests 3 and 5 (Fig.21).

Nest number 1- hatching success 33%

Nest number 4- hatching success 33%

Nest number 3- hatching success 75%

Nest number 2- hatching success 25%

Nest number 5- hatching success 100%
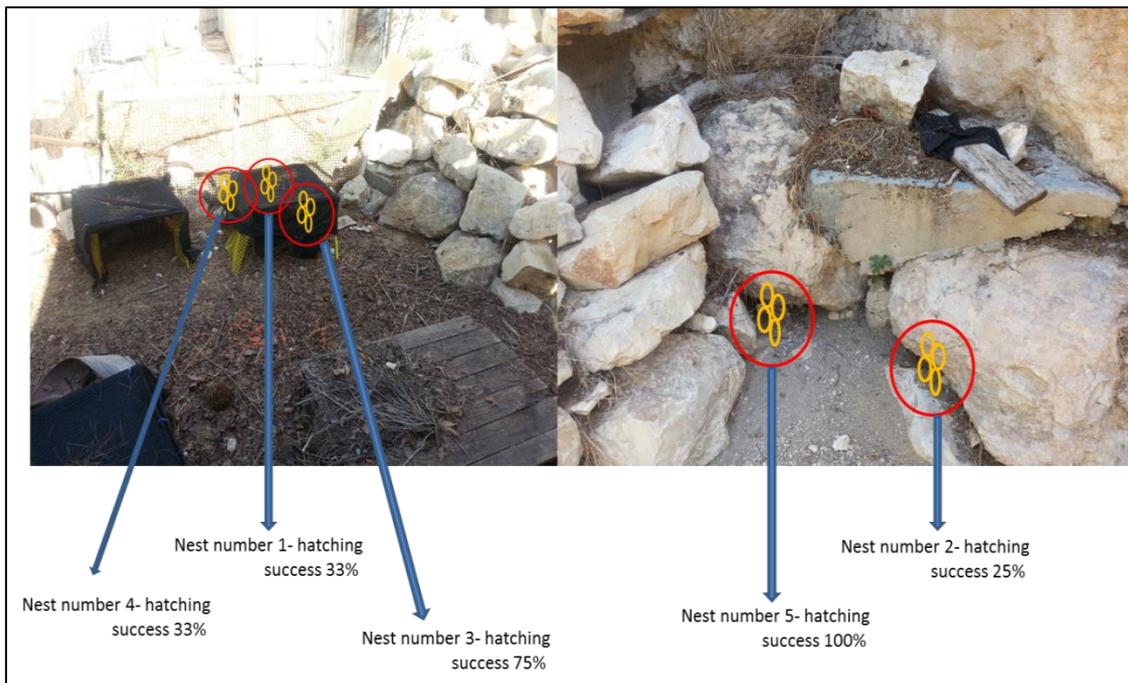
Fig.21. Nests locations and hatching success. The nests number is by order of nesting date.

The soil temperatures during the first period of 24 hours after egg layings varied considerably among nests (Table 4). During the incubation period the soil temperature adjacent to the nest reached above 50°C in two cases (Fig.22, control 2 and control 5). However nest temperatures did not reach this level. The average temperature of all nests for all incubation period was 28.76±1.67°C, and did not increase above 30.5°C (the pivotal point of *T. graeca*), however all nests experienced a maximum temperature that exceeded the pivot temperature (Fig.22).

Table 4- Nesting date, mean, minimum and maximum soil temperature 24 hours after nesting, nests depth and hatching success.

Nest number	Nesting date	Mean temperature at egg laying day	Minimum temperature at egg laying day	Maximum temperature at egg laying day	Nest depth	Hatching success
1	27/04/14	24.54	22.21	27.91	8	33%
2	30/04/14	22.13	21.32	23.96	12.5	25%
3	21/05/14	24.37	22.36	26.49	8.8	75%
4	07/06/14	26.23	24.18	29.48	11	33%
5	09/06/14	26.49	24.39	30.27	9	100 %
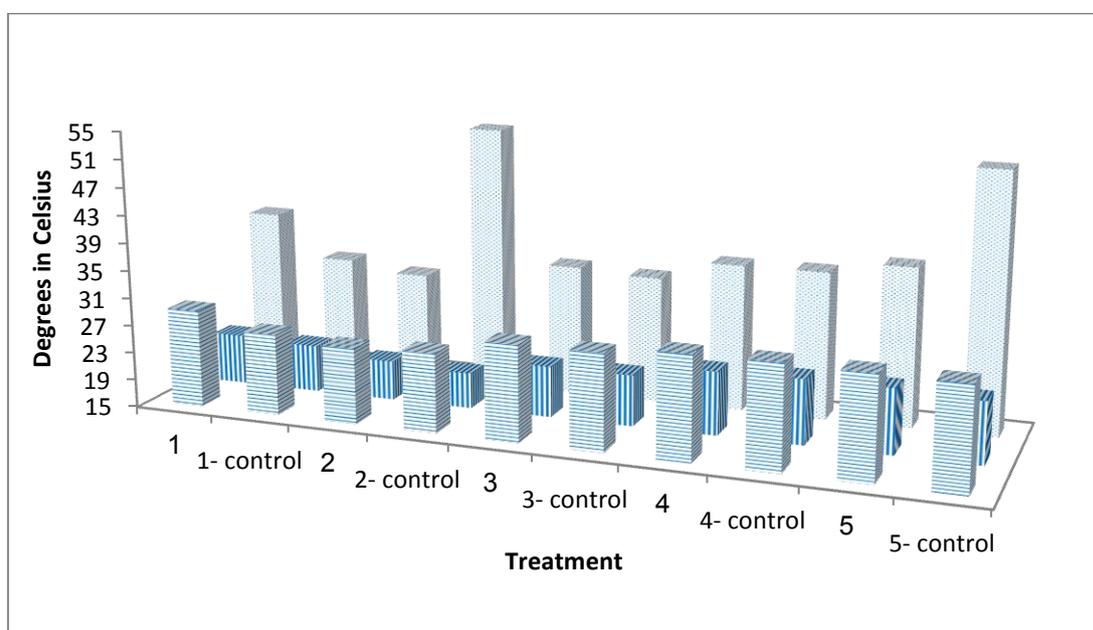
Fig.22. Minimum (vertical lines), Maximum (dots) and average (horizontal lines) temperatures of five nests and controls, for the entire nesting period.

