Hibernation experience of *Testudo hermanni* and *Testudo graeca* in natural and very close to natural conditions in Bulgaria

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Introduction

Over the last couple of decades, *T. hermanni* and *T. graeca* have become one of the most common pet-tortoises all over the world. It is mostly due to illegal export from east European countries in the near past, and later to their largely spread captive breeding. Other reason is that they are "survivors" and can adapt to conditions, sometimes very different from their natural.

A significant number of studies have been conducted in the wild and in captivity. Most of their biology and ecology have been well investigated. However the most unknown aspect remains the period of hibernation. It is well known that, as a main part of their life cycle it plays significant role in connection with right development, successful reproducing, good health and longevity.

Recently the techniques of artificial hibernation have been well developed and seem to give good results. They are based on data collected about various species, which do hibernate in the wild. The relatively large area of distribution of *T. hermanni* and *T. graeca*, suggests that in different parts of the habitats, they are exposed to different specific weather conditions during winters, which supposedly resulted in specific adaptational techniques for overwintering. Very little is known about how they reflect the severe environmental conditions within their range like subzero temperatures for prolonged periods, which are often presented during winters in some regions in the Mediterranean's. Every bit of data from different regions of the habitats is essential and will contribute to better understanding and knowledge about the nature's mechanism to protect its cold-blooded creatures.

In general, it is known that in most cases *Testudo hermanni and Testudo graeca* hibernate from the middle of October, until the end of March. In periods of warm weather, "awakening" can be observed (Stubbs, 1989a,b). Most critical factors are freezing and desiccation. Habitat selection and excavating burrows below the freezing level are generally the major method of coping with cold. Dehydration may be a significant mortality factor among terrestrially overwintering reptiles, especially when hibernating within the frost zone, where water potential within a frozen soil matrix is low (Costanzo et. al, 2001). Desiccation can be avoided by selecting a burrow with high relative humidity, by minimizing water loss associated with respiration (largely accomplished by the depressed metabolism associated with hibernation) and by minimizing exposure of portions of the integument through which water is most readily lost (Ultsch, 2006). Combination of subzero temperatures with wet conditions could lead to lethal ice formations (Bidmon, 2001).

They belong to the large group of terrestrial species, which pass the winter underground in burrows that they excavate or that are preformed. The optimal temperature is 5°C, with safe tolerance between 0 and 10°C (Highfield, 1984). Prolonged periods of subzero temperatures, often cause severe health damage or death due to freezing of body fluids. Long term kidney damage, anorexia, and loss of fertility are being sited as some of the negative consequences when there is a lack of hibernation in captivity of these temperate zone species.

Land tortoises present in Bulgaria are the subspecies *Testudo hermanni boettgeri* Mojsisovics 1889 (*T.h.*) and *Testudo graeca ibera* Pallas 1814 (*T.g.*). There are no particular studies about hibernation in the Bulgarian part of their habitat.

Brief characteristic of the area and husbandry conditions

The area of the study is a natural tortoise habitat for *T.h.* and *T.g.* situated in the easterly part of the Balkan range, comprising a territory of approximately 80 sq. km. with 0 to 250m. altitude. The climatic conditions are typical for the Black Sea coastal climatic zone with an average annual precipitation of 550-600 mm. and an average annual temperature of 12 degrees C. Vegetation consists of mixed broadleafed forests featuring cerris oak, elm, ash, oak, hornbeam, etc. The region is very favorable from an ecological perspective. There are no mineral resources nor industry, the human population density is low, and the region remains undisturbed by resort construction (Ivanchev, in print) (Pic.1a).

In the year 2002, in the northwestern part of the habitat, in the outskirts of Banya village was founded a small private specialized research and conservation center for both species, which has been the only one of its kind in Bulgaria by now. Its territory is about 2000 sq.m. and 180m. altitude. It is part of a slope with southern exposure, well protected from wild and domestic animals, practically isolated from human presence. There is enough vegetation to serve tortoises needs of natural food, so extra food is not given on a regularly basis, but water access is permanently available. The characteristics of the center are kept as close as possible to the natural ones, which makes it a favorable place for various observations in conditions very similar to the wild. (Pic.1b-f).

