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The re-evaluated conservation status of the mountain populations of the highly endangered Cyprus grass snake, *Natrix natrix cypriaca* (Hecht, 1930), with miscellaneous natural history notes

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Abstract. The conservation status of the populations of the highly endangered Cyprus grass snake, *Natrix natrix cypriaca* (Hecht, 1930), in the Troodos Mountains was re-evaluated in 2008. Results suggest that only two streams continue to be inhabited by the snake. The total number of grass snakes in the Troodos area is estimated to be around 90–100 specimens. There has been a severe decline in the percentage of juveniles in both subpopulations in comparison to earlier studies in 2002 and 2005. There are a number of threats to the survival of the grass snake at all sites. A pair was observed mating relatively late in the season in July, which was only the third mating ever observed in *N. n. cypriaca*. One male caught by hand displayed a mild form of akinesis, which is the first record of this defensive behaviour in the Cyprus grass snake. Six specimens had tail damage, which is thought to have resulted from interaction with humans.

Key words. Colubridae, Cyprus, Troodos Mountains, population size, age structure, sex ratio, akinesis, mating, tail loss.

Introduction

Scientifically described in 1930 by Hecht as a new subspecies endemic to Cyprus, the Cyprus grass snake (*Natrix natrix cypriaca*) was believed to be extinct by the early 1960s. Then, in 1992, a population was rediscovered at the Xyliatos Dam in the Troodos Range (Wiedl & Böhme 1992). During 1994–1996, this population was investigated as part of a PhD research programme (Blosat 1998) and was estimated to consist of 454–650 individuals. Four additional populations were found, one of which occurs outside the Troodos Range in the eastern part of the island.

When the status of these populations was evaluated in 2002, it became clear that they were highly endangered (Blosat 2002). A further monitoring study in 2005 (Blosat 2005) confirmed that the Xyliatos population was most likely extinct, while the other three Troodos populations and those in the eastern part of the island were highly endangered. The Cyprus grass snake is currently listed in Annex IV of the Flora-Fauna-Habitat (FFH) Directive (Directive 92/43/EEC) and additionally in Annex II, the highest protection category, as a 'priority species'. The IUCN Red List does not currently list *Natrix natrix cypriaca*, although intraspecific units are normally included if these are threatened. It is strongly recommended to upgrade the Cyprus grass snake to the IUCN Red List category 'critically endangered' (for reasons see Blosat 2008, Baier et al. 2009). Blosat (2008) and Baier et al. (2009) summarise in detail the status of the Cyprus grass snake as of 2005, the threats to these four relict populations, and required conservation measures.

Two of the measures necessary for the survival of the Cyprus grass snake (Blosat 2008: 266) are that "the already known *Natrix* sites should be monitored on a regular basis" and that "more research on this rare subspecies" should be done "which includes a survey of all permanent stream valleys in Troodos mountain range". Therefore, a fourth field survey (following on from those of 1994–1996, 2002 and 2005) on the Cyprus grass snake was carried out in 2008. The purpose of the present paper is to report on this study with respect to (i) the survey of as many as possible stream valleys in the Troodos Range in search of unknown populations, (ii) the investigation of the population status (population structure and size) of the three remaining Troodos populations, (iii) key factors threatening each of these three populations as of 2008, and (iv) miscellaneous observations on the natural history of the Cyprus grass snake (akinesis, tail injuries, mating behaviour). The field survey was also aimed at investigating the interesting behavioural ecology of the Cyprus grass snake. The latter results will be reported elsewhere.

Material and methods

Fieldwork was carried out by F.B. between 6 April and 3 September 2008. The current distribution of the Cyprus grass snake in the Troodos Range is restricted to the Pitsylia region in the northeast of this mountainous area. In order to survey this region for possible additional populations, water courses were identified from a map and field observations and divided into different sample transects. Areas were visited at least once and usually for several hours. Streams, which were the main suitable habitats, were investigated on foot in segments, depending on their accessibility. They were checked for the presence/amount of water, how they were vegetated, and whether there were any cultivation or other possible disturbances along the stream.

