Volume 27 (July 2017), 245-251

Herpetological Journal

FULL PAPER

Published by the British Herpetological Society

Food habits, habitat use and density of *Emys orbicularis persica* from Jelilabad, Azerbaijan

Luca Luiselli

Department of Applied & Environmental Biology, Rivers State University of Science & Technology, P.M.B 5080, Port Harcourt, Rivers State, Nigeria

IDECC – Institute for Development, Ecology, Conservation and Cooperation, via G. Tomasi di Lampedusa 33, I-00144 Rome, Italy.

Emys orbicularis persica is one of the ecologically least known subspecies of the widespread European pond turtle. Populations of this subspecies were studied in springtime at two extended wetlands of Azerbaijan, and data on density, habitat use, and food habits were collected. These turtles exhibited a mono-peaked diel activity pattern, with peaks during midday hours. Highest mean estimated densities were found in reed-bed habitat (9.51 individuals × ha-1) and in the open water habitat (lake) (9.12 individuals × ha-1), with much lower values in seasonally inundated grasslands (6.0 individuals × ha-1) and no turtles being found in temporary ponds. Density of reeds did not influence the selection of micro-habitat by turtles. Sex-ratio was even, and females attained larger size than males. Diet was carnivorous and relatively specialised, with large larvae of aquatic beetles (*Hydrophilus piceus*) accounting for by far the main prey item. In this regard, the food habits of the Azerbaijan turtles appeared more specialised than those of other *E. orbicularis* populations from elsewhere.

Key words: Azerbaijan, diet, habitat use, natural history, population density, turtle

INTRODUCTION

The European pond turtle *(Emys orbicularis)* is widespread in Azerbaijan, where it may be found in almost all freshwater bodies excluding high mountain regions (IUCN, 2016). In this country, the turtle subspecies is *E. orbicularis persica* Eichwald 1831, which has a wide distribution in Northern Iran to Turkmenistan, Armenia, Azerbaijan, Georgia, Iran, and Dagestan, and is one of the least known subspecies of *E. orbicularis* in terms of ecology and natural history (Lenk et al., 1999; Ananjeva et al., 2006; Arakelyan et al., 2008, 2011; Rastegar-Pouyani et al., 2008). Only fragmentary notes presented in non-peer-reviewed literature are available for Azerbaijan populations of *E. orbicularis* (e.g., Scott Wilson Ltd., 2007; AzerRoadServices, 2012). In addition, *E. o. persica* was proposed for inclusion in the draft Red Book of Azerbaijan (Azerbaijan National Academy of Sciences, 2004). However, the scarce knowledge on the ecology of these turtles may impede a good management programme for the species at the country scale, and therefore robust field data on Azerbaijan populations are needed (Azerbaijan National Academy of Sciences, 2004).

In this paper, the results of a short-term field study on the general ecology of *E. o. persica* populations in Azerbaijan are presented, with emphasis on analysing density, habitat use, body size and food habits. These results are important because they represent the first relatively comprehensive field data on this little known subspecies of *E. orbicularis*.

MATERIALS AND METHODS

Study areas

The study was carried out at two almost adjacent wetlands situated in the surroundings of Jelilabad (Akchala and Mahnudchala wetlands), along the drainage of the Taxta River (Azerbaijan) (Fig. 1). Overall, these wetlands are fed from a number of sources at spatially variable point locations (Scott Wilson Ltd., 2007). Mahnudchala wetland is predominantly sourced from the Azizbeyov Canal irrigation system, whilst Akchala wetland is mainly sourced by the Mugan Canal (Scott Wilson Ltd., 2007). Highest water salinity was observed in Akchala wetland (Scott Wilson Ltd., 2007), where the main habitat type consisted of reed-bed vegetation (*Phragmites australis*, *P. communis*, *Scirpus lacustris* and *Typha angustifolia*). Akchala wetland was characterised by scarce open water availability and low water depth (about 20-50 cm), with very dense reed vegetation (Fig. 2(a) and 2 (b)). Total surface of this wetland was 15,000 ha. At Mahmudchala wetland, open water habitat type was widespread and included both drainage channels and a lake (Fig. 2(c) and (d)). The vegetation at this site was similar to that at Akchala, but with less vegetation density and more flowing water. Overall surface of this wetland was 8,000 ha.