The study

The survey comprises four hibernation periods – from the fall of 2002, until the spring of 2006, 46 specimens in total from both species were observed.

The body mass data was collected during 2003 –2006. 34 specimens *T. h.* were weighed to the nearest gram - 17 adults (6 males and 11 females), 8 two years old, 5 one year old and 4 hatchlings. Some of the adults were measured more than one season. With the same precision 10 adults and 2 juveniles *T. g.* were weighed. Body mass prior hibernation was determined during the second half of October. The end of the fasting period was followed, which in the region starts from the end of September - beginning of October and continues until the final burying under the ground – mostly during the second half of November.

Almost all the body mass data after hibernation was taken in the actual day of awakening, very rarely on the next or the day after the next, but always before the first feeding.

Body mass after hibernation was measured after washing the tortoise with very slightly warm water, in order to clean the soil, which would normally appear all over it and to void urine and feces. It is important that this procedure is not confused with “giving a bath”, because drinking water will actually add weight.

With one day accuracy was determined the duration of hibernation 20 times in total for *T. h.* and 8 times for *T. g.* Considerably more are the registered dates of entering and coming out of hibernation (without determination of the exact hibernation duration), of specimens from both species, in the center and in the observed wild population.

For initial day of hibernation was considered the day when the tortoise permanently burrows under ground. For final day - the day when the specimen actually leaves the burrow

and makes its first steps out (Pic.2), as it often stays one third or half buried (Pic. 3), without actually showing activity for few days (up to 10 observed).

Temperatures were monitored during the winter periods 2003-2006, with thermochron I-button thermologers, tuned to take data every three hours. Outside temperature was measured on the surface of the ground, right above the hibernaculum. Inside temperature was measured in the burrow, approximately on the level of tortoise tail and the thermologer was placed after the final burrowing under the ground of the observed specimen.

During the winter period of 2003-2004 temperature was monitored in a winter refuge of a female *T.h.*– specimen F1, from 26.12.2003 – 8,30am to 12.04.2004 – 15,45pm. During winter 2005-2006 in the burrows of female *T.h.* - F2, from 14.12.2004 – 9am to 18.04.2005 – 12am and male *T.h.* - M, from 12.12.2004 – 18pm until 10.04.2005г. – 9am. Temperature data during winter 2005-2006 is from the burrow of two years old juvenile *T.h.* - J, from 30.11.2005 – 18 pm until 10.04.2006 – 10 am. (Table1).

Specimen	SCL mm.	MW mm.	MH mm.	W g.	Hib. Start	Hib. End	Duration days	Body mass loss	
								g	%
F1	189	135	89	1290	13.11.03	12.04.04	150	50	3.97
F2	186	140	95	1420	19.11.04	18.04.05	150	120	8.45
M	162	130	86	880	21.11.04	10.04.05	140	60	6.80
J	77	64	38	97	20.11.05	10.04.06	140	7	7.20

Table1.Specifics about the specimens, hibernation duration and body mass loss.

Unfortunately due to insufficiency of thermologers, temperatures were not monitored in *T.g.* hibernaculum. As far as no particular differences between *T.g.* and *T.h.* hibernating sites and techniques were observed, most probably the results would be similar.

Prior hibernation period and hibernation sites.

Usually by the end of September – beginning of October, gradually tortoises will start to eat less, bask less, and appear sluggish. Individuals generally bask and feed at or near their burrow entrances (which later might be used for overwintering) only during the warmest hours of the day. In warm sunny days, occasionally courtship behavior can be observed during October. If the weather is not favorable there could be no daily activity at all. Through that period feeding slows down until it stops. Occasionally tortoises can be observed pinching little bit from the weeds around them, few days, or even the same day they go underground.

The final burrowing under the ground in most cases is performed during times of maximum daytime temperature, and when favorable weather is maintained at least for few consecutive days. This will contribute to accumulate enough body energy,
required for excavating the burrow. Some times, after few days under the ground, specimens are observed to come out again, taking off impetuously to completely different spot, where they burrow again. It almost seems like “they know exactly what they are doing”. Others, which burrowed long before the rest of the specimens from the colony, no mater that the

weather conditions remain favorable, don't come out anymore and stay permanently underground. In general there could be up to 30 days period between the first and the last burrowing during the same season.

