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Preliminary results on biological aspects of the grass snake, *Natrix natrix* in the southern coastal area of the Caspian Sea

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Abstract. *Natrix natrix* is, together with *N. tessellata,* the only representative of water snakes found in Iran. The lack of ecological studies on this species in Iran stimulated the current preliminary research on some basic biological traits. From April to July 2008, a total of fifty five snakes were collected from two stations in the southern part of the Caspian Sea coast, Iran: Gomishan Wetland and Sari. Basic morphometrics were compared between sexes and stations and gonadal development was compared between stations and seasons. At the two stations, adult sex ratio was not different from 1:1 and juveniles composed a predominant portion of the sample. Both sexes had unimodal length class size with a peak at 40-45 cm snout-vent length (SVL). Body sizes were unusually small for the species and no SVL sexual dimorphism was detected although males were relatively heavier than females for the same SVL. Snakes of both sexes attained larger size with better body condition at Sari than in Gomishan. Males and females carried more mature gonads in summer. However, in Sari males developed relatively larger testes earlier in the season and both sexes displayed less synchronic reproduction at this station. These results are best explained by local variations in habitat, trophic availability and degree of environmental disturbance.

Keywords. *Natrix natrix*, sexual dimorphism, reproduction, Iran.

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INTRODUCTION

In Iran, the water snakes in the genus *Natrix* are represented by two species: the dice snake *Natrix tessellata* (Laurenti, 1768) and the grass snake *Natrix natrix* (Linnaeus, 1758). The former is a medium-sized (up to 1.2 m total length), semi-aquatic snake that prefers relatively open, and often rocky shore habitat of any aquatic system where it hunts mostly fish (Mebert, 2011a). It is widely distributed across the north, northwest, west, southwest and south of the country, whereas the second has a very limited distribution range (Firouz, 2000; Latifi, 2000; Bagherian and Kami, 2009). The second semi-aquatic snake, the grass snake, attains a slightly larger total length (occasionally > 1.5 m), and is typically associated with lentic water habitats, such as ponds and lakes, but inhabits also streams, rivers and grasslands where is forages predominantly on anurans (Beebee and Griffiths, 2000; Latifi, 2000; Szczerbak, 2003). The grass snake has a huge distribution, ranging from Britain, Iberian Peninsula and North-western Africa in the west to Lake Baikal and Northwest China in the East (Thorpe, 1981; Orlov and Tuniyev, 1987; Szczerbak, 2003). In Iran, it is found in large numbers in the Northern provinces, including Gilan, Mazandaran and Golestan (Latifi, 2000; Bagherian and Kami, 2009). Currently, the grass snake is regarded as a serious pest in some fish farming areas and causes harm to the industry by feeding on juvenile fish, especially in open and uncontrolled farms like lakes (Patimar, pers. comm.).

Despite their abundance, the ecology of snakes is less well known compared to other reptiles (Greene, 2001; Pough et al., 200l). Nevertheless, the natricine snakes are among the better studied (Gregory, 2004), and *N. natrix* is no exception (e.g., Thorpe, 1975, 1984; Madsen and Shine, 1993a, 1993b; Mertens, 1995; Filippi et al., 1996; Blosat, 1998; Borczyk, 2004, 2007; Consul et al., 2009). However, biological studies providing quantitative data for this species in Iran are still lacking. Therefore, the aim of this preliminary study is to stimulate the research on associated topics, by providing more detailed biological information, namely on basic morphometrics and reproduction, for the Iranian populations of this species. We focused on interpopulational, intersexual and seasonal variation, as well as on habitat features and conservation status.