Searches were conducted regularly in the four areas in the Troodos Range known to be inhabited by grass snakes: Stream 1 (see Blosat 2008 and Baier et al. 2009 for illustrations of this stream) was visited 17 times, Stream 2 (Fig. 1) 11 times, Dam 1 (Fig. 2) eight times, and the farm ponds (Fig. 3) four times. The streams were sampled over their entire lengths consecutively in segments, but only a short transect of each stream was visited repeatedly in order to estimate population sizes. Captured snakes were measured, weighed and photographed. Their sex, colour morph (normal, melanistic or picturata) and approximate age were noted. Specimens up to 30 cm total length were considered juvenile, up to 50 cm in total length subadult and over 50 cm in total length adult. Snakes were marked according to Blanchard & Finster (1933) and Brown & Parker (1976), respectively. This procedure took no more than 5 min. Snakes were released afterwards exactly where they were caught. The locality was recorded with a GPS device. Snake specimens which were already marked when caught were processed in the same way except marking.

The population size in the respective area was estimated on the basis of the mark-recapture (M-RC) data, which were analyzed according to the Darroch model (= M_t) (Darroch 1958) as included in CAPTURE, which is incorporated in the software MARK (White & Burnham 1999). The Darroch model supposes a closed population. Since the M-RC data used in the analysis were collected during a fairly short time span (August/September), the population is assumed to be demographically closed (i.e., no deaths or births). While it is very unlikely that there is any exchange of individuals between the two streams or other subpopulations, migration to and from the sample transect at each stream cannot be ruled out. However, the transects at both streams spanned most of the remaining ponds in which grass snakes seem to be concentrated during the summer months (FB, unpubl. data). Thus, there is no reasonable evidence for a violation of the geographical closure assumption. A further assumption of the Darroch model is that all animals have the same probability of capture on any particular trapping occasion, but that probability can change from one occasion to the next (Otis et al. 1978).

This paper is aimed at following on from the earlier monitoring by Blosat (1998, 2002, 2005, 2008). To ensure methodological consistency and a comparability of the results, her M-RC methods were also applied to the data (Lincoln-Petersen-Index both in the original version and in the modified version of Bailey for recapture rates below 10, e.g., Donnelly & Guyer 1994; Schnabel method, Schnabel 1938). The Darroch model relies on the same assumptions as the Schnabel method, but avoids some problems of this approach, and is nearly identical to the Lincoln-Petersen-Index when two sampling occasions ($t = 2$) are used (Otis et al. 1978). Thus, estimates of population sizes from the Darroch model should not significantly differ from estimates based on the Lincoln-Petersen-Index, the Bailey modification or the Schnabel method; this assumption was tested using the Chi square test.

Results and discussion

Survey of the Pitsylia region for unknown populations

Dam 1 in the upper northern Troodos foothills was confirmed to be inhabited by grass snakes. Two specimens were caught and about six more were observed while swimming. HJW (unpubl. data) found grass snakes at this place in 2007, i.e., six adult specimens were observed swimming. The dam is usually a conflux of Stream 1, but was not so in the dry season of 2008.

During one of two visits of a dam (Fig. 4) situated at a lower level of the northern Troodos foothills (here called Dam 2), a melanistic female was observed swimming close to the bank. A worker had already observed a grass snake swimming in a pool on the construction site in 2007 when the dam had just been built (B. Blosat, pers. comm.). The dam collects water from Stream 1. It is assumed that this specimen migrated to the dam in spring when the stream

Figure 1. Stream 2 in September 2008; note the rubbish tip originating from the road along the stream.

Figure 2. Dam 1, which was found to be inhabited by Cyprus grass snakes in 2007.

Figure 4. Dam 2, in which a melanistic female Cyprus grass snake was observed swimming, thus confirming at least the occasional occurrence of grass snakes in this reservoir (previously unpublished).