Behavioral patterns of males and females before and after maturation

According to a discriminant analysis test there was not a behavioral variable (movements number of: upper limbs, lower limbs, head, head circles and "madness" behavior. See details in Appendix 6) that contributed to the discrimination between the two adult sex groups (Wilks' Lambda= 0.476, p=0.098). Additionally, there was no correlation between either male's or female's body weights and the summation of all the movements (p=0.894, N= 9 and p=0.218, N= 8 respectively).

In the discriminant analysis between hatchlings males and females, the number of head

movements (see details in Appendix 7) separates the two groups, with males having higher number of head movements (Wilks' Lambda= 0.809, p=0.029, discriminant score for females=-0.622 and discriminant score for males=0.350). The number of upper and lower limb movements was removed from the stepwise statistics. The probability to accurately predict hatchlings sex based on this model is only 64%, while the chance to accurately predict the sex without the model is 54%.

Discussion

Yearly activity patterns

Locating tortoises during the research gave an indication for activity times. The scarcity in observations not necessarily indicates for inactivity periods. However, when the radio-transmitted tortoises did not move from the same location for several days or more, it is an indication for inactivity times. The yearly inactivity pattern of the radio-transmitted tortoises is a mirror image for the pattern of activity found in the tagged tortoises during the first year of the research. Geffen and Mendelssohn (1989) reported that the Egyptian tortoise *Testudo kleinmanni* in Israel decreases activity during the summer months, and that the highest numbers of active tortoises were seen during winter and spring. Díaz-Paniagua and coauthors (1995), show in their research of the same species (though a different subspecies) in Spain that between February and May both sexes are in maximum activity. In their studies males precede females in decline of activity starting in June and lasting until October, while females decrease activity one month later (July), when they are still engaged in nesting and post nesting behavior. Their results are similar to my observations on the local subspecies of *T. graeca.* My study is the first to describe a long term of summer-autumn hiatus of *T. graeca* in Israel. Contrary to the tortoises in Spain, and to the reports in Israel according to Arbel (1984), in Ramat Hanadiv the tortoises did not go into hibernation. In general, there are more reports on hibernation in turtles and tortoises (Ultsch, 1989; Litzgus et al., 1999; Mazzotti, 2004; Hochscheid et al., 2005) than on aestivation. Díaz-Paniagua and coauthors (1995) suggests that the summer period of inactivation of *T. graeca* in Spain is aestivation. However it is not clear whether there is a physiological basis for it. In my research I assume that the inactivation of the tortoises is aestivation, but it will need further physiological experimentation to confirm it. There are some recordings on aestivation for other turtles and tortoises. For example: the desert tortoise, *Gopherus agassizii*, which spends more time aestivating in burrows when summer drought continues and feeding is diminished. This species also hibernate in the winter (Nagy and Medica, 1986). The Virginia chicken turtles, *Deirochelys reticularia*, inhabit swales that have seasonal water fluctuations, and some turtles will leave drying wetlands and aestivate on land during the summer months (Buhlmann, 1995).

It is generally assumed that ectothermic species are not active in the winter. However, the 2014 winter and spring were the most active period for tortoises in Ramat Hanadiv, especially between January and March. In this period the soil temperatures began to rise and the precipitation was low (about 24 mm, Appendix 2). These conditions were probably favorable to the tortoises because there were plenty of fresh seedlings and it was not too cold for the tortoise's activity. These conditions were apparently also good for starting the breeding season that began in January 2014 (Fig.10), earlier than what described for the Israeli population of *T. graeca* (Arbel, 1984). The literature (Willemsen and Hailey, 2003; Pellitteri-Rosa et al., 2011) describes a courtship season in the fall, but I have not noticed it in this study, though I had only a single fall (2013) period in the field.

Roques and coauthors (2004) revealed that *T. graecas'* females from Spain able to store sperm from single or multiple matings and can use it to fertilize subsequent clutches of eggs. In the case of my study, if the mating events were mostly in January and nestings occur as described in the literature (and from observation from nesting experiment in Oranim) from April to June, one can assume that the females in Ramat Hanadiv have the capacity to store sperm. I suggest that in a future scenario of global warming the females could advance the laying date, and thus might prevent the exposure of eggs to extreme soil temperatures (a behavioral-phenological response to climate change).

Selecting favorite micro-habitats

During the casual observations on non-radio transmitted individuals, tortoises were usually spotted in open areas or beneath vegetation. However, when I started to follow the radio-transmitted tortoises the spotting method could be dissociated from relying on sound and sight, and I stared to have increasing sightings of the tortoises in micro-habitats like shrubs and under rocks. The radio-transmitting method revealed that tortoises can be found most of the day in shrubs' boundaries. The tortoises use the shrubs for diverse activities, such as basking (or any thermoregulation activity) at the margins, sleeping, resting, mating and probably aestivating near the roots. I suggest that compared with the bare soil, the stable ambient conditions (temperatures, radiation and humidity) around the shrub make the shrub a favorable micro-habitat for the tortoises. Similarly, in Sinai peninsula the Egyptian tortoise, *Testudo kleinmanni*, used smaller shrubs in the fall, winter, and spring seasons for basking, thermoregulation, and concealment and used shrubs with a cover diameter greater than one meter during the aestivation season (Attum et al., 2013).

