Amphibian & Reptile Conservation 5(1):98-104.

Notes on reproduction and conservation of *Testudo graeca ibera* Pallas 1814 (Reptilia: Testudinidae) in Zagros, western Iran

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***Abstract.*—During longtime fieldwork in the Zagros Mountains, we studied tortoises of the western Iranian plateau. In this paper we focus on *Testudo graeca ibera*. We present the first information about mating behavior, timing of mating, egg shape, and hatching of this subspecies. In general, our results on reproduction in *T. g. ibera* are different from previous reports. Additionally, we report anomalous reproductive behavior in *T. g. ibera*.**

Key words. *Testudo graeca ibera*, mating, eggs, Zagros Mountains, Iran

Citation: Sadeghi R, Torki F. 2012. Notes on reproduction and conservation of *Testudo graeca ibera* Pallas 1814 (Reptilia: Testudinidae) in Zagros, western Iran. *Amphibian & Reptile Conservation* 5(1):98-104(e45).

Introduction

Testudo graeca includes two subspecies on the Iranian plateau: *T. g. ibera*, distributed in western Iran, and *T. g. zarudnyi*, distributed on the eastern Iranian Plateau (Anderson 1979; Torki 2010). We realize that the nomenclature of southwest Asian tortoises is in flux, as there seems not to be a recognized consensus as yet; here we use the conventional taxonomy of the older literature.

Jasser-Hager and Winter (2007) reported results regarding incubation in tortoises, including a Greek population of *T. g. ibera*. Information on reproduction of this species in Iran is very rare (Pritchard 1966). In this paper we focus on husbandry of *T. g. ibera* on the western slope of the central Zagros Mountains, western Iranian plateau.

Materials and methods

To study reproduction in *T. g. ibera*, we worked in the natural habitat from 2002-2010 in this region. After egg deposition by one female specimen under natural conditions, we transferred all eggs into our lab. Thus, our results are based on our observations under natural and laboratory conditions.

Results and discussion

Mating activity time

Mating of *T. g. ibera* in the Zagros population occurred from early spring to late summer. Pritchard (1966) observed copulation in the Zagros population of *T. graeca* in late August and early September, whereas Nikolsky (1915) recorded mating in April and May in the Transcaucasian area. Mating behavior for *T. g. ibera* in Greece was observed during two time periods: March-April and late autumn (Jasser-Hager and Winter 2007). In contrast, we did not see any tortoises in natural habitats in the central Zagros Mountains during mid- and late autumn. Temperatures during this time are low and most species of herpetofauna are going into hibernation (e.g., Torki 2007a, b). Thus, there is a difference in timing of mating and courtship between the Zagros and Greek populations of *T. g. ibera*.

Most mating occurred in shady places, such as under trees or other vegetation, large stones, etc. Jasser-Hager and Winter (2007) reported maximum mating of *T. g. ibera* in Greece during morning hours. Maximum mating in the Zagros populations mostly occurred near mid-day, from 11:00 to 15:00. Mating in *T. g. ibera* usually occurred after feeding.

Mating behavior

Based on our observations (from 2002-2010) of 35 pairs (female: 35; male: 35) of *T. graeca*, we classified courtship behavior into four phases as follows:

1. *Aggressive phase*: aggression is the first step in courtship; in this step, the male attacks the rear of female's carapace and females attempt to escape during this phase. In our observations, this behavior occurred repeatedly several times. The duration of this phase differed among specimens; in general, duration was between 10 and 50 minutes. Biting occurred during this phase; the male bit the limbs, neck, or head of the female. Duration of the aggressive phase was related to (a) agility of male, as agile males were successful with a decreased aggres-

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sive phase; and (b) place of mating, as minimum duration occurred in uneven terrain (such as in mountains) and maximum duration occurred in flatter terrain (such as agricultural land).

2. *Submission phase*: after the aggressive phase, the female remains in one place and the male can start the mating step. The duration of this phase was related to the terrain; in even places, the duration of this step was less than in uneven terrain, as the male has to rest for a few minutes on rougher ground.