The observed freshwater fauna was similar in the two study sites, with several fish species being observed (*Esox lucius*, *Rutilus rutilus*, *Nemachilus angorae lenkoranensis* and *Tinca tinca*), as well grass snakes (*Natrix natrix*), frogs (*Pelophylax ridibundus*) and very abundant populations

Correspondence: Luca Luiselli (l.luiselli@ideccngo.org, and lucamlu@tin.it)

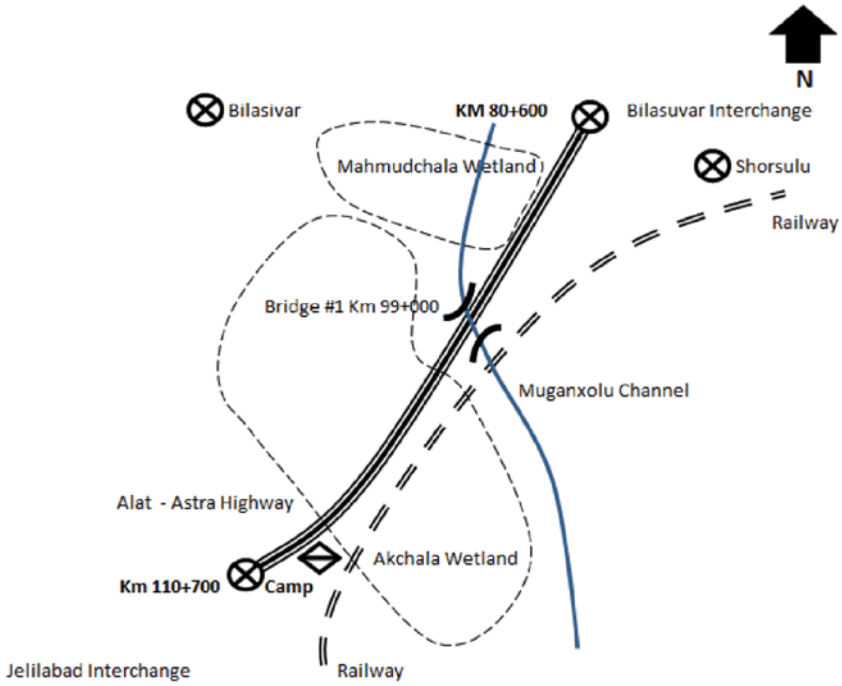

Figure 1. Map of the Jelilabad area in Azerbaijan, showing the location of the two studied wetlands.

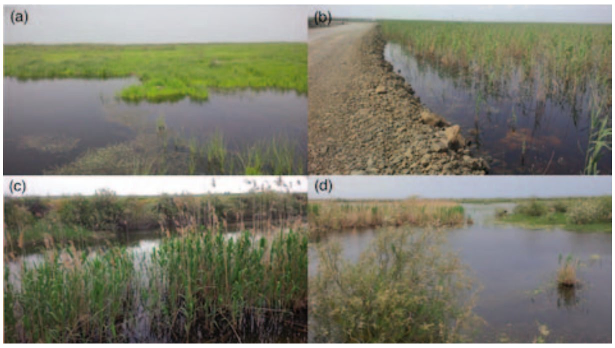

Figure 2. Habitat types at the study areas: Akchala wetland (figures (a) and (b)) and Mahmudchala wetland ((c) and (d)). All photos: Luca Luiselli

Figure 3. Phenotypic variability of *E. o. persica* at the study area. All these turtles were captured within a 100 m transect at Akchala wetland. Microhabitat of capture is also shown. All photos: Luca Luiselli

of large Dytiscidae (*Dytiscus* and *Cybister* spp.) and *Hydrophilus piceus* beetles. The study species (*E. o. persica*) was the only freshwater turtle found at the study area; *Mauremys caspica* was observed in a drainage channel about 10 km north of Jelilabad (Tom Kirschey, personal communication) but apparently does not occur at the two studied wetlands. Turtle individuals exhibited a remarkable phenotype variability at the study area, with dominant coloration ranging from black to light brown to yellowish (Fig. 3).