It is noteworthy to mention the fact that some times during October and November, in a few days periods overnight temperatures fall below zero and the daily temperatures don't exceed 5-6°C. In such short periods, followed by good weather again, tortoises don't seem to bother, even during the subzero temperatures and stay on the surface of the ground. Such event was observed on October the 27th, 2003 when the snow was falling during the night and the temperatures dropped to -1°C. On November the 19th, 2005 when there was snow fall, which resulted in 10cm snow cover (lasted 24 hours) and on the following 20th and 21st the overnight temperatures were falling to -2°C, some specimens remained on the surface, covered with snow (one of the most stressful views during my tortoise experience, but I seemed to be the only one worried). In such periods tortoises seem to become comatose the same way they can be found during the "real" hibernation period. During such periods, most of the observed tortoises spend in shallow depressions (Pic.4), termed pallets and described by (Kazmaier et al. 2001), for one of the North American species – *Gopherus berlandieri*. Similar type of refuges are commonly made and used during the whole active season. When the temperatures increase again, they showed the normal for the season activity pattern, which is a proof of their remarkable sense for the weather changes.

Overwintering refuges within the observed wild population are mostly prepared on south and southeast slopes. Preferable spots seem to be at the foot of the forested slopes, where black and brown soils are mixed with long standing deposit of leaves and other decomposing organic left over (Pic.5), which provides increased heat. It has to be pointed out that a number of individuals were observed to hibernate successfully in completely different spots – bare slopes, slopes with other exposure, burrows prepared in more sandy and clay grounds etc. Burrows in clay grounds can become potentially hypoxic, but turtles are known to be very tolerant of hypoxia (Ultsch, Anderson, 1988).

The size of the burrow prepared from adult tortoise might vary between 20-27cm in length, 12-17cm width and 13-16cm depth and it is closely correlated with the size of the specimen. The hibernation camera is a few centimeters wider than the actual size of the specimen, just enough to allow it to turn around. The soil depth above the carapax is usually not more than 10cm. In comparison with the common Mediterranean species *Agrionemis horsfieldii* (six specimen are living in separate enclosure in the territory of the center), which in September start digging 30-50cm deep tunnels, *T. h.* and *T.g.* seem to remain on the primary depth, in a "den" type of burrow during the whole period of the hibernation (Pic.6).

In order to determine the preferred hibernating spots, some experiments were carried out in the center. In 2005 an area of approximately 4-5sq.m. was prepared by filling with various plants and weeds left over, dry autumn leaves, cut grass and other debris. When it gradually started to rot it was mixed with the soil under and around it. The result was that many tortoises from both kinds prepared their winter burrows in it. Later, during the following summer it became a favorite overnight shelter and hiding place from the midday heat (Pic.7).

Another experiment was with a number of artificial burrows, dug in order to imitate abandoned tortoise or other animal burrows, which are often seen in the natural habitat landscape. No matter that they were often used overnight, none of them were later

used for hibernation. In my opinion burrows, used prior hibernation, are rarely used during the winter. Tortoises seem to prefer "fresh", new made burrows for overwintering.

Tortoises have been observed to hibernate successfully all over the territory of the center. It has to be kept in mind that the whole territory is with favorable south exposure. In fact, the small vineyard in the eastern part of the center was more frequently used for hibernation. Some tortoises dig their burrows among the vine roots.

Hibernation duration

The mean hibernation duration of *T.h.* is 147 days. The longest hibernation period was 157 days (7.11.2004-13.04.2005), the shortest period – 139 days (22.11.2004-10.04.2005). It is interesting to point out, that both are observed during the same winter. The earliest observed date of hibernation start was on October 31st 2003, the latest on December 13th 2005. The earliest and the latest hibernation end dates were April 3rd 2004 and April 29th 2003.

Data about *T. g.* shows mean hibernation duration of 139 days. The longest registered period was 167 days (27.10.2004 – 12.04.2005) and the shortest - 109 days (30.11.2004– 19.03.2005). Again both periods are during the same winter. The earliest date of hibernation start – October 27th 2004, latest – December 13th 2005. The earliest observed date of hibernation end – March 19th 2005, the latest – April 20th 2005.