3. *Copulation phase*: copulation occurred between 5 and 21 times for each pair. The rate of coupling was related to duration of the first phase, which may reduce energy of the male for mating. The duration of each copulation was between approximately 10 and 70 seconds. The duration of this time was in inverse relationship to the duration of the aggressive phase; if the duration of the aggressive phase was short, then duration of the mating step was longer (because males have maximal energy for mating); in contrast, if duration of the aggressive step phase was longer, then duration of the mating step was short (presumably because males were tired due to running and did not have sufficient energy for as many copulations).

4. *Resting*: the resting step occurred in most specimens, because both sexes, especially males, expend much energy for successful mating. After mating, the male and female rest close together. The duration of the resting time is related to duration of mating; duration of the resting step was minimal when mating occurred in the morning and maximal when mating occurred in the afternoon, possibly because individuals must sleep, or perhaps because afternoon temperatures are higher.

Douglas et al. (1994) reported courtship behavior in the Desert tortoise (*Gopherus agassizii*) and described several phases for courtship behavior: *trailing*, *biting*, *rear ram*, *soliciting*, *mount*. These phases occurred for *T. graeca* and the aggressive phase in this study is the same as the *trailing*, *biting*, *rear ram*, and *soliciting* phases of Douglas et al. (1994). Douglas et al. (1994) reported the final step of courtship behavior as follows: mount by male while female does head-swing. Head-swing of female occurred in *T. graeca* during the copulation phase. In this study we reported a resting phase; also, this phase is outside of mating behavior, but we cite this phase in mating (or courtship) behavior because, the resting phase occurred as the result of all previous phases of this study. We see this phase in other reptiles, such as *Lacerta media* and *Laudakia nupta*.

Anomalous mating behavior

Both in captivity and in the natural habitat, we saw several unsuccessful mating or courtship attempts in *T. graeca*: (a) Unsuccessful mating: males sometimes directed mating behavior toward an inappropriate part of the fe-

Figure 1. Mating of *Testudo graeca ibera*, Zagros populations. *Photo by Farhang Torki.*

male body, such as dorsolateral or anterior of females. (b) Anomaly: male specimen attempted to mate with other animals (not females of *T. graeca*), for example, under captive conditions, a male *T. graeca* attacked and repeatedly showed mating behaviors toward *Mauremys caspica*. (c) Male-male courtship and mating: under captive condition some male specimens showed courtship and mating behaviors with other males.

In general, mating anomalies occurred only in male specimens. Therefore, in this study we report anomalous mating behavior in *T. graeca* for the reason that males of *T. graeca* showed courtship and mating behavior toward other animals, materials, etc. (e.g., inappropriate parts of female body). We observed maximum anomalous behavior under captive conditions.

Hatching

During our fieldwork in the Zagros Mountains, we observed one female during egg laying. Egg laying occurred on 13 May 2010 at 1630 h in even terrain. The female excavated a nest cavity during less than 10 minutes. She laid four eggs (Fig. 3) during ten minutes, and covered the eggs in five minutes. The nest cavity is shown schematically in Fig. 4. Egg shape was oval (Fig. 3) as is true for most tortoises, such as other subspecies of *Testudo graeca* and *Indotestudo forstenii* (Kruger 2007; Jasser-Hager and Winter 2007; Struijk 2009). Hiley and Loumbourdis (1988) reported egg size, shape, and weight of *Testudo graeca* from northern Greece (Table 2). Our comparison with this population showed that eggs of the Iranian *T. graeca* population have greater length, width, and especially, mass. Jasser-Hager and Winter (2007) reported that eggs average 25 g (range 14-33 g) for a northern Greek population. Both reports about egg weight of *T. graeca ibera* in Greece record less weight than in the Zagros population.