MATERIALS AND METHODS

Study areas

The study was carried our near the south-eastern coast of the Caspian Sea, in northern Iran (Fig. 1). Initially, sampling was conducted at three stations: Gomishan wetland (37°09’07.3”N and 53°54’32”E), Ghaz Harbour (36°47’41.0”N and 53°56’43.0”E), and Sari (36°32’42.9”N and 53°06’41.9”E). Ghaz Harbour was subsequently removed from the analysis due to low sample size. The Gomishan Wetland is a protected brackish water body at the south-eastern corner of the Caspian Sea coast with an area of approximately 20,000 hectares. The main wetland area contains salt marsh vegetation and seasonally-inundated flats of *Salicornia* mixed with *Halostachys* and *Halocnemum* grasses. The Sari station is located in the vicinity of fish farms near the city of Sari, the Capital of Mazandaran Province, at about 130 km from Gomishan Wetland. At this station, the rainy season lasts about seven months with an annual precipitation of more than 1,110 mm, enabling green

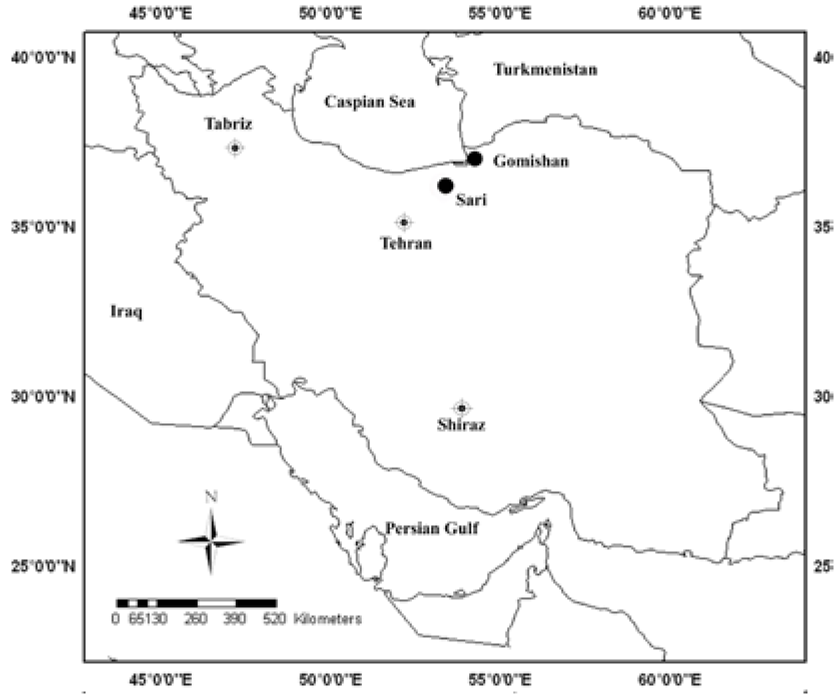

Fig. 1. Study sites in the south-eastern coast of the Caspian Sea, Iran.

and lush vegetation typical for a Hyrcanian forest habitat. All specimens at this station were collected along streams in the woodland and the area around a large fish pond (Scott, 1995; Kiabi et al., 1999). Following physicochemical measurements were recorded at both stations once, using a HACH portable device: temperature (°C), pH, salinity (‰) and total dissolved solids TDS (g/l) during the first week of May.

Field sampling

Field methods were the same for both stations. Sampling was carried out from April to July 2008, the first and fourth week of each month, in the morning from 08:00-10:00 hrs. We recorded diet through anecdotal observation of prey capture or occasional regurgitation (but no methodical investigation), as well as types of primary microhabitat and behaviour. All specimens were collected by hand and transferred to the Zoology Laboratory of Environmental Sciences Research Institute, Shahid Beheshti University, sacrificed humanely with the permission by the institutional authority and fixed in 10% formalin. Individuals were considered adults when longer than 30 cm SVL according to Mertens (1995). Adult specimens were weighted to the nearest gram (body mass, BM). The following measurements were recorded: SVL (snout-vent length) and LCT (length of complete tail from vent to tip of tail). In addition, RTL and LTL (right and left testes lengths) in males and ROL and LOL (right and left ovarian lengths) in females were measured to assess their reproductive status. All linear measurements were recorded to the nearest centimetre. No other variables, like the number of follicles or their sizes could be recorded due to methodical constraints.