Figure 3. The only farm pond still satisfying basic needs (enough water, vegetation, sufficient frog abundance) of the Cyprus grass snake.

still carried water. Both the observation of a specimen in the Visakia dam by Blosat (2008) and the present one illustrate the migratory activity of the Cyprus grass snake. This snake will migrate to new habitats if these are appropriate. This attribute can be helpful for conservation, e.g., by facilitating migration to artificial habitats in the vicinity of existing ones. The Xyliatos dam was visited four times at different hours of the day, but no sign was found of the presence of grass snakes.

Survey of the three known Troodos populations

Population structure

Sex ratio and size classes: There is no significant deviation from a 1:1 sex ratio among the captured specimens both from Stream 1 ($\chi^2 = 0.077$, df = 1, $P = 0.78$) and Stream 2 ($\chi^2 = 2.571$, df = 1, $P = 0.11$). Thus, there has been no negative shift in the sex ratio when compared to the studies from 2002 and 2005, and there does not seem to be a risk of an imbalance of sexes, which would lead to reduced reproduction success (especially if there were fewer females).

The size distribution of all captured specimens from Stream 1 is shown in Figure 5 and from Stream 2 in Figure 6. At Dam 1, one adult female (total length 89.5 cm) and a subadult specimen (total length 36.7 cm) were caught, and the farm ponds yielded three adult specimens (male: total length 61.5 cm; females: total lengths 77.0 and 82.0 cm, resp.).

Age was then inferred based on the total length as defined in the Material and methods section above. Tab. 1 shows the relative representation of each age (juvenile, subadult, adult) among the captured specimens. Data from Blosat (2002, 2005) are included to compare the development of this parameter for each population over time.

Figure 7 graphically illustrates the relative age distribution for Stream 1, Figure 8 for Stream 2, and Figure 9 for the farm ponds.

It is clear from the graphics that the specimens caught in 2008 were almost exclusively adults. The percentage of juveniles and subadults has declined heavily since 2002 (e.g., Stream 2: 21.2% juveniles in 2005, 6.7% in 2008; Stream 1: 22.2% subadults in 2005, 0% in 2008). Since juvenile specimens have a secretive life style, they are usually more difficult to find and thus are caught in lower numbers. However, not a single juvenile was caught at Stream 1 in 2008 and only one juvenile at Stream 2 (6.7%). Therefore, we assume that reproduction of the Troodos populations of the Cyprus grass snakes decreased between 2002 and 2008 and almost came to a halt in 2008. In addition to the numerous threats listed below, another reason for this change in the age structure might be the recent climatic conditions. Both the seasons 2006 and 2007 had annual precipitation below normal, which came to a trough in 2008, when the second lowest average annual precipitation was recorded since 1901 (54% of normal) (Annual Reports of the Meteorological Service for 2006–2008). Such a low amount of precipitation might be responsible for a negative shift in the reproduction rate of the population.

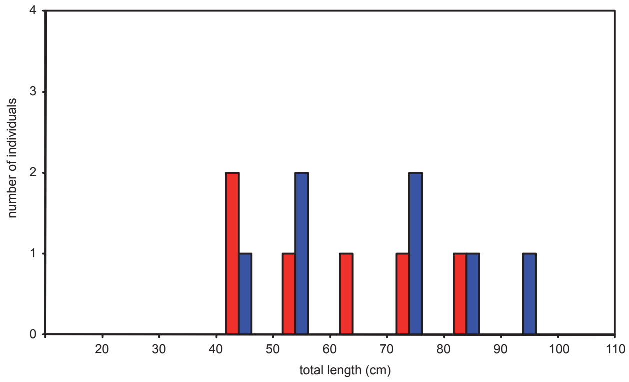

Figure 5. Distribution of size classes among the specimens from Stream 1 in 2008 (red: males; blue: females).