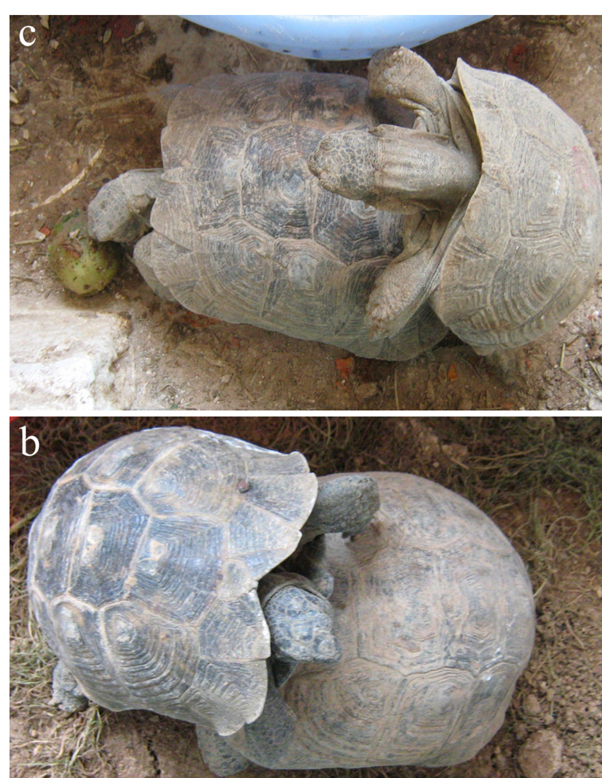

Figure 2. Anomalous mating behavior of *Testudo graeca ibera*. (a) Mating behavior of *T. g. ibera* with *Mauremys caspica*; (b) Mating behavior of *T. g. ibera* with anterior body of other specimen; (c) Mating behavior of male *T. g. ibera* with another male. *Photos by Farhang Torki.*

We transferred eggs from natural habitat to laboratory conditions. We preserved one egg and provided a nest cavity for the other eggs. We inserted the three eggs into the cavity and covered them with soil. No further care of the eggs was provided. We only covered eggs with soil (similar to natural conditions; see Fig. 4). All environmental conditions of the laboratory were similar to those of the natural habitat. We did not touch the eggs, because handling stops egg development, as our experiments with other reptiles had confirmed. Therefore, we transferred all eggs using paper or wood. Duration of incubation of eggs varied from 72 to 76 days (Tab. 1; Fig. 5). In comparison, Jasser-Hager and Winter (2007) reported the incubation period for *Testudo graeca* in Greece as between 54 and 89 days (average 62 days), for *T. hermanni* boettgeri between 49 and 72 days (average 56 days), and for *T. horsfieldii* between 54 and 102 days (average 68 days).

We preserved one egg the first day and measured thickness of the shell. We recorded shell thickness for other eggs after hatching. Our results show that the egg shell has a maximum thickness during the first day after laying (middle of egg: 0.25 mm) and has minimum thickness at hatching (middle of other eggs: 0.12, 0.10, and 0.07 mm). Decreased shell thickness is probably important for easy hatching and/or drawing essential elements from the shell. After egg-laying, the egg shell was soft and flexible; this is in contrast to the following days, especially at hatching. During this time, egg shells were stiff and breakable.

Juvenile specimens have a circular shape, with carapace length and width and plastron length and width being similar (Table 1). This is true for other tortoises, es-

Table 1. Measurements and information on four eggs of *Testudo graeca ibera* after egg-laying (13 May 2010) and after hatching (24-27 July 2010). The third egg did not hatch.