The shrubs species *Pistacia lentiscus*, *Phillyrea latifolia*, *Calycotome villosa* and *Sarcopoterium spinosum* where I found tortoises in their margins are the dominants shrubs in Ramat Hanadiv (Hadar et al., 1999; Levin et al., 2013). However there is not enough data currently to calculate whether the tortoises selected them randomly or they are favorable shrubs.

An exception to the common near shrub activity of most tortoises was seen in male 78 and female 74 as they were often active in open areas. Both individuals' home range predominantly occupied a sparse stand of pines which probably provided them relatively stable abiotic conditions beneath the canopies.

The study area in Ramat Hanadiv has 4 coniferous stands and they were usually avoided by the radio-transmitted tortoises except for the sparse stands (see home ranges of individuals: 119, 127, 2 and 28 and yellow and black circles, Appendix 3). Tortoises can probably thermoregulate better under sparse than under dense trees. Working on a different poikilothermic species, Todd and Andrews (2008) showed that the highest relative abundance of several species of snakes (*Cemophora coccinea*, *Diadophis punctatus*, *Lampropeltis triangulum*, *Storeria occipitomaculata*, *Tantilla coronata*, *Virginia valeriae*) occurred in thinned forest stands (and not in dense forest). The preferable habitat for tortoises probably also depends on the local climate. For example, Hermann's tortoise, *Testudo hermanni*, that can be found in more northern Mediterranean countries selects grassland and shrub habitats, avoids forests, and will use forest edges only randomly (Rozylowicz and Popescu, 2013). The Wood turtle, *Clemmys insculpta*, a north American semi-aquatic species seems to select mixed forest stands that were relatively young, short, had low arborescent cover, moderate cover of the upper shrub layer, and low total canopy closure (Arvisais et al., 2004).

Arbel (1984) described that in Israel *T. graeca* is common in the Mediterranean region and prefers rocky habitats. The absence of tortoise observations from the rocky areas (Appendix 4) can be related to ground and above ground characteristic like the difficulties to dig in the stony ground (for nesting or aestivate for example). Moreover, the temperatures perhaps are less regulated in rocky soil and the micro conditions under or near the vegetation in the rocky habitat are less stable than under shrubs' canopy in non-rocky habitats.

Biotic conditions affecting the distribution of tortoises in Ramat Hanadiv

The results from all 10 different models revealed that tortoises' habitat preference is influence dominantly from land cover variables such as vegetation characteristics and not from relief variables like slopes. The vegetation characteristic that contributed the most to the models was cv20 which means that heterogeneous vegetation cover serves as a suitable habitat. It is well known that animals favor heterogeneous habitats (MacArthur and Wilson, 1967). Structurally complex habitats may provide more niches and diverse ways of exploiting the environmental resources and thus increase species diversity. In most habitats, plant communities determine the physical structure of the environment, and therefore, have a considerable influence on the distributions and interactions of animal species (MacArthur and Wilson, 1967; Tews et al., 2004). The effect of habitat heterogeneity on a specific species may differ across spatial scales. Tortoises' habitat preference in the study area are varied, some were in garrigue (dense or sparse), and some were in sparse stands of trees. Those differences between habitats created contrasts between sub-habitat preferences of individuals. For example, individual number 2's home range was in the garrigue, and consequently its model revealed a preference for a uniform vegetation height (low values of cv20). In contrast, individual 78's home range was in a sparse stand of pines, and consequently its model revealed preference for areas with large height differences between treetops, understory shrubs, and open patches (high values of cv20). When combined with the two other important variables (h_cv and p_ground5), the sub-habitat requirements of tortoises' becomes apparent: heterogeneous areas with diverse vegetation types, including isolated trees, shrubs, open patches (probably for thermoregulation activities) and avoidance of areas with dense woody vegetation. Areas with high variation in vegetation height (denoted by cv20) may be characterized by easy access to open and closed patches that provide tortoises and poikilothermic organisms, in general, a suitable sub-habitat. The preference for areas with an open vegetation structure is likely to be linked to the thermoregulation requirements of tortoises (Anadón et al., 2006b)

In terms of model evaluation and performance, the AUC's values I obtained are relatively low compared to other studies that focused on much broader spatial scales. This may be because at a large scale, the entire study area of Ramat Hanadiv is in general a good habitat for tortoises, but not uniformly, as we can see from all observations collected during the study period in Ramat Hanadiv (Appendix 4). The predictive variables we used for generating

the MaxEnt model cannot isolate tortoises' habitat preference better than that, and probably there are more suitable variables that could contribute to those models, for example: temperature and precipitation, but there is a lack of such specific data for the study area.

Most species distribution models are used to understand and predict species distributions across broad geographical extents, from regions to continents. This study focuses on a very small spatial extent of about 133 hectares. It is therefore difficult to compare the results of this model to those of other models that focus on a much larger scale. At broader scales, climate (rain, temperature) has the strongest impact on the distribution of species in general (Warren et al., 2001; Parmesan and Yohe, 2003; Pearson and Dawson, 2003; Perry et al., 2005; Anadón et al., 2006a; Thuiller, 2007), and on *T. graeca* in particular but for a different sub-species in North Africa, where a presence-only model indicates that distribution is mainly related to rainfall, specifically rainfall values in the wettest and coldest quarter of the year (Anadón et al., 2012).