Statistical analysis

The juveniles were excluded from the statistical analyses. For adults, the overall sex ratio was assessed using Chi-square (χ^2) test (Zar, 1999). Measurements were log-transformed and normality and homoscedasticity were ensured prior to the analyses. A two-way ANOVA was performed to compare adult SVL differences between stations and sexes. For the other length measurements like tail length (LCT) and lengths of testes (TLs) and ovaries (OLs), two-way ANCOVAs with station and sex as factor and SVL as covariate were run. In the same way, gonad measurements were compared through two-way ANCOVAs with station and season as factors and SVL and BM as covariates. No pholidotic variables could be recorded. All statistical analyses were performed in STATISTICA 10 (Statsoft, 2011) and significance level was set at 0.05.

RESULTS

In early May, Gomishan Wetland exhibits higher values of water physical features than equivalent measurments show from Sari, including water temperatures, pH, salinity (%) and TDS (g/l) (Table 1).

A broad range of potential prey types for *N. natrix* was observed at all sites. The scattered observations indicated the consumption of amphibians, such as *Pelophylax ridibundus* and *Rana macrocnemis*, one legless lizard, *Anguis fragilis*, and some cultured fish (*Barbus* sp., Cyprinidae).

The number of specimens collected by station, season and class is shown in Table 2. At the two study sites, the grass snake was relatively common. In total, 55 grass snakes including 18 males, 21 females and 16 juveniles were collected from both sites during two field seasons. The overall sex ratio was not significantly different from 1:1 ($\chi 2 = 0.23$, df = 1, $P > 0.05$). Juveniles (< 30 cm SVL) constituted a large percentage of the collected specimens, 29% and 28% in spring and summer, respectively. The total length of adult males

Table 1. Physical traits of water, temperature (T), PH, salinity and total dissolved solids (TDS) in the two sampling stations from the south-eastern Caspian Sea, Iran.

Station	T(°C)	PH	Salinity (‰)	TDS (g/l)
Gomishan Wetland	21.9	8.97	28.7	22.2
Sari	16.9	8.2	1.3	20.4

Table 2. The number of Iranian *Natrix natrix* collected by station, season and class.

	Gomishan Wetland			Sari		
	males	females	juveniles	males	females	juveniles
Spring	5	5	1	4	7	8
Summer	4	4	4	5	5	3

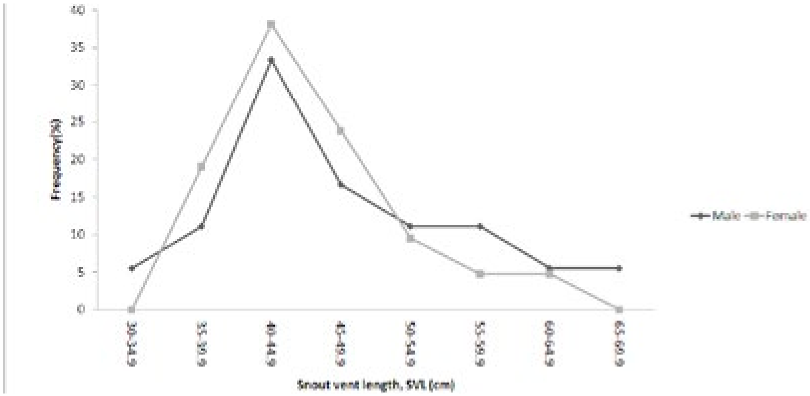

Fig. 2. Length frequency distribution for grass snake *Natrix natrix* from the south-eastern Caspian Sea, Iran.

ranged between 46.0 and 85.0 cm and adult females between 46.0 and 76.7 cm. The snout-vent length frequency distribution of snakes from the two stations indicated that the dominant length class was 40-45 cm for both sexes, representing 33% of males and 38% of females for both stations lumped (Fig. 2).