Measurements	1st egg	2nd egg	3rd egg	4th egg
Length (mm)	45.9	45.1	44.9	43.4
Width (mm)	33.8	34.4	34.9	31.6
Weight (g)	28	28	30	26
Hatching date	24 Jul 10	25 Jul 10	-	27 Jul 10
Time of day	sunset	afternoon	-	sunset
Carapace length	40.8	35.8	-	35.7
Carapace width	37.1	35.2	-	34.8
Plastron length	34.9	32.1	-	31.6
Plastron width	33.2	31.1	-	30.7

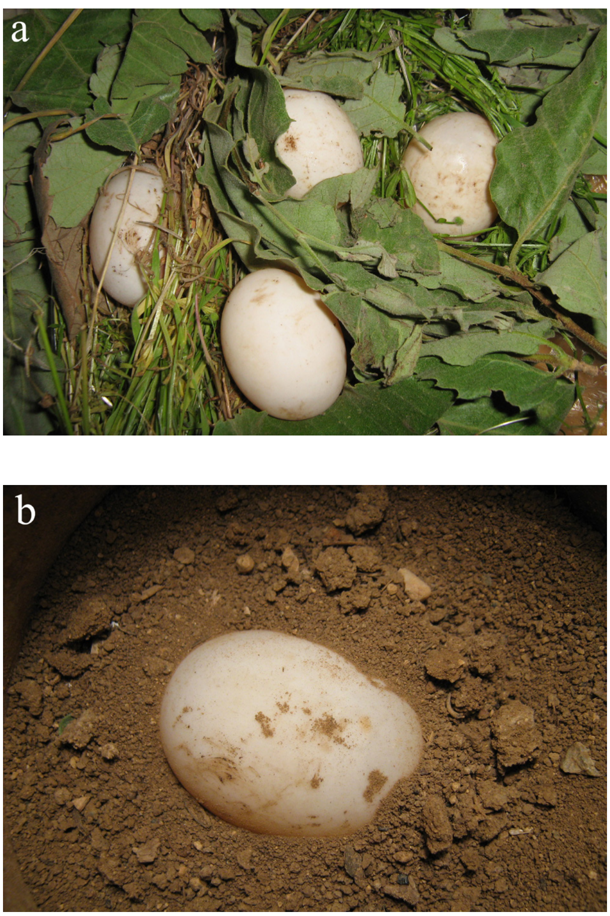

Figure 3. (a) Four eggs of *Testudo graeca ibera*, after oviposition, (b) One egg of *T. g. ibera*, under captive conditions. *Photos by Farhang Torki.*

pecially for other subspecies of *Testudo graeca* (Kruger 2007; Jasser-Hager and Winter 2007). Plastrons of hatchling specimens were covered by yolk sacs. After hatching, the yolk sac was distinct from plastron of juveniles (Fig. 6). The bodies of juveniles during the first days after hatching are soft. The plastron and especially the carapace of juveniles harden after more than one month.

Conservation

Several factors pose threats to *T. graeca* in the Zagros Mountains; we classified these factors as follows.

Natural threats

(a) Drought indirectly and directly affected survival of *T. graeca*, especially juvenile specimens. (1) *Directly*: physical activity of *T. graeca* was reduced during high temperature, especially during mid-day (especially in summer). Temperature during recent years has increased (IMO). Therefore, daily biological activity of *T. graeca* was reduced. This is true for juvenile specimens. Juvenile specimens must obtain more food. Hence, during high temperatures, physical activity of juvenile specimens is strongly reduced. Therefore, some juvenile specimens are not successful in obtaining food and survival of juveniles is endangered due to drought. (2) *Indirectly*: Drought occurred during several recent years. Density and longevity of vegetation during droughts is reduced (our observation). Therefore, the rate of food production is reduced during the warm season (summer). Juvenile specimens could not obtain enough food. Some adults and juvenile specimens could not store enough fat for hibernation periods; this occurred due to loss of food in natural habitats.