MaxEnt is capable of performing relatively well even with small numbers of observations, and produces useful results compared to other methods (Hernandez et al., 2006). In this study, low number of observations per individual was useful enough to predict suitable habitats for tortoises in Ramat Hanadiv. If future researches explore habitats preference of other *T. graeca*' populations in Israel, then the climate variables could be included in the models and probably provide an effective predictor for tortoises' distribution in Israel.

The above models don't take into account the soil type variable, since when using this variable it becomes the dominant one in the model, and thus masks the other variables. I therefore analyzed the soil preference separately.

Tortoises' distributions on different soils
The results (from the first year observations) revealed that tortoises had a nonrandom preference for soil type in their sub-habitats. Tortoises' observations on dark rendzina contributed above the expected by chance and those on grumosol below the expected by chance observations to the total observations. I suggest that soil type it is an important element in habitat preference for tortoises because of the soil' characters such as regulating extreme temperatures, absorption of radiation and as a substrate for digging. There are almost no studies on the relationship between habitat preference and soil type, and

moreover about the impact of global increasing temperatures on the potential regulatory capacity of different soils. Andersen and coauthors (2000) imply that soil composition can be important determinants of habitat suitability for desert tortoises, and for burrowing animals in general.

Tortoises' home ranges in Ramat Hanadiv

The size and location of tortoises' home ranges in Ramat Hanadiv revealed important information on this population. First, there is a high degree of overlap between individuals' home ranges, mostly between male and female ranges, but also between the ranges of the same sex, which suggests a lack of territoriality in this species (Harless et al., 2009). Burt (1943) argues that only those that protect some part of the home range, by fighting or aggressive gestures, from others of their kind, during some phase of their lives, may be said to have territories. Acording to Willemsen and Hailey (2003) there is no fighting behavior among *T. graeca* males during the courtship season, which means that they probably do not hold a territory. In the Israelis' Egyptian tortoise *T. kleinmanni* there are overlaps between male home ranges, between female home ranges and also overlaps between male and female home ranges (Geffen and Mendelssohn, 1988). Second, both sexes have home range sizes which are similar to those of other populations of the species or the order: *T. graeca* population in southeast Spain (Anadón et al., 2006b), *T. hermanni* in a rural Romanian landscape (Rozylowicz and Popescu, 2013) and western box turtle, *Terrapene ornata luteola*, in New Mexico (Nieuwolt, 1996). Although no significant difference was found between sizes of *T. kleinmanni*s' male and female home ranges, the home range size of males (34.9±24.6 ha) was twice the size of females (15.7±86 ha) (Geffen and Mendelssohn, 1988). I did not examine daily or seasonal movement patterns of males and females (a common method for this purpose is the thread trailing method developed by Breder, described in Díaz-Paniagua et al., 1995). This might reveal more patterns of tortoises' activities at different time scales throughout the year, in particular movement differences between males and females. Finally, most of the radio-transmitted individuals tended to remain in the same area throughout the year and did not move far from their home range, except for females 75, 118 and 120 that moved much further from their home range compared to other radio-transmitted individuals. I assume that this was part of nesting behavior, but I did not succeed in observing them laying eggs.

Several radio-transmitted individuals that their home range was adjacent to a dense stand of

pine trees (males number 2, 28, 127 and female number 119) avoided entering those stands, probably because tortoises cannot thermoregulate well there.

The introduction of cattle into some parts of the study area could be the cause for home range shifts for some individuals. The ecological costs of livestock grazing can be dramatic. Examples of such costs include loss of biodiversity; lowering of population densities for a wide variety of taxa; disruption of ecosystem functions, including nutrient cycling and succession; change in community organization; and change in the physical characteristics of both terrestrial and aquatic habitats (Fleischner, 1994). In the current research I did not compare between areas with and without grazing, and therefore I cannot evaluate the impact of this management practice on tortoises' presence. Further study will be needed to examine the influence of different grazing intensities on tortoises' presence.

I selected the α-hull method for evaluating home range size because it provides a more detailed description of the habitat's shape, and it is capable of breaking the hull into several discrete hulls when it spans an uninhabitable region (Burgman and Fox, 2003). Other methods like kernel methods, do not converges to the true area represented by the data as the number of data points increase (Getz and Wilmers, 2004) and moreover these methods frequently fail to capture the kinds of hard boundaries common to many natural systems (Getz et al., 2007).

Effect of incubation temperature on sex determination

The results of the current incubation experiments support the previous results from Pieau's experiments in the 70's (Pieau, 1971; Pieau, 1972; Pieau, 1975). The repetition of Pieau's experiments was important to establish that the sex of the common sub species in Israel, *T. gracea terrestris*, is also determined by temperature (TSD mechanism) during the incubation period, and to find whether it has the same pivotal point as the sub species in France. Identical pivotal points in different populations (and sub species), inhabiting different climates, may suggest that this trait did not (yet) undergo selection. My experiment, same as Pieau's experiments, shows that this species has the TSD Pattern Ia with one sex formed at low temperatures and the other at high temperatures, but it did not rejected the possibility of TSD Pattern II with one sex formed at both high and low temperatures and the other or often both sexes formed at intermediate values. There are reports for TSD Pattern II only in turtle species (*Chelydra Serpentine* and *Chrysemys picta*; Janzen and Paukstis, 1991; Ciofi and Swingland, 1997), but not for tortoises. This question waits to be examined in all

subspecies of the *T. graeca*.

The laparoscopy was the only method other than post-mortem examinations to identify hatchlings' sex correctly. This procedure for small sized animals can lead accidently to their death because of complications such as the tearing of blood vessels. Better sexing methods, such as the use of hormones from egg shells are sought.