Descriptive statistics of the morphometric variables and ANCOVA comparisons are shown in Table 3. Snakes attained longer SVL in Sari than in Gomishan but tests failed to detect significant differences either between sexes or interactions between sex and station (Table 3). Moreover, relatively to their SVL, males were heavier than females and individuals from Sari were heavier than those from Gomishan, although there was no variation of sexual dimorphism between stations (Table 3, Fig. 3). Finally, no differences in tail length relative to SVL were detected either between sexes, between stations or their interaction (Table 3).

The smallest adult male with thickened muscular tail root was 35 cm SVL. Relative to SVL, both ovaries were longer in summer than in spring. Whereas right ovaries showed no differences between the stations, thus following the same seasonal pattern (ANCOVA station $F_{1,15} = 1.07$, $P = 0.32$; season $F_{1,15} = 49.74$, $P < 0.0001$; station*season $F_{1,15} = 0.01$, $P = 0.92$), left ovaries were slightly longer in grass snakes from the Gomishan site when compared to those from Sari (ANCOVA station $F_{1,16} = 7.34$, $P = 0.02$; season $F_{1,16} = 63.44$, $P = 0.0000001$; station*season $F_{1,16} = 1.46$, $P = 0.24$). The same patterns persisted when both SVL and BM were used as covariates to account for body condition either for the right testis (ANCOVA station $F_{1,14} = 0.000001$, $P = 0.99$; season $F_{1,14} = 54.15$, $P < 0.0001$; station*season $F_{1,15} = 0.16$, $P = 0.69$) or for the left testis (ANCOVA station $F_{1,14} = 9.96$, $P = 0.007$; season $F_{1,14} = 70.09$, $P < 0.000001$; station*season $F_{1,15} = 0.63$, $P = 0.44$). The smallest male with thickened efferent ducts, indicating sexual maturity, was 32 cm SVL. Relative (to SVL) length of the testes was significant higher in Sari than in Gomishan, but seasonal variation, spring being lower than summer, was slight and only detected in the left testis (ANCOVA right testis station $F_{1,13} = 34.27$, $P < 0.0001$; season $F_{1,13} = 6.18$, $P = 0.03$; station*season $F_{1,13} = 3.18$, $P = 0.10$; left testis station $F_{1,13} = 10.73$, $P = 0.006$; season $F_{1,13} = 0.42$, $P = 0.53$; station*season $F_{1,13} = 0.48$, $P = 0.50$).

Table 3. Descriptive statistics of the morphometric variables for the two analysed populations of adult *N. natrix* from Iran and ANCOVA comparisons for site and sex; all but the first using SVL as covariate. BM: body mass; SVL: snout-vent length; LCT length of complete tail. Numbers indicate mean ± SE, range and sample size.

variable	Gomishan		Sari		ANCOVA (station, sex, station*sex)		
	males	females	males	females	F	df	P
SVL	40.32 ± 1.60	40.33 ± 1.14	54.20 ± 2.41	48.67 ± 2.04	1.73	1,35	0.20
	32-47	47-35	45-67	38-62	35.12	1,35	< 0.0001
	9	9	9	12	1.98	1,35	0.17
BM	59.00 ± 3.26	49.78 ± 2.57	82.87 ± 3.78	74.25 ± 4.67	7.17	1,34	0.01
	47-72	60-36	67-98	51-112	4.51	1,34	0.04
	9	9	9	12	1.83	1,34	0.18
LCT	12.49 ± 0.65	11.44 ± 0.47	12.86 ± 1.07	12.68 ± 0.37	0.96	1,34	0.33
	10-15	14-9	9-18	11-15	0.03	1,34	0.87
	9	9	9	12	2.27	1,34	0.14