(b) *Predators*: based on our observations and life history of *T. graeca*, we divided predators of *T. graeca* into three types, as follows: (a) egg predators, including some snakes (*Eryx*) and scincid lizards; (b) predators of young, including birds (crows, ravens, etc.), and mammals (some carnivores; Fig. 7c); (c) and predators of adult *T. graeca*, such as birds (eagles) and mammals (some carnivores). Eagles grab adults and fly to high altitudes (more than

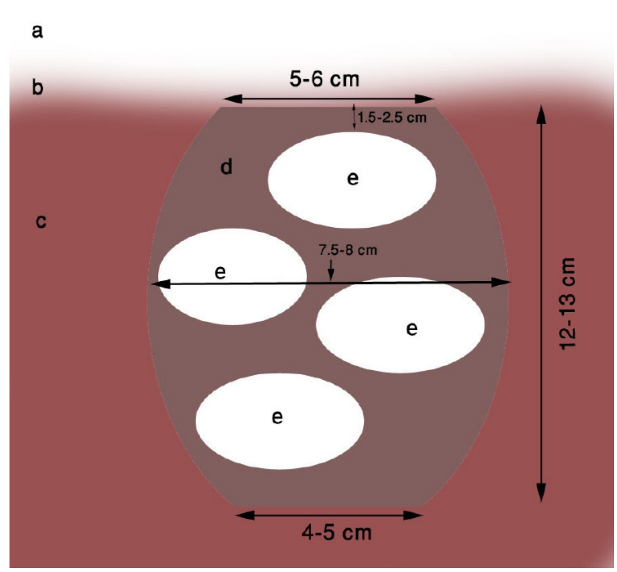

Figure 4. Schematic of egg-site in *Testudo graeca ibera*, Zagros Mountains. Abbreviation: a: air; b: surface; c: soil; d: hollow egg-site; e: eggs.

Table 2. Egg size and weight comparison of northern *Testudo graeca ibera* between two populations: western Iran (Zagros) and northern Greece.

	Hiley and Loumbourdis 1988	Present study	Population fitness: Iran and Greece
Year assessment	1985-1986	2010	
Location	northern Greece	western Iran (Zagros)	
Weight (g)	17.5 ± 2.0	28 ± 0.80	1.6
Length (mm)	35.4 ± 2.0	44.8 ± 0.50	1.2
Width (mm)	29.2 ± 1.9	33.68 ± 0.7	1.1
Shape	1.22 ± 0.1	1.32 ± 0.02	1.1