Effect of nesting location and temperature regimes in the nest on eggs' survival.

While incubation at a constant temperature can provide useful information on the mode of sex determination, it may have limited applicability for discerning how offspring's sex may be affected by temperature under natural conditions of incubation (Bowden et al., 2014). The semi-natural incubation experiment revealed that under natural conditions, the nest temperatures can reach temperatures that are high above the pivotal point, without reducing the viability of the eggs.

The average nest depth in the semi-natural conditions of this study was almost 2 cm dipper than the recorded depth from Spain (Díaz-Paniagua et al., 2006). This, unlike the pivotal point can be a local adaptation as nest depth is a variable that could potentially be manipulated by TSD species in response to climate change to maintain balance in offspring sex ratio (Georges, 2013). However, the depth is limited to the length of the hind leg of the female, and at least in the *Chrysemys picta* it did not affect sex ratio (Refsnider et al., 2013).

The mean incubation temperature of 28.76°C was similar to those observed in *T. graeca* population in Spain, and it is a value frequently recorded for development of reptile eggs (Díaz-Paniagua et al., 2006). This average temperature of all nests was below the pivotal point. It is probably not a good predictor for hatchling sex ratio when nest temperatures fluctuate (for example: mean daily temperature in natural nests of freshwater turtles with temperature-dependent sex determination is known to be a poor predictor of hatchling sex ratios, Georges, 1989). Under the current study, it is yet to be found how the temperatures affected the sex.

In two of the nests, adjacent soil temperatures increased to very high levels, yet the nest temperatures remained relatively low and not far high above the pivotal point. Extended exposure to temperatures below the low thermal limit (LTL) or above the high thermal limit (HTL) can result in embryonic death (Bowden et al., 2014). The difference between the nest

temperatures and the adjacent soil temperatures implies that the female select carefully the specific nest location to ensure adequate incubation conditions. A meticulous nest site selection is a wide spread behavior among reptile species. For example, the snapping turtle, *Chelydra serpentina,* in northwestern Illinois selected nest sites with shorter vegetation, more open sand, and fewer cacti than random locations (Kolbe and Janzen, 2002); Female striped mud turtles, *Kinosternon baurii*, were found too, to have a nonrandom nest site selection, ovipositing their eggs closer to grass tussocks or other herbaceous vegetation and in areas of less bare ground (Wilson, 1998). Janzen (1994b) offered that female turtles might use a more reliable cue to predict incubation conditions during the thermosensitive period, such as shade cover.

Behavioral patterns of males and females before and after maturation

In the first year of the research at Ramat Hanadiv I noticed that when I picked up adult males for measurement routines they were very active and tried to escape with fast moving their limbs. It was in contrast to the relatively docile behavior of adult females under the same conditions. These observations led me to perform a behavioral experiment that examined behavioral differences between the sexes. Tortoises need to have the ability to return to upright position ('righting ability') after being overturned. The ability to perform righting can make the difference between life and death, especially in suboptimal thermal conditions or in the presence of predators (Golubović et al., 2013). Falling on the carapace is a common situation when the male climbs on the female's back during the courtship. Also, every individual can turn over as a result of climbing a steep rock or when they are placed on their back by an animal or a human. The mechanism to turn over is not known yet, but it demands the use of limbs, tail and head as anchors. I observed this behavior occasionally in the field and during the experiment. Unlike other studies (Bonnet et al., 2001; Freedberg et al., 2004; Mann et al., 2006; Golubović et al., 2013) that measured the time it takes an individual to turn back on his feet, here I explored the difference in movement components between sexes while they were upside down and did not succeed to turn back. It is most likely, that the time of year (mid May) when this experiment was carried out was not optimal, as it is a month where males reduce their activity to minimum (Fig.7). I assume it is the reason for not finding a difference between sexes in the movement components while they perform righting behavior. This experiment should be repeated in the spring, when both sexes are active. In contrast to the adult's experiment, and though the model quality was not high, but

significant, I found that the number of head movements in hatchlings can be a predicted variable for discriminating between hatchlings' males and females. In the future this experiment should also be repeated, and conducted as soon as they hatch, when sex hormones that determine the sex during incubation may still be high.

Conclusions

This study describes for the first time a long term summer-autumn activity hiatus of *T. graeca* in Israel that might be regarded as an aestivation. Furthermore, apparently land tortoises in Israel do not hibernate, as it is commonly believed. In fact, during this study the winter and spring were the most active period for tortoises in Ramat Hanadiv, probably due to favorable climatic conditions for exothermic species like tortoises.

The tortoises in Ramat Hanadiv are not territorials and there is an overlap among home ranges. Models for predicting their distribution in Ramat Hanadiv revealed that they prefer heterogeneous areas with a variety of different vegetation types and open areas. However, they avoided areas of high vegetation cover. The tortoises significantly had a nonrandom preference for soil type in their sub-habitats. Tortoises had a non-random occurrence on dark rendzina soil, where they were mostly found in shrub margins, probably because of the stable micro-climatic conditions these shrubs provide.

Sex determination of *T. graeca* in Israel follows the TSD pattern Ia mechanism, which means that incubation in constant temperatures above 30.5°C (pivotal point) during the thermosensitive period leads predominantly to females development and below this temperature predominantly to males. In naturals nests the temperatures regime can rise significantly above the pivotal temperature without affecting the embryos' survival. The effect of the later on sex determination in *T. graeca* is not known yet, due to difficulties in sexing newly hatched tortoises. However, this study revealed the potential of using a behavioral method to distinguish between the sexes, and shows that this approach deserves further studies.