Field protocol

The field study was performed during springtime from 25 April to 7 May 2016. At each of the study wetlands, both line transects and random exploration surveys were carried out by slowly walking along the banks of the wetland, with several transects being walked in the water. There were good visibility conditions (transparent water) in most parts of the wetlands, thus turtle sighting was not difficult. When a given turtle individual was observed, its behaviour, date and hour of observation, and precise site of observation were recorded.

In order to analyse habitat preferences of turtles, we categorised available habitat at two spatial scales: macro-habitat (hereby simply defined as habitat) and micro-habitat.

Macro-habitat types were categorised as follows: 1) Reed-bed habitat; 2) Open water habitat (channel); 3) Open water habitat (lake); (4) Seasonally inundated grasslands; 5) Ponds.

Habitat 1) was characterised by extended areas of *P. australis, P. communis*, *S. lacustris* and *T. angustifolia*, occupying over 70% of the area surrounding a given transect (Figure 2a and 2b). Water depth was generally

20-50 cm, and there were very few emerged trunks where turtles can bask.

Habitats 2) and 3) were similar to each other, in that about 20-30% of the area surrounding a given transect was covered by reeds (same species as above). Water depth was 20-100 cm. The difference between these habitats was that the former was a drainage channel (Fig. 2c) and the latter was a lake (Fig. 2d). In these two habitat types, turtles had plenty of basking sites, mostly situated along the banks of the channel or of the lake.

Habitat 4) consisted of small (often less than 25 m^2 surface), scarcely interconnected, temporary ponds with muddy soil and grassy vegetation along the banks, that are regularly used by cattle and other domestic animals. These ponds were situated in the surroundings of Jelilabad along the Alat-Astara Highway, i.e. at the peripheral side of the Akchala wetland.

Habitat 5) was characterised by extended grasslands (with *Halocnemum strobilaceum*, *Halostachys caspica*, *Festuca* spp., *Polygonum hydropiper*, *P. amphibium*, *Eleocharis meridionalis*, and *Sasola crassa*) that are dry from July to November. During the flooded period, water depth is about 5-15 cm. Soil and water salinity were the highest among all habitat types surveyed in the present study.

Microhabitat types of the site of each turtle were expressed in terms of percent of water body invaded by reed-bed vegetation, with four categories: (1) = 0-20%, (2) = 21-40%, (3) = 41-60%, (4) = > 61%. For each place, these percentages were defined by eye.

Line transects were walked slowly by maintaining constant velocity (7.5 minutes per 100 m on average, range = 7-9 minutes). For each transect, the GPS coordinates of the extremes were recorded. Turtles were not disturbed during the line transects in order to minimise the risk that other individuals escaped before being noticed. Otherwise, turtles were captured by hand or hand-net during the random surveys in order to process them for morphometric measurements and for any food items (see below).

All captured turtles were sexed (according to the presence/absence of male secondary sexual characters, i.e. tail shape, concave plastron; Auer and Taskavak, 2004), and measured for over-the-curve carapace length, over-the-curve carapace width, plastron length and plastron width. When the turtles were too small for sexing, they were recorded as juveniles (Auer and Taskavak, 2004). They were also carried to the laboratory in Jelilabad Tera Camp, and individually caged till defaecation occurred. Faeces were then separately placed into alcohol for later dissection and examination under binocular microscope. This methodology allows easy identification of food items eaten by turtles in the wild (Luiselli and Amori, 2016). A few direct observations of foraging free-ranging turtles were also made.