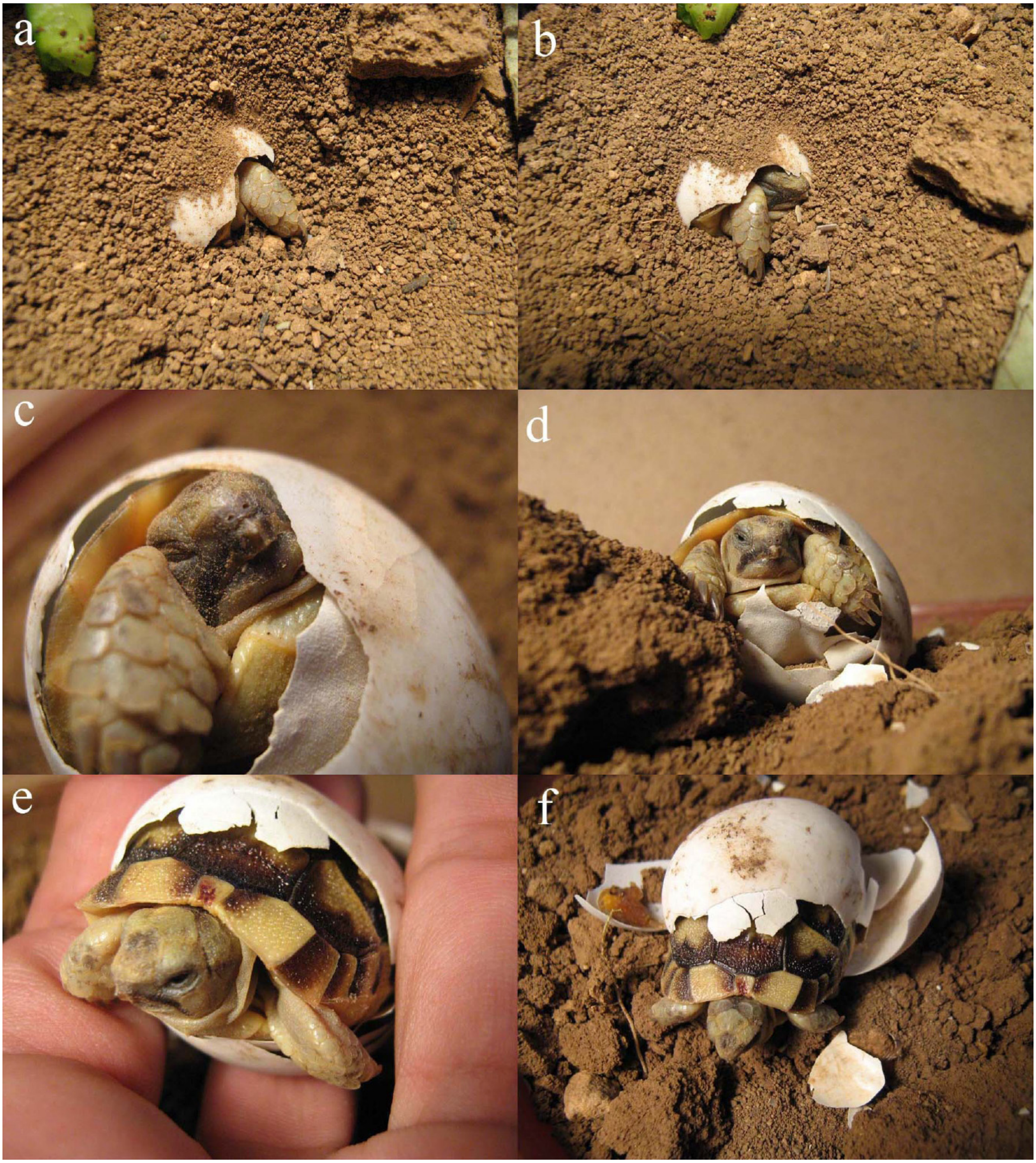

Figure 5. Hatching of *Testudo graeca ibera*. a-f: arrangement of broken egg shell (during hatching). *Photos by Farhang Torki.*

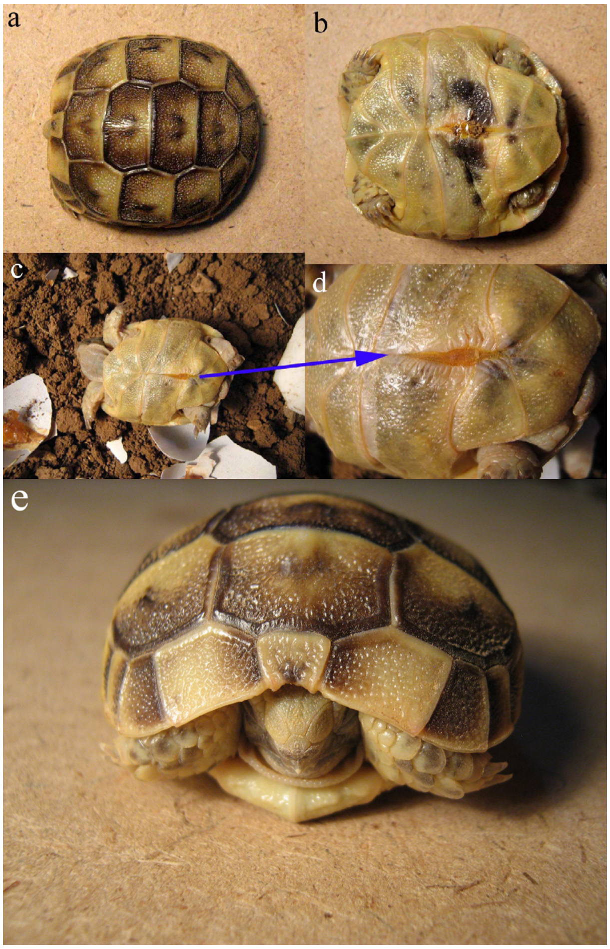

Figure 6. Hatchling specimens of *Testudo graeca ibera* under captive conditions. (a-b) carapace and plastron of juvenile specimens (after one week); (c-d) plastron of juvenile specimens (one day old), showing narrow yolk sac; (e) juvenile specimens after one month. *Photos by Farhang Torki.*

100 m) and release them. Due to this action, the shell of T. *graeca* is broken and eagles easily eat adults. Several predators such as birds, dogs, and wolves eat *T. graeca*; this occurs due to loss of food in the natural habitat.