Ectothermic organisms respond to temperatures not just in the breeding period, but in each aspect of their existence. The survival challenge under warming climate will be even greater because of the potential lack of available food and water, strong radiation and a shortage in suitable habitats. All of these factors, together, can affect the reproductive capabilities of such organisms. In species with TSD mechanisms the expected warming climate will not only

affect the reproductive capabilities, but could directly affect the sex ratio.

This short-term study did not directly deal with the hazards of global warming on *T. graeca* in Israel. However, it shows the sensitivity of the sex determining mechanism to incubation temperatures and that tortoises have particular micro-habitat preferences. This currently preferred micro-climate may change under increasing global temperatures leading to skewed sex ratio, and thus endangered the species' future.

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Appendices

Appendix 1- Dorsal, ventral and lateral photos of tortoise number 80.
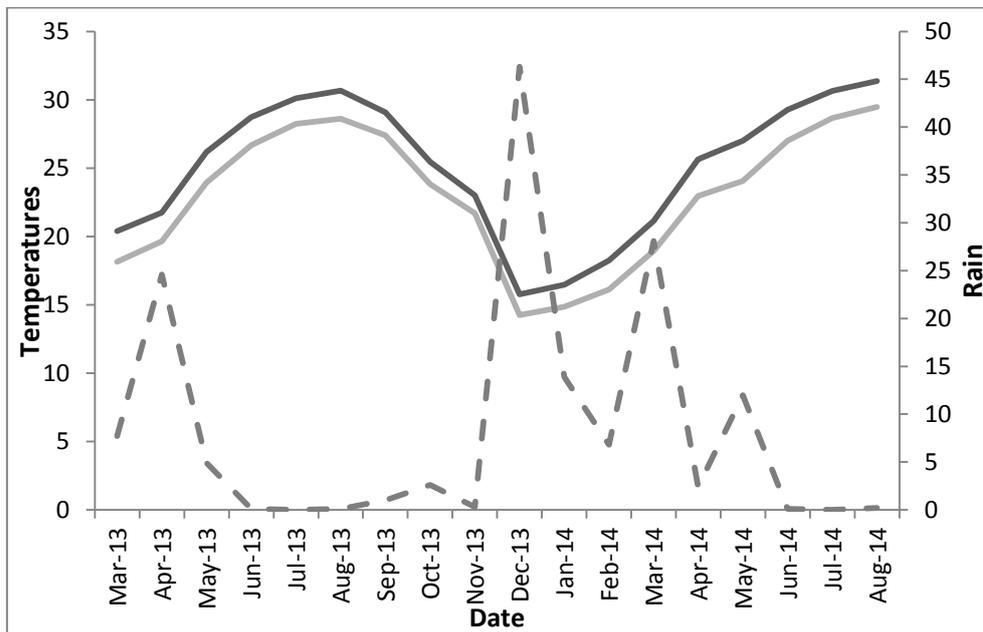
Appendix 2- Meteorological data from Ramat Hanadiv. Temperatures are in Celsius, in dark line is the average of maximum soil temperature, and in light line is the average of minimum soil temperature. Broken line is rain in mm.

Transmitted tortoises

Female 105	Male 127	Female 74
Male 107	Male 140	Female 66
Male 113	Male 2	Male 60
Female 118	Male 28	Female 75
Female 119	Male 49	Male 78
Female 120	Female 55	Male 79

Appendix 3- Home ranges of all radio-transmitted tortoises. Black circles are dense coniferous stands in the study area. Yellow circles are sparse coniferous stands in the study area.
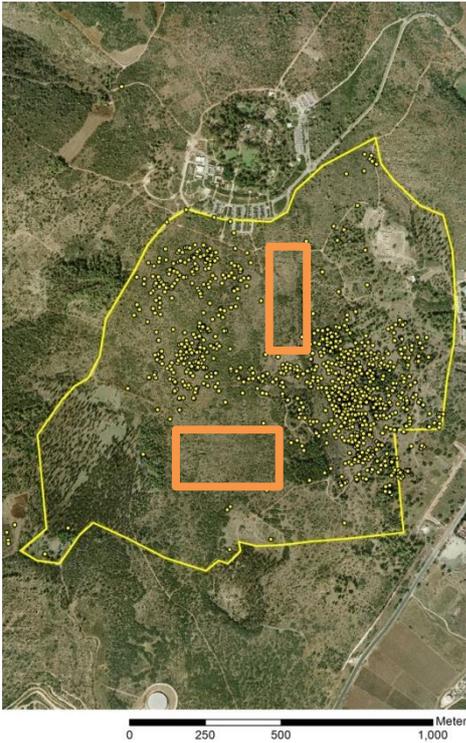
Appendix 4- All tortoise observations from the study area at Ramat Hanadiv. Orange rectangles are rocky areas with almost no observations.

ID	Gender	Home range
55	Female	14980.55
66	Female	4606.15
74	Female	15129.29
75	Female	8431.98
105	Female	13562.75
118	Female	9466.225
119	Female	4597.17
120	Female	12297.83
2	Male	25307.85
28	Male	16101.72
49	Male	21185.44
60	Male	17781.2
78	Male	4837.87
79	Male	1297.845
107	Male	3525.69
113	Male	29945.27
127	Male	16621.68
140	Male	12368.59

Appendix 5- Home ranges of radio-transmitted tortoises in Ramat Hanadiv. The values are in square meter.