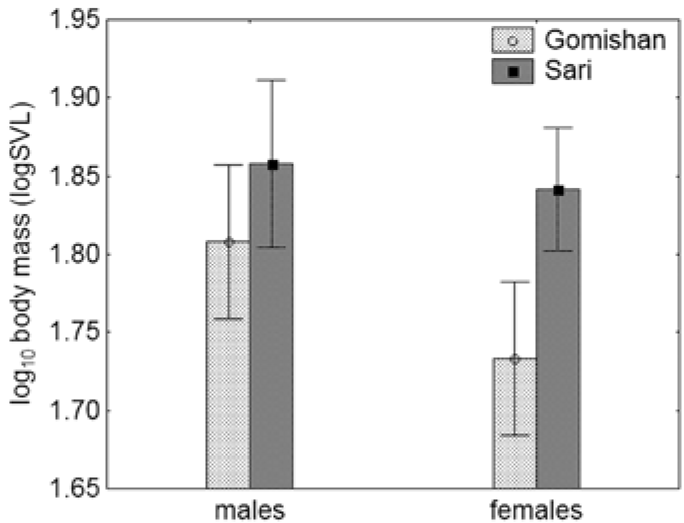

Fig. 3. Variation of the relative body mass (relativized to SVL) between sexes and stations in adult *Natrix natrix* from the south-eastern Caspian Sea, Iran. Vertical bars denote 95% confidence intervals.

DISCUSSION

This study presents the first ecological picture of the grass snake of Iran, and thus, augments our knowledge on the biology of the widespread Palaearctic water snake, *Natrix*

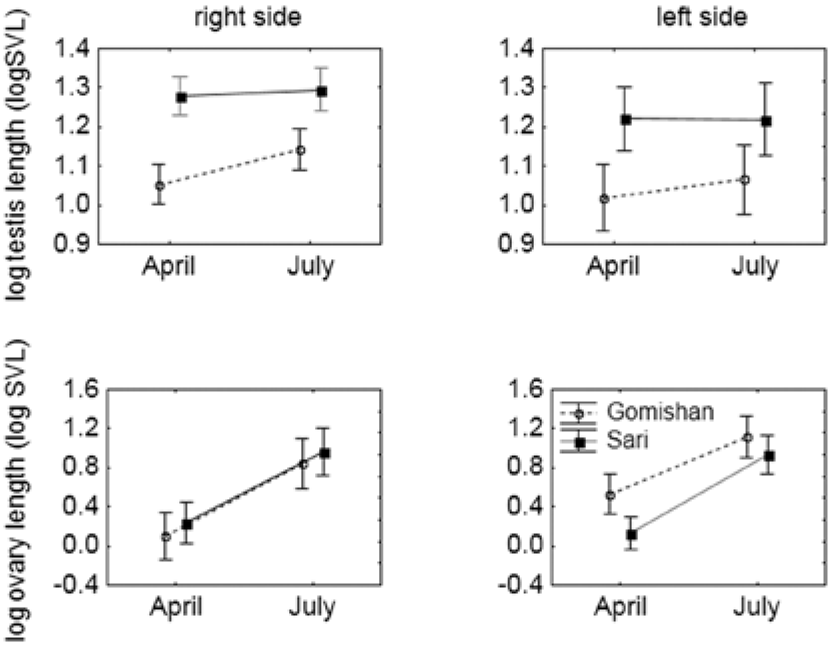

Fig. 4. Variation of the gonad length between spring (April) and summer (July) in adult male and female *Natrix natrix* from the south-eastern Caspian Sea, Iran. Vertical bars denote 95% confidence intervals.

natrix. The results and conclusions have still a preliminary character, yet they warrant their relevance due to virtually non-existing information of that species from the south-eastern corner of it global distribution. The incidental observations on diet are in accordance with the generally recorded wide food niche based on amphibians, fish, and rarely lizards, as was reported from other areas (e.g., Reading and Davies, 1996; Luiselli et al., 1997; Kabisch, 1999). This distinguishes the grass snake from the syntopic and more piscivorous dice snake, *N. tessellata*, which at the same sites consumed only fish and has never been found as far from water as *N. natrix* (Ahmadzadeh et al., 2011). Similar food niche differences have been detected in other syntopic populations, as in Italy (Capula et al., 2011), Greece (Ioannidis and Mebert, 2011), and Croatia (Janev Hutinec and Mebert, 2011).