Table 1. Estimated population density of turtles in the reed-bed and open habitat types, calculated from line transects and using DISTANCE 6.2 software. Symbols: AIC = Akaike's Information Criterion- an index of the relative fit of competing statistical models. The lower the AIC, the more parsimonious the model (other things being equal); Symbols and abbreviations: T = transect number; MOD = mean distance of turtles from observer (in m); SD_{MOD}= Standard deviation of MOD (in m); D = estimated density (number of individuals per ha); D_{LCI} = Density (Lower confidence interval); D_{UCI} = Density (Upper confidence interval); D_{CV} = Density (coefficient of variation); K = number of parameters of the model; ESW = effective strip width; EDR = effective detection radius.

T	Length (m)	No of turtles observed	MOD	SD_{MOD}	D	D_{LCI}	D_{UCI}	D_{CV}	ESW/ EDR	AIC	K
					Reed-bed habitat						
1	800	7	3	1.41	8.751	3.63	21.098	0.372	LCI5	24.53	1
2	300	4	4.1	1.06	12.123	2.997	49.036	0.461	5.5	15.64	1
3	500	4	1.75	2.06	10.001	2.994	33.408	0.393	4	13.09	1
4	400	3	2.67	2.08	10.001	1.605	62.315	0.445	5	11.66	1
5	500	5	3.6	1.82	6.668	1.717	25.899	0.447	6	16.33	1
					Open water habitat channel						
6	500	7	4.86	3.01	7.435	3.067	18.027	0.374	9.41	34.22	1
7	500	5	5.8	5.26	4.167	1.674	10.375	0.338	12	26.85	1
8	500	3	5.33	7.5	2.143	0.393	11.697	0.41	14	17.83	1
					Open water habitat lake						
9	500	11	4.27	6.83	16.929	8.929	32.097	0.289	6.5	59.13	2
10	500	6	6.67	5.99	4.395	1.97	9.803	0.32	13.65	35.14	1
11	500	6	4.67	4.27	6.042	2.445	14.931	0.363	9.93	30.72	1
					Ponds						
12	350	0	0	0	0	0	0	0	0	0	0
13	450	0	0	0	0	0	0	0	0	0	0
					Seasonally inundated grassland						
14	400	1	0	12.5	0	0	0	0	0	0	0
15	300	3	0	5.556	0	0	0	0	0	0	0
16	350	0	0	0	0	0	0	0	0	0	0

Statistical analyses

Diel activity patterns were determined by assigning each turtle sighting to its defined time interval, with two-hour intervals from 0700 to 1900 hours (Baku standard time). Differences in turtle frequency of observations among different time intervals were analysed using an observed-versus-expected χ^2 test.

In each habitat type, estimated density of turtles (number of individuals × ha^{-1}) was calculated by 'distance sampling analysis', using DISTANCE 6.2 (Buckland et al., 2001; Thomas et al., 2007), a dedicated software utilised with free-ranging animal populations (e.g., see Katsanevakis, 2006). DISTANCE produces a detection function g(x) describing the probability of detecting an object (an individual turtle in our study case) located at distance x from the line transect under survey. For each transect, the following were also calculated (i) the 95% confidence intervals of the density estimate, (ii) the coefficient of variation of the density estimate, (iii) the model fitting by using an Akaike Information Criterion (AIC) score.

Morphometric differences between sexes were analysed by Student t-tests, and linear relationships between different morphometric variables by Pearson's correlation coefficient. All parametric tests were used only after having verified the normality and homoscedasticity of the used variables by Kolmogorov-Smirnov test. Even/uneven sex-ratio and intersexual differences in frequency of consumption of food items were analysed by observed versus expected χ^2 test, with P-values generated after 9999 Monte Carlo permutations. In the text, means are indicated with ± 1 Standard Deviation (S.D.).

RESULTS

Diel activity patterns and density estimates by macro-habitat

Field observations on turtles (n = 131) were concentrated essentially during morning hours, with a peak at 1100-1300 local time (Fig. 4). Overall, a mono-peaked diel activity pattern was recorded (Fig. 4), with a significantly uneven frequency of observation by time interval (χ^2= 41.54, df = 5, P < 0.0001).