(c) *Ectoparasites*: the main ectoparasites of *T. graeca* in this region are several taxa of Acari (*Acarina*, ticks; Fig. 7d). Ticks attach to carapace, plastron, and limbs. We see most ticks on soft parts, such as joints of scutes or limbs.

Human threats

(a) *Habitat destruction*: habitat destruction occurs due to several important factors. (1) *Ashayer* (nomadic herders): the lifestyle of some peoples in the Zagros Mountains is similar to that of other herders elsewhere; they do not build homes, but live together in nature. Ashayer, for migratory periods of their lives, only use natural material; for example: they cut trees for fire. Ashayer and their animals, such as goats, are in competition with most wild animals, such as *T. graeca*, for food resources. (2) *Building roads*: many animals are killed on roads during day and night. We could see several corpses of *T. graeca* on roads or near roads (Fig. 7b). All specimens were killed due to various vehicles. Based on our observation on one road in northern Lorestan Province, more than 20 corpses of *T. graeca* were seen on roads or near roads; all specimens were killed by vehicles. (3) *Recreation*: some areas are good places for recreation. People play a negative role during recreation, for example, some people bring juvenile specimens of *T. graeca* home and some people release their trash and other waste into the environment. Some wastes, such as oils and grease, are released into the natural habitat of *T. graeca*. These materials have a negative role in the survival and life of *T. graeca*, especially juveniles.

(b) *People's beliefs* (outlandish stories): this factor occurred during past years in the Zagros Mountains, but we could not see or hear any reports about this threat in recent years. Mostly people killed turtles for some purposes, such as to make love potions, increased milk production of cows, etc. These are ancestral beliefs, and today no one pays attention to these outlandish stories.

(c) *Agriculture*: (1) destruction of eggs and juvenile specimens by agricultural elements during planting and harvest; (2) killing tortoises by plough (agriculture elements); (3) chemical materials; these are important threats to most animals, because most farmers use chemical materials for their farmland. In some cases some farmers release the runoff of chemical materials out of their farmland into the habitat. Poison is distributed to nature and *T. graeca* (and other animals) are affected directly or indirectly by these poisons. (d) Fire (Fig. 7a): During recent years, human-caused fire has occurred in the central Zagros Mountains. Due to fire, the habitat of *T. graeca* and other animals is damaged. In some cases we could see corpses of some animals such as *T. graeca*, killed due to fire. Most fires occur after harvest; this time is synchronous with hatching of most reptiles, such as *T. graeca*. In addition, due to fire, juvenile reptiles cannot obtain an abundance of necessary fat. Therefore, these specimens cannot live through their hibernation period (and die in mid-hibernation).

Acknowledgments.—This study was supported by Islamic Azad University, Boroujerd Branch, Iran. We wish to thank S. C. Anderson (USA) for editing our manuscript.

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Figure 7. Natural (a, b) and human (b, c) threat factors in *Testudo graeca ibera*. (a) Fire: human-caused fire in natural habitat of *T. g. ibera* and agriculture land; (b) Building roads: one specimen killed on road by cars; (c) Predators: one specimen killed by birds; (d) Exoparasites: one species of Acari on plastron of *T. g. ibera*. *Photos by Farhang Torki.*

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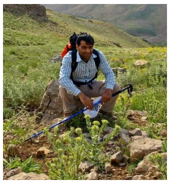
Received: 25 October 2011
Accepted: 18 December 2011
Published: 21 April 2012



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