The balanced sex ratio at the two populations show a normal pattern as generally described for snakes in Parker and Plummer (1987). Luiselli et al. (2011) calculated a near equal sex ratio in *N. tessellata* from Italy, whereas sex ratio for another natricine snake, *Nerodia sipedon*, was slightly elevated with 1.5:1 and 1.3:1, respectively (Feaver, 1977; King, 1986), and locally much higher with 2.7 for the closely related *N. maura* (Hailey and Davies, 1987). The mature to immature ratio found here (2.5:1) was clearly lower than in a population in central Germany (1.1:1, Mertens (1995). Although this could be the result of a high recruitment, the effect of a low sample size can also be hypothesised.

Concerning adult snakes, Mertens (1995) found the 60–70 cm SVL size class the most numerous in males in central Germany. Similarly in other German populations, Waitzmann (1991) found the size class 70–79 cm total length for adult males to be dominant (incl. 20–25% tail length), whereas Kühnel's (1993) sample contained mostly smaller adult males in the class 50–60 cm total length (tail included). On the other hand, females displayed bimodal size distribution with one peak in medium sizes and another in the very large specimens (> 70 cm). The pattern for females in the studies by Waitzmann (1991) and Kühnel (1993) was similar to those of males, but in 10 cm higher size class, i.e., with 80–89 cm and 60–70 cm being the most frequent snakes. This reveals a strong geographic variation on body size, possibly resulting from local environmental effects on growth. Unlike Mertens's finding, the size distribution of Iranian grass snakes is unimodal in both sexes with small modal values (40–45 cm). Two results are remarkable in reference to the adult sizes and their frequencies at both Iranian sites: first, no sexual size dimorphism was detected in tail length relative to SVL; and, second, males yielding slightly larger SVL than females. Both results contrast not only with other studies on the same species in other parts of its range (Thorpe, 1984; and refs. in Kabisch, 1999, in Blanke et al., 2008; and in Pleguezuelos, 2010), but also with other *Natrix* species (Schätti, 1982; Gruschwitz et al., 1999; Santos et al., 2000; Mebert, 2011b), as well as with other natricines (King, 1989; Shine, 1994; Mebert, 2010). Regarding sexual dimorphism, in most snakes, females attain a larger body size (SVL) than males, whereas males have longer and thickened tail roots (Shine, 1994; Pough, 2001). However, in this study, statistical analyses were not able to detect differences between sexes either for SVL or for relative tail length. Significantly, specimens from both study sites exhibited a rather small size compared to other populations in Germany (e.g., Wisniewski, 1958; Waitzman, 1991), but were similar to those sampled from a sub-population of Kühnel's (1993) study sites in Berlin, northern Germany. The interpretation of the last author suggested that a visibly sharp decline in green frogs, the principal prey, had resulted in the temporary survival or local persistence of smaller individuals only. Such conditions may apply temporarily to the Iranian sites as well. Certainly, it could be hypothesised that both results (lack of sexual dimorphism and small size) from the Iranian grass snakes are linked, that is, that the overall reduction in body size has minimised the sexual size dimorphism to the degree of becoming statistically undetected. However, Kühnel's study (1993) across a greater area in Berlin found a significant sexual dimorphism with approximately the same number of grass snakes and a similar average size. In addition, males tended to be more robust than females for the same size in both populations studied. In contrast, Capula et al. (1994) found female *N. natrix* on Sardinia exhibiting higher body conditions than males. At this stage of the study, we can only speculate about the causes for the larger males or the lack of large females in the samples, including a) an artefact of low sample sizes (~ 5 adult snakes per season and sex), b) that the sampled males are older than females in the area, c) show a more rapid growth rate at the same age as females due to earlier emergence and feeding after hibernation, or c) occurrence of a negative selection against females, such as: 1) suffering a higher incidence of predation due to their larger size, 2) dying before reaching a large size/age due to increasing toxic load from the copious amounts of herbicides and pesticides applied in the agriculture surrounding the sites, 3) migrating outside the sites for oviposition.