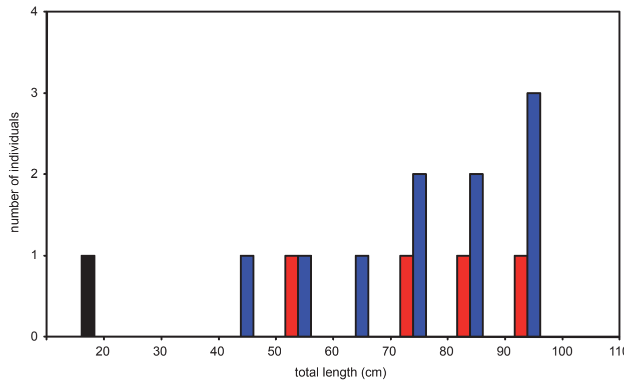

Figure 6. Distribution of size classes among the specimens from Stream 2 in 2008 (red: males; blue: females; black: unsexed).

Population size: At Stream 1, 13 individuals (6 males, 7 females) were recorded, of which five were recaptured once, two twice and one specimen three times. At Stream 2, 15 individuals (4 males, 10 females, 1 young) were recorded, of which three were recaptured once. Of the four farm ponds mentioned by Blosat (2008), only two had water and only one presented suitable conditions (enough water, vegetation, sufficient frog abundance) for the occurrence of the grass snake. As mentioned above, three individuals (1 male, 2 females) were recorded in this pond, none of which were recaptured. In Dam 1, two individuals were registered (1 female, 1 subadult), neither of which was recaptured.

Only the numbers of captures and recaptures at the two streams were sufficiently high for estimates of their population sizes. Using a month as a sample unit (and discarding any recapture within a month), the transect (433 m along the stream bank) investigated at Stream 1 has an estimated population of 16 specimens (SE: 3.13). The 95% confidence interval is 14–28 specimens. This estimated population size is not significantly different from estimates produced with the methodology of Blosat (1998, 2002, 2005, 2008), i.e., the Lincoln-Petersen-Index (χ^2 = 0.118, df = 2, P = 0.94), the Bailey modification (χ^2 = 0.001, df = 2, P = 0.99), or the Schnabel method (χ^2 = 0.001, df = 2, P = 0.99). Since all ponds inhabited by grass snakes at this stream were sampled, the population size at Stream 1 is likely limited to a maximum of 20–30 specimens.

Using a survey as a sample unit (due to a short sampling period for this stream), the transect (1305 m along the stream bank, divided into two subtransects separated by a distance of 1557 m) investigated at Stream 2 has an estimated population of 24 specimens (SE: 13.05). The 95% confidence interval is 15–79 specimens. This estimated population size is not significantly different from estimates produced with the methodology of Blosat (1998, 2002, 2005, 2008), i.e., the Lincoln-Petersen-Index (χ^2 = 0.667, df = 2, P = 0.72), the Bailey modification (χ^2 = 0.364, df = 2, P = 0.83), or the Schnabel method (χ^2 = 1.570, df = 2, P = 0.46). The density of specimens is then 18.39 specimens per km of stream length. It is not possible from this rate to infer the

Table 1. Distribution of age classes in the four populations over three periods of monitoring.

Age class	2002 (Blosat 2002)	2005 (Blosat 2005)	2008 (present study)
Stream 1			
juveniles [%]	–	0.0	0.0
subadults [%]	–	22.2	0.0
adults [%]	–	77.8	100.0
Stream 2			
juveniles [%]	–	21.2	6.7
subadults [%]	–	42.4	0.0
adults [%]	–	36.4	93.3
Farm ponds			
juveniles [%]	0.0	0.0	0.0
subadults [%]	20.0	50.0	0.0
adults [%]	80.0	50.0	100.0
Dam 1			
juveniles [%]	–	–	0.0
subadults [%]	–	–	50.0
adults [%]	–	–	50.0