Most individuals from both species are going into hibernation during the second half of November and during the first 20 days of April are coming out of it. All individuals come out of hibernation in approximately 30 days period. Similar data - two months is the period between the first and the last individual reported for the Desert tortoise - *Gopherus agassizii* in some parts of its range, where it hibernates similar – late November to mid March for about 151 days (Nagy, Medica, 1986).

Often in the center are brought specimens with unknown origin, supposedly from areas with relatively different specific climatic conditions. No difference was observed in their hibernation behavior, like earlier, or later hibernation beginning or ending, different shaped burrows etc.

Body mass loss

The minimum relative body mass loss for *T.h.* of only 1% during 145 days of hibernation, was determined for a two years old neonate. The maximum of 19% was determined for one year old juvenile, during hibernation lasting 153 days. Body mass loss among adults differ between 1.0 – 16.0%. The mean results for *T.h* . are: females – 4.7% (from 15 measurements), males – 6.2% (9), one year old – 12.7% (5), two years old – 6.2% (8), hatchlings – 9.1% (4).

The range of body mass loss results for *T. g.* is 1.0-10.6%. The means are: females – 5.5% (7), males – 4.6% (7), two years old – 11.0% (2).

It is noteworthy that during different hibernation periods, body mass loss of a particular specimen from either of the species might differ up to 8%.

The data suggests that relative body mass loss of hatchlings and juveniles is greater than of adults, perhaps because dehydration is facilitated by their higher surface area per unit body mass. Most probably body mass loss is correlated with body size, rather than age and sex. However further investigation is needed in this direction.

It might be interesting for some keepers to mention that in my refrigerator hibernation experience, in conditions considered optimal– temperature range of 3-6°C and relative humidity 60-80%, for 60 days period, one and two years old juveniles *T.h.* and *T. g.* (Pic.8 c,d) lose about 7-9% of their weight. In the same conditions for the same period of time 5-7 years old *Agrionemis horsfieldii* subadults (Pic.8 a,b), lose 3-4%.

Temperatures

Winters in some regions of Bulgaria are characterized with a lot of snow precipitation and low temperatures. Obviously tortoises are forced to cope with some extreme conditions during hibernation. Temperature range 2-6°C is considered optimal, while temperatures over 8-10°C will often lead to certain complex chemical and biological processes and initiate release into the bloodstream of a chemical called glycogen, which has been stored in the liver and will lead to awakening (Highfield, tortoisetrust). However little is known about the physiological and ecological adaptations for the winter survival of *T. h.* and *T. g.* in the wild, such as freeze avoidance through supercooling, and freeze tolerance.

There is some documentation of freeze tolerance of North American Box turtles: *Terrapene carolina* recovered from freezing in laboratory studies at a body temperature as low as –3.6°C, for up to 3 – 4 days, during which 44-58% of the body water froze. *Terrapene ornate* experience hibernacula temperatures as low as -8°C, up to 54 days (Ultsch, 2006). Hatchlings of some terrestrially hibernating North American species *Chrysemys picta bellii* are known to recover from supercooling to temperatures as low as –10 to -15°C, others like *Kinosternon flavescens* and *Trachemys scripta* supercooled to –6 or below (Costanzo et. al, 2001).

According to my measurements (Table 2), temperatures in *T.h.* hibernaculum range between –5 and 15°C. During the whole hibernation period in 2004-2005, inside the hibernaculum of F2, subzero temperatures were with total duration of 18 days. For 13 days and 6 hours (between 31.01.05- 3.00 and 14.02.05- 9.00) the inside temperature remained below zero, ranging between –4.0 and –0.5°C and average of -3°C. In March another four shorter periods of constant subzero temperatures were detected. They lasted respectively 4 days, 15 hours, 6 hours and 3 hours. On 12th of March 2005 in 12am was recorded the lowest burrow temperature of -5°C and the lowest environmental temperature of –26.5°C for the whole three years period of temperatures monitoring. Despite these periods, F2 came out of hibernation in good shape and showed no difference from the normal behavior pattern. During the following season F2 oviposited twice (Pic.9 a), four eggs each clutch, six from which hatched successfully (Pic.9 b) while other years it developed only one clutch of 3 to 4 eggs.