Sex	I.D	Date	Time	Sum upper limbs movements	Sum lower limbs movements	Sum head movements	Sum head circles	Number of crazies
m	49	15/05/2014	12:21:00	176.00	214	138	21	3.00
m	113	15/05/2014	09:53:00	145.00	185	137	10	6.00
m	78	15/05/2014	10:25:00	220.00	364	144	22	8.00
m	140	15/05/2014	10:48:00	194.00	226	193	21	8.00
m	28	15/05/2014	11:39:00	107.00	153	77	6	4.00
m	107	15/05/2014	11:59:00	288.00	325	216	8	8.00
m	127	16/05/2014	11:00:00	308.00	361	231	16	12.00
m	2	16/05/2014	11:20:00	271.00	330	210	20	11.00
m	60	16/05/2014	12:30:00	119.00	116	86	8	2.00
f	55	15/05/2014	12:43:00	162.00	182	148	12	8.00
f	120	15/05/2014	09:30:00	30.00	49	31	0	0.00
f	74	15/05/2014	10:15:00	168.00	175	125	3	6.00
f	105	15/05/2014	10:33:00	267.00	275	182	16	12.00
f	119	15/05/2014	11:16:00	230.00	210	193	16	11.00
f	118	16/05/2014	11:39:00	312.00	313	207	28	12.00
f	75	16/05/2014	12:50:00	193.00	205	170	15	7.00
f	169	16/05/2014	12:40:00	187.00	151	129	8	12.00

Appendix 6- Data of adult tortoises' behavioral experiment of in Ramat Hanadiv. The summation of movements is for five minutes.

ID	Sex	Sum upper limbs movements	Sum lower limbs movements	Sum head movements	Sum head circles	Number of crazies
11	female	76	84	49	1	2
14	female	13	51	16	0	1
1	female	2	92	5	0	0
3	female	30	65	30	7	3
9	female	49	72	54	1	3
17	female	4	6	11	0	0
20	female	50	51	30	4	3
19	female	84	89	62	5	4
17	female	21	6	31	0	0
11	male	78	75	54	6	4
13	male	63	62	50	0	3
7	male	103	88	82	3	5+
10	male	11	23	16	3	0
14	male	9	8	13	0	0
1	male	88	107	66	1	3
2	male	106	142	115	6	5+
3	male	67	110	37	1	0
4	male	72	85	67	1	2
5	male	66	88	69	7	4
7	male	20	25	22	0	0
8	male	53	53	61	0	2

16	male	51	66	31	1	1
6	male	65	83	55	2	4
5	male	115	88	87	2	5+
12	male	115	88	87	2	5+

Appendix 7- Data of hatchlings' behavioral experiment in Oranim. The summation of movements is for three minutes.

"גלגל החיים" של המין *Testudo graeca* בישראל: השפעת הטמפרטורות על קביעת הזוויג ודפוסי פעילות שנתיים, העדפות בתי גידול וניצול שטחי המחייה.

מאי ברנהיים

תקציר

זוחלים הם אחת הקבוצות הרגישות ביותר לאבדן מינים כתוצאה משינויים סביבתיים מעשי ידי אדם כמו שינויי אקלים. ישנן ראיות מוצקות להשפעות ההתחממות הגלובלית על הביולוגיה של מינים מקבוצות שונות כמו שינויים בגבולות התפוצה, מעבר לקווי רוחב גבוהים יותר, ושינויים פנולוגיים, כמו לדוגמא הקדמת זמני הנדידה של ציפורים באביב. שינויי האקלים עשויים להשפיע במיוחד על מיני זוחלים עם מנגנון ה TSD (Temperature-dependent Sex Determination) בו יחס הזוויגים מושפע בתקופת השליש השני של ההתפתחות העוברית מטמפרטורת ההדגרה של הביצים. אפיון בתי הגידול המועדפים למחייה על ידי הזוחלים, ואיך הטמפרטורה משפיעה על זוויג האבקועים תתרום רבות לניהול ממשקים של אוכלוסיות תחת תרחשי שינויים אקלימיים. צבי יבשה רגישים במיוחד לשינויים בטמפרטורת הסביבה עקב יכולות נדידה (פיזור) נמוכות, קיטוע בתי הגידול, ועקב כך שרוב המינים נמצאים כבר תחת סכנת הכחדה. בצב היבשה המצוי , *Testudo graeca*, מנגנון קביעת הזוויג תלוי בטמפרטורת ההדגרה (TSD). תחום תפוצתו סובב את מדינות הים התיכון באופן לא רציף עם שלוחה בצד המזרחי עד איראן. עונת החיזורים וההזדווגויות חלה בין מרץ לאפריל, ואילו ההטלות מתרחשות מאוחר יותר בין סוף אפריל ליוני. משך ההדגרה הינו כשלושה חודשים, והאבקועים (צבים צעירים) בוקעים בין אוגוסט לספטמבר. כיום אין מידע משטחים טבעיים בישראל על אתרי ההטלה של הנקבות, ובנוסף כמעט ולא קיים מידע על דפוסי הפעילות והעדפת שטחי המחייה של המין בארץ. מטרות המחקר היו לזהות בקרב צבים בוגרים את דפוסי הפעילות השנתיים, שטחי המחייה, מיקרו בתי הגידול המועדפים למחייה ותנאים ביוטיים המשפיעים על תפוצת המין ברמת הנדיב. בנוסף, ללמוד את השפעות טמפרטורת ההדגרה על קביעת הזוויג במין *T. graeca* בישראל, והשפעות מיקום הקנים ומשטר הטמפרטורות בהם על שרידות האבקועים. לבסוף, לזהות דפוסי התנהגות של זכרים ונקבות שקשורים ביכולת ההתהפכות של צבים ככלי להבחנה בין הזוויגים. המחקר התקיים בשני אתרים: במכללת אורנים בקריית טבעון, שם נערכו ניסויי הדגרת ביצים של המין *T. graeca* וניסויי התנהגות בקרב אבקועים, ובפארק רמת הנדיב שם סקרתי וסימנתי 181 פרטים ומשדרתי 18 צבים בוגרים. מודלי MaxEnt נערכו בכדי לחזות את תפוצת הפרטים ממין זה בשטח המחקר. התוצאות מראות שבין ינואר לאפריל 2014 שני הזוויגים נמצאים בשיא הפעילות כולל חיזורים והזדווגויות. הזכרים מקדימים את הנקבות בהורדת הפעילות החלה במאי 2014 ועד אוקטובר, ואילו הנקבות מורידות פעילות החל מיוני. מחקר זה מתאר לראשונה הפסקת פעילות ממושכת (שנעה בין שבועות לחודשים) בעונות הקיץ ותחילת הסתיו של מין זה בישראל. המודלים