The estimated population density of turtles by macro-habitat type is presented in Table 1. Overall, a better model fitting the data was obtained for the line transects made in the reed-bed habitat type rather than in the two open water habitat types. Highest estimated densities were in the reed-bed habitat (*mean* = 9.51 individuals × ha^{-1}, S.D. = 2.0, *range* = 2.59-38.35) and in the open water habitat (lake) (*mean* = 9.12 individuals × ha^{-1}, *S.D.* = 6.8, *range* = 4.45-18.94), with much lower values in seasonally inundated grasslands (*mean* = 6.0 individuals × ha^{-1}, *S.D.* = 6.26) and in open water (channel) (*mean* = 4.58 individuals × ha^{-1}, *S.D.* = 2.7, *range* = 1.71-13.37). No turtles were found in the temporary ponds during line transects, although these turtles also inhabit this habitat type (two shells of squashed animals were found along the road crossing this habitat type near Jelilabad).

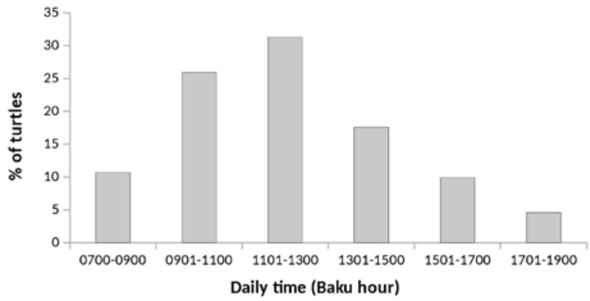

Figure 4. Patterns of diel activity of turtles at the study areas in Azerbaijan. Total n = 131.

Table 2. Micro-habitat use by turtles at the study areas in Azerbaijan. Micro-habitat categories are expressed in terms of percent of water body invaded by reed-bed vegetation (see methods for more details).

Micro-habitat type (% water body invaded by reeds)	Number of turtles	% use
0-20	21	17.9
21-40	36	30.8
40-60	33	28.2
>61	27	22.7
TOTAL	117	

Micro-habitat use

In the studied sample of turtles (n = 117), there was no significant departure from an even distribution of observations across micro-habitat types (χ^2= 4.538, df = 3, P = 0.21; Table 2). Thus, it may be concluded that the density of reeds was not an important element in determining the micro-habitat use by turtles at the study areas, although the potential effects of reeds on the prey density and micro-thermal exigencies of pond turtles need to be assessed with precision.

Sex-ratio and intersexual body size differences

A total of 76 individuals were captured and measured. Sex ratio of captured individuals (M:F = 1.11:1) did not differ significantly from equality (χ^2 = 0.10, df = 1, P = 0.87). Since carapace length was significantly positively correlated with plastron length (r = 0.97, r^2 = 0.94, n = 76, P < 0.0001), plastron width (r = 0.94, r^2 = 0.88, n = 76, P < 0.0001), and carapace width (r = 0.96, r^2 = 0.93, n = 76, P < 0.0001), only carapace length was considered for further analyses on intersexual body size differences. Females significantly exceeded males in terms of body size (carapace length – females: *mean* = 193.1 mm, *S.D.* = 23.7; males: *mean* = 182.1 mm, *S.D.* = 20.1; t = 2.353, df = 74, P < 0.05), showing a nearly identical minimum size (123 versus 121 mm) but a considerably larger maximum size (220 versus 200 mm). Juvenile turtles, which live a cryptic lifestyle for the first few years and are therefore harder to find, were not observed during field surveys.

Table 3. Summary of the diet (expressed in terms of number of turtle individuals having eaten a given food item) of *E. o. persica* at the study area.