In comparison with F2, during the same winter the temperatures in the burrow of M have not dropped bellow zero. It is noteworthy to mention that the burrows of these two specimens were only six meters away from each other, excavated in visually the same type of soil and not differing considerably in depth or shape. Obviously there are other factors, generating the significantly bigger temperature variations in the hibernaculum of F2 compared to these of M.

Subzero hibernaculum values were also detected during the winter period 2005-2006 - 30 days in total, with longest periods of constant below zero values of 8 and 7 days during January. The lowest temperature was -4°C, with environmental minimum of -23°C (Table2). In the early spring after this winter 5 adult *T.h.* were found dead in their burrows in

the center. This winter was unusual. In January after a significant rainfall, temperatures dropped rapidly to -20°C, which had disastrous effect to some trees in the whole region – some of hot loving and almost all the fig trees in the Costal area froze to death. After careful examination, it became clear that, due to the heavy rainfall, preceding the temperature drop, the Ground water horizon covered their burrow area. Most probably the result of devastating humidity levels in the particular spot, combined with subzero temperatures simply killed these tortoises. Dead specimens were in very poor condition in regard to deterioration, indicating that deaths have occurred in midwinter (Pic.10). In the spring, after coming out of hibernation, were observed some individuals with symptoms of sight damage, respiratory problems and reluctance to eat. In a month period with favorable weather and provided warm water baths every other day, most of them fully recovered, with an exception of a two years old *T. h.*, which died. However the two years old specimen J, in the burrow of which temperatures were monitored, came out of hibernation in perfect shape. Its refugia was excavated in the roots of a almond tree, which probably provided better drainage of the surface and underground waters. Except the specimens mentioned above, no other tortoises with post hibernation health problems were observed.

The temperature data of both 2004-2005 and 2005-2006 winters, confirms the conclusion made by Bidmon (2001), that subzero temperatures are not as devastating as they are when combined with high levels of water present in the soil.

The winter period of 2003-2004 could be described with no extreme temperatures in the refuge of F1. There are no values below 1 °C in the burrow, and an environmental minimum of -21°C (Table2).

		December			January		February			March			April			December-April		
Winter/ Specimen	**Min**	**Max**	**ean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**ean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
03-04/out	0.5	11.0	2.8	-4.0	12.5	2.2	-21.0	24.5	3.3	-9.5	32.5	8.6	-6.0	30.0	12.9	-21.0	32.5	5.5
03-04/F1	2.5	6.5	4.4	2.5	7.5	4.3	1.0	11.0	4.5	3.0	13.5	7.9	6.5	19.0	10.8	1.0	19.0	6.1
04-05/out	-19.0	16.0	0.0	-17.0	18.0	-0.4	18.5	21.5	-3.9	-26.5	38.5	2.8	-10.5	40.0	8.2	-26.5	40.0	0.4
04-05/F2	1.0	9.5	5.3	-1.5	11.0	4.8	-4.0	9.5	1.3	-5.0	14.0	5.4	3.5	15.5	9.6	-5.0	15.5	4.9
04-05/M	4.0	10.5	6.8	2.0	10.0	6.4	1.0	9.5	4.0	1.0	13.5	6.9	5.0	14.5	9.1	1.0	14.5	6.3
05-06/out	-19.5	23.0	0.8	-23.0	20.0	-6.1	-17.5	30.5	1.5	-8.0	26.5	4.9	4.5	27.0	11.6	-23.0	30.5	1.1
05-06 /J	-3.0	13.5	5.6	-4.0	10.5	-0.1	-3.5	12.5	3.1	-0.5	15.0	6.3	9.0	14.5	12.0	-4.0	15.0	4.3

Table2. Temperature data (°C)

The temperature data in general shows that the hibernation period comprises 44-70 days with temperatures in the range between 2 and 7.5 °C. Approximately the same duration have periods in the range of 8 - 15°C. Considerably less - 6 – 16 days the temperature is in the range of 0 – 1.5°C. Subzero temperatures, not falling below -5°C, for periods up to 30 days in total, can be in most cases tolerated without further problems. Whether hibernating *T. h.* and *T. g.* use either supercooling or freeze tolerance has yet to be established, but either could be survival mechanism during exceptionally cold winters with lack of snow cover, or during periods in fall and spring when tortoises are threatened with sudden cold snaps.