לחיזוי התפוצה של מין זה חשפו שהצבים מעדיפים בתי גידול הטרוגניים במבנה וצפיפות צומח (לדוגמא שטחים עם שיחים מפוזרים, בני שיח, כתמים חשופים ועצים בודדים) שמאפשרים גישה לשטחים פתוחים לצורכי פעולות שקשורות לתרמורגולצייה ולשטחים סגורים לצורך הסתתרות ומנוחה. המין אינו מראה טריטוריאליות, שטחי המחייה של זכרים ונקבות חופפים ואין הבדל מובהק בגדלם בין הזוויגים. גודל שטח המחיה הממוצע עבור נקבות הינו 1517.38 ±10383 מ"ר ועבור זכרים 2998.05 ±14897.31 מ"ר. הצבים מופיעים באופן לא אקראי על קרקע מסוג רנדזינה כהה, ובדרך כלל הם ימצאו בשולי שיחים לצורכי פעולות של תרמורגולצייה, שינה, מנוחה והזדווגות. מיקרו בית גידול זה כנראה מספק להם תנאי אקלים יציבים באופן יחסי לסביבה. שישים ושישה ביצים מעשרים תטולות חולקו באופן שווה להדגרה בשני אינקובטורים בעלי טמפרטורה קבועה: 29.1±0.1°C ('טמפרטורה נמוכה') ו 31.8±0.1°C ('טמפרטורה גבוהה'). הזוויג של עשרה פרטים מתוך שמונה עשרה שבקעו מהאינקובטור בטמפרטורה גבוהה ושל שישה עשר פרטים מתוך עשרים ואחת שבקעו מהאינקובטור בטמפרטורה נמוכה זוהו בהצלחה בלפרוסקופיה (הליך שאינו דורש להמית את הפרטים, בו אנדוסקופ מוחדר לחלל הבטן לצורך זיהוי הגונדות: שחלות או אשכים). נמצא שקביעת הזוויג במין T. graeca בישראל הינה במנגנון ה (Ia)TSD . טמפרטורת הדגרה מעל ל - pivotal point 30.5° (בה יחס הזוויגים 1:1) מובילה באופן מובהק להתפתחות נקבות, ומתחת לטמפרטורה זו להתפתחות זכרים באופן מובהק. בניסוי משלים שבדק את השפעת הטמפרטורות על שרידות העוברים בתנאים טבעיים הוכנסו לשטח מגודר טבעי למחצה במרץ 2014 ארבעה זכרים וחמש נקבות. הקרקע הכהה כוסתה בשכבה של חול בהיר כדי שניתן יהיה לזהות על פני הקרקע עדויות לבניית קנים (הקרקע הכהה הופיעה על פני המשטח הבהיר). הנקבות חפרו חמישה קנים מסוף אפריל ועד תחילת יוני. כל הקנים הוטלו תחת מחסה טבעי, כמו סלע, או מלאכותי (מבנה רשת מוצל). תשעה אבקועים בקעו בסה"כ מכל חמשת הקנים לאחר תקופת הדגרה שנעה בין 84- 125 ימים. נמצא שבקנים הללו היה מנעד טמפרטורות שהאמיר באופן משמעותי מעל ל pivotal point מבלי לפגוע בשרידות העוברים. טמפרטורות קרקע גבוהות מ 50°C שנצפו בקני ביקורת סמוכים ללא ביצים, אך לא בקנים עצמם, מצביעים על כך שייתכן שהנקבות בוחרות באופן ספציפי ומדויק את מיקום הטלת הביצים בכדי להבטיח תנאי הדגרה אופטימליים. השפעת תנודתיות הטמפרטורה בקנים טבעיים על קביעת הזוויג במין זה טרם התגלתה, בעיקר בגלל חסר בשיטות אמינות ויעילות לזיהוי הזוויג בקרב אבקועים בתנאי שדה. על אף כל זאת, מחקר זה מראה את הפוטנציאל בשימוש בשיטות התנהגותיות בכדי להבחין בין הזוויגים כגון מדידת תנועות הגפיים והראש כאשר הצבים הפוכים על השריון הגבי שלהם. בקרב אבקועים נמצא שמספר תנועות הראש שונה באופן מובהק בין זכרים לנקבות. מחקר זה אומנם לא עסק באופן ישיר עם הסכנות של תהליכי התחממות גלובלית על T. graeca בישראל, אך הוא מראה את הרגישות של מנגנון קביעת הזוויג לטמפרטורות ההדגרה, ושתחת מגמת עלייה רציפה, טמפרטורות הסביבה יכולות להוות סכנה לבריאות האוכלוסיות ממין זה.

"גלגל החיים" של המין *Testudo graeca* בישראל: השפעת הטמפרטורות על קביעת הזוויג ודפוסי פעילות שנתיים, העדפות בתי גידול וניצול שטחי המחייה.

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