Prey type	males (n = 40)	% of males	females (n = 37)	% of females
Hydrophilus piceus larva	27	67.5	30	81.09
Hydrophilus piceus adult	1	2.5	1	2.70
Odonata larva	1	2.5	1	2.70
Sympetrum larva	6	15.0	3	8.10
Coleoptera indet	1	2.5	1	2.70
AdultTenebrionidae	1	2.5	0	0.00
Adult *Dytiscus*	1	2.5	2	5.40
Adult Dytiscidae	1	2.5	0	0.00
Dytiscus larva	0	0.0	4	10.81
Nepa sp	2	5.0	3	8.10
Cladocera	5	12.5	3	8.10
Hirudinea	1	2.5	1	2.70
Undetermined fish	5	12.5	3	8.10

Diet

Including faeces and a few direct observations of turtles while foraging, diet data were collected from a total of 77 turtles (40 males, 37 females). The dietary diversity of the turtle population was accurately described by the examined sample, as a plateau in the cumulated number of prey items was reached after about 40 individuals (Fig. 5). The summary of the turtle diet, expressed in terms of number of turtle individuals having eaten a given food item, is presented in Table 3. The diet composition appeared relatively specialised, with large-sized larvae of aquatic beetles (especially of *Hydrophilus piceus*) being found in the great majority of the individuals (Table 3). Other larvae (for instance of Odonata: *Sympetrum* sp.), were also taken. Vertebrate items were uncommon, with fish remains being found in less than 10% of individuals. Males and females did not differ significantly in terms of frequencies of individuals containing a given prey type (observed versus expected χ^2= 3.3, Monte Carlo *P* = 0.342).

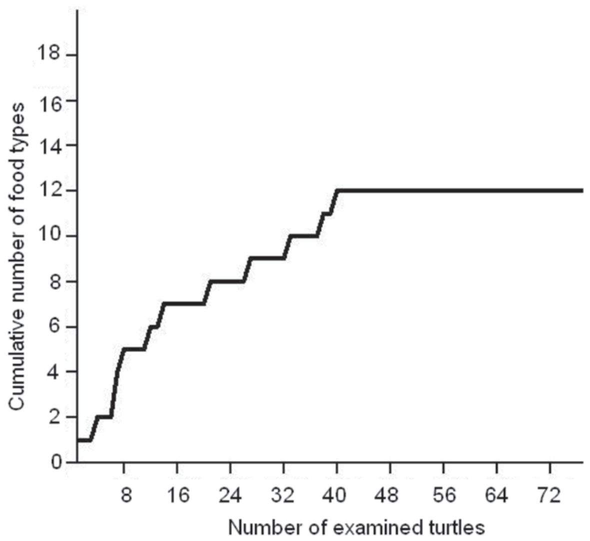

Figure 5. Diversity accumulation curve for the diet habits of turtles at the study area (males and females being pooled, total n = 77). Note that the plateau phase in cumulated dietary diversity was clearly reached.

No amphibian remains were found in turtle faeces (Table 3). However, it should be remarked that these prey species are more difficult to identify from faeces than arthropods or fish, and, in addition, at the time of our surveys, frogs had not yet deposited their eggs and thus their tadpoles were not yet available. Turtles may possibly shift on foraging much on tadpoles later in the season, when this latter food resource would become readily available. In addition, no evidence of eating plants was seen, and no plant fibres were identified in the faeces.

DISCUSSION

Data on density and microhabitat preferences collected in this study showed that *E. o. persica* populations prefer the extended reed-bed habitats with perennial water to the temporary ponds and wetlands that desiccate during the summer months. Although the percentage cover of reeds was not a significant feature of micro-habitat preference for turtles, it was evident that the numbers of observed turtles decreased substantially outside this type of micro-habitat, especially in temporary water sites. Indeed, density decreased substantially from over nine (in the reed-bed habitat with perennial water) to about four or even less (in the temporary water-bodies) individuals × ha^{-1}. Comparisons of the observed densities with other conspecific populations from elsewhere are complicated by the different methodologies employed by the different studies. Line-transect counts, i.e. one of the same methodologies as used in the present study, provided relatively similar estimates for different subspecies of *E. orbicularis*: in Italy (subspecies *galloitalica*): densities varied between 3 and 6.13 individuals × ha^{-1} (Mazzotti et al., 2007). Considerably higher densities were recorded when baited trapping nets were used, but these latter estimates may be biased by the attraction factor as bait may have attracted turtles from outside their usual home ranges (Mazzotti et al., 2007). Indeed, very high densities were reported in Switzerland (64 individuals × ha^{-1}; Mosimann and Cadi, 2004), Hungary (128-242 individuals × ha^{-1}; Balazs and Györffy, 2006), and Turkey (up to 130 individuals × ha^{-1}; Ayaz and Cicek, 2011).