Grass snakes from Sari attained larger sizes with better body condition than those from Gomishan, which could be the result of a stable food source as provided by the fish farms. Overall, the various studies on *N. natrix* populations indicate that body length in both sexes can vary greatly among sites and possibly even fluctuate within a site depending on periodically varying food availability. Finally, the number of adult snakes in this study is too small for any further conclusion on the high frequency of small females and lack of sexual dimorphism of tail length. Hence, only increasing the sample size will likely produce a conclusive answer in this context.

Regarding the reproductive organs, across its wide range, grass snakes are active from the beginning of March (in the south) or May (in the north) until September or November, respectively (Kabisch, 1999; Szczerbak, 2003). Reproductive activity lasts from May to mid-July. In May, after a quiescent period of 7-8 months, females start vitellogenesis followed by an increase of the length of both ovaries. All females collected showed some reproductive activity with enlarged ovaries in the summer, when likely maximum levels are reached, as oviposition is normally from June to August (Kabisch, 1999; Meyer et al., 2009). In contrast, males showed equal gonadal activity over a longer period, the increase of testis length being nearly equal in spring and in summer. This is likely due to their postnuptial spermatogenesis, i.e. spermatogenesis and growth of testes occurs in summer and continues into the fall, a period that was not measured in this study. Such a spermatogenetic pattern was confirmed very recently for grass snake populations from the same area in Iran (Faghiri et al., 2011). This is also known for the other *Natrix* species (for *N. tessellata:* see Rutishauser, 1996; and for *N. maura:* see Santos et al., 2000; Santos et al., 2001). Since climate is very similar between both field stations, the difference of testicular growth between populations derive from other local ecological factors. The larger testes in grass snakes from Sari may be the result of a larger energy input early in the season due to a higher food availability at the fish farms, hence, being less seasonal than in Gomishan. The growth pattern of the ovaries seems less clear, but female grass snakes in Gomishan may start earlier and be less synchronic than those of Sari which will be all peaking in summer. This kind of local environmental constraints have also been reported for *N. maura* in a river delta from Spain (Santos and Llorente, 2004; Santos et al., 2005).

Generally, *N. natrix* inhabits in a wide range of aquatic habitats: riverbanks, swamps and mountain streams up to 2400 m a.s.l. (Madsen, 1984; Mertens, 1995; Beebee and Griffith, 2000; Szczerbak, 2003). Comparing the two stations in this study, the Gomishan wetland is a more open habitat type, situated in close proximity to the Caspian Sea, with sparse submerged vegetation, and higher values of physicochemical characters, a biotope and habitat structure typical for piscivorous *N. tessellata* (see refs. in Mebert 2011a). This contrasts to the wooded site at Sari, which represents a typical Hyrcanian forest habitat. If the conclusions of other studies on *N. natrix*, summarized in Kabisch (1999) and Blanke et al. (2008), can be extended to Iran, the forests of Sari with an abundance of anuran prey are expected to provide more suitable habitat structures and ecological resources for *N. natrix* than the Gomishan Wetland. Wooded areas combined with the fish farms at Sari, yielding an additional, easy accessible food source, could be the factors producing markedly higher population densities of *N. natrix*, composed by larger individuals in better condition, which are able to start reproduction earlier. These results are in correspondence to those published for *N. natrix* (e.g., Mertens, 1995; Beebee and Griffith, 2000; Szczerbak, 2003) and similar for *N. maura* (Pleguezuelos and Feriche, 2003; Santos et al., 2005, 2011).

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