Occasionally winter activity was observed during warmer days in winter months. This activity can be described as exposing some parts of the shell, the head and the limbs outside the burrow. Some times it lasts only a few hours, or could be seen for a few days. As the temperatures drop again the tortoise retreats permanently back inside the burrow. This event occurs most often in March, but once a male *T. g.* was observed to come out completely or in the described manner, for few hours in three consecutive days period, during

the first half of January. Similar outside activity during winter months in days when temperatures exceed 21°C, and usually during periods in which such temperatures are reached on two or more consecutive days, is reported for one of the North American species – *Gopherus polyphemus* (Douglas, Layne, 1978).

Waking up

In my opinion the period close to the hibernation end is the most dangerous for the tortoises life and health. Short periods with high ambient temperatures, followed by considerable decrease, especially during March are not exceptional in the region. During such periods, some tortoises may be mislead to come out of their burrows and might easily become victims either of a predator attack, or freeze to death. Due to their poor post hibernation physiological condition, it might be impossible to find the way back to the burrow (even if it is not more than one meter away), to excavate a new one or make it to another refuge.

In the early spring of 2003, in the middle of March, there was considerable increase in temperatures, which lasted for a week and than followed by very cold snap and snow fall, creating snow cover of about 10-15cm. From March 28th in a week period seven dead adults *T. h.* were found in the field. Most of them were not more than one meter away from their burrows. Three of them seemed untouched, but four had parts of the limbs and head missing, which leads to the conclusion that scavenging may have been involved. Some authors (Metcalf, Metcalf, 1979) suggest that perhaps some carnivores become used to scavenging dead tortoises during winters. Badger (*Meles meles*), jackal (*Canis aureus*), fox (*Vulpes vulpes*), are most common predators in the region and along with the present wild boars (*Sus scrofa*), might be often involved in tortoise mortality.

In the beginning of April, when the temperature in the hibernaculum stays around 10°C (Table3) the first movements are observed. The tortoise will stay half buried and bask in burrow entrance during the warmest part of the day (Pic.11). It will hide back overnight. Most often in a week period it will permanently come out and begin to feed. First activity, among male individuals is often associated with courtship behavior, rather than feeding, if a female is present in their field of vision.

Specimen	Min t°C	Max t°C	Mean t°C
F1	7	14.5	11.0
F2	8	15.5	11.6
M	5	14.5	9.0
J	9	14.5	12.0

Table3.Results from the temperature measurements during the last ten days of the hibernation periods.

Conclusion

The observations show that in the studied area both species of land tortoises overwinter in 15 –20 cm. deep burrows. Hibernation period is with mean duration 147 days for T.h. and 139 days for *T.g.* during which *T.h.* loose 1.0-16.0% and T.g. 1.0 – 10.0% of their body mass. Mean burrow temperatures are in the range 4.3 – 6.1°C, while the average

environmental ones are in the range 0.4 – 5.5°C. Considerable tolerance to subzero temperatures up to 13 days intervals with constant burrow temperatures bellow zero was observed. Slight activity during warm winter days, especially by the end of the hibernation period was observed.

Combination of observations in the wild and in enclosure, which is a part of the natural habitat is giving great opportunities of studying *T.h.* and *T.g.* biology and ecology in Bulgaria. The results might contribute to captive breeding and especially to all kinds of programs of tortoises research, preservation and reintroduction. Further studies have to be supplied with more technical equipment in order to obtain greater amount of relative data.

A lot still has to be done to further investigation of *T.h.* and *T.g.* biology and ecology in Bulgaria. The conditions in Eastern Balkan range where there still exist stable populations of both species are favorable for various studies. Main long-term guarantees for the future of *T.h.* and *T.g* in the region are their highest protection by the Bulgarian legislation, the existence of 42.3ha protected area Iracly (Pic.12), and the prospective for including considerably larger part of the whole region in the directive of the EU - Natura 2000 for establishing protected areas.

Acknowledgments

I would like to thank Mr. Hans-Jurgen Bidmon and Mr. James Buskirk for their support and for making me believe that what I do deserve to be done.

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