A clear preference for permanent water bodies, as seen in the present study, was also documented by Ficetola et al. (2004) for Italian *galloitalica* populations. The characteristics of basking sites are important for determining the suitability of given microhabitats for *E. orbicularis* (Ficetola et al., 2004; Vignoli et al., 2015), but basking site characteristics were not analysed in the present study.

In terms of body size and sex-ratio, the studied turtle population widely confirmed previous studies on other conspecific populations. In particular, females were also significantly larger than males in populations from central Italy (Zuffi et al., 2006) and Anatolia (Bayrakci and Ayaz, 2014), with a sex-ratio almost invariably being even (Balazs and Györffy, 2006; Bayrakci and Ayaz, 2014).

Our study also demonstrated a carnivorous and relatively specialised food preference in *E. o. persica*, with large larvae of aquatic beetles being the dominant fraction of their diet. Although invertebrate items are frequently eaten also by French and Italian populations of this species (Arvy and Servan, 1998; Lebboroni and Chelazzi, 1991), it has been observed that adults may feed extensively on vegetal matters (Ottonello et al., 2005). Although a considerable variation in diet composition has been observed among the various populations studied to date (Ficetola and De Bernardi, 2006), it has been argued that populations inhabiting canals tend to feed on terrestrial arthropods more often than populations inhabiting lakes (Ottonello et al., 2005). This pattern was not confirmed by the present study, with individuals from canals, lakes and marshes feeding prevalently on aquatic larvae (L. Luiselli, unpublished data). Overall, the populations of *E. o. persica* under study appeared among the most carnivorous of the various conspecific populations studied to date, as no plant fibres were seen in their faeces. This fact is a little surprising because some populations of *Emys* eat more vegetable matter as they mature (Ficetola and De Bernardi, 2006).

Main threats for turtles at the study area

From the point of view of the potential management implications for this turtle population, it is noteworthy that at Akchala reed-bed habitat, potential basking spots are very few, and certainly present a limited resource for the large number of turtles present in the area.

Road-killing and fishing nets were the main threat for turtles at the study area. Indeed, six individuals squashed on the road (*mean* = 0.43 turtles × day^{-1}) were observed during the study period, including adult females. Female turtles are attracted to gravel shoulders during the nesting season, especially in sites where extended ponds/ aquatic habitats reduced the potential availability of their oviposition sites. Since the wetland habitat at the site is very extended but apparently with scarce availability of good spots for nesting, it is likely that many female turtles may try to deposit their eggs in the rock-filled (up to 40 cm above water level) areas surrounding the roads. This explains the high frequency of dead specimens observed on the road during the present study.

Another main threat is represented by fishing nets, which can kill a considerable number of individuals within short time intervals in the open water habitat. One fishing net was explored on 28 April 2016 at Mahnudchala wetland and 7 dead individuals were found. Interviewed fishermen also reported that turtles can die in high numbers in these fishing nets, though they are not the target of their catches. It is recommended that local authorities will monitor carefully the incidence of these threats for turtle populations, and that appropriate management actions can be taken.

ACKNOWLEDGEMENTS

This study was performed under authorisation of the Government of Azerbaijan, as a part of a impact assessment study for the realisation of the Alat-Astara Highway, Jelilabad intersection to Shorsuulu intersection. Funds were provided by Asian Development Bank and Tera International Group. Sincere thanks are expressed to Dr Asil Gezen, Eng. John Bentley, and Eng. Andrew Taylor (all from Tera International) for helpful collaboration during the field studies, and to Roger Meek and two anonymous reviewers for their helpful comments on the submitted draft.

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Accepted: 29 November 2016