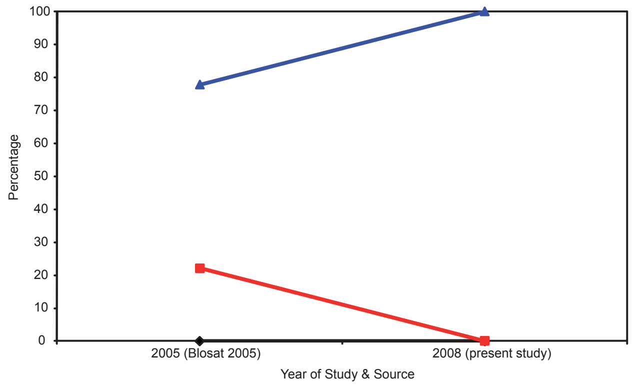

Figure 7. Development of the age structure (percentage) in the population at Stream 1 (blue: adults; red: subadults; black: juveniles).

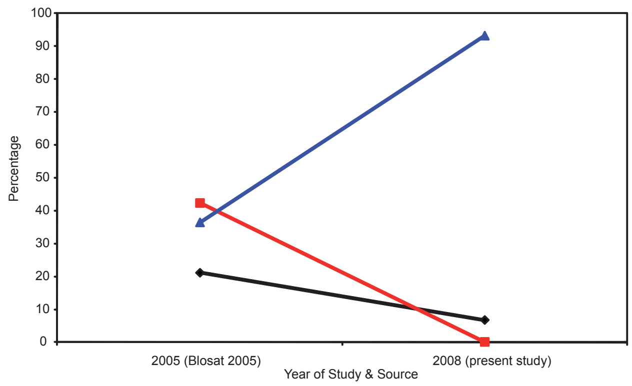

Figure 8. Development of the age structure (percentage) in the population at Stream 2 (blue: adults; red: subadults; black: juveniles).

population size of the whole stream, since the abundance of snakes along the stream is heterogeneous and depends on the number and quality of ponds (pers. obs.). The total number of snakes living at Stream 2 might be more than twice as high as estimated for the sampled transect, i.e., about 60 specimens.

At Dam 1, two specimens were recorded and more were observed. From our observations in 2007 and 2008, the number of specimens observed at this locality is estimated to be about 10. Three individuals were caught at the farm ponds. While 20 specimens were caught at this location in 2002 by Blosat (2002), this number dropped to only two recorded by Blosat (2005) in 2005. She concluded that "it can be stated that a reproductive subpopulation is not existent at this habitat any more" (Blosat 2005: 32). With only three specimens caught in 2008 (all of which were adults), we can confirm her conclusion: although we found specimens of both sexes, their number is far too low to form a viable population. This subpopulation is therefore practically extinct.

In conclusion, the estimated remaining number of specimens of the Cyprus grass snake in the Troodos area as of 2008 comes down to around 90–100 specimens.

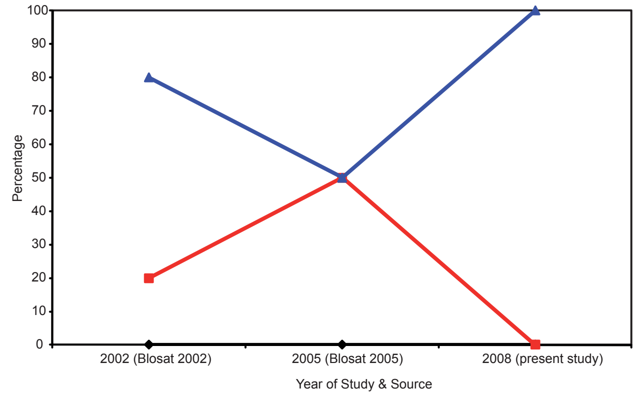

Figure 9. Development of the age structure (percentage) in the population at the farm ponds (blue: adults; red: subadults; black: juveniles).

In addition, the breeding stock of grass snakes from the Snake George Reptile Park, Pegeia, Cyprus, was reluctantly released back into the wild when the park had to close down. In warm January 2009, eleven specimens (five adult males, four adult females, two subadults) were released at Stream 1, and six specimens of the 'Paralimni type' (five adult males and one adult female) were released at the site near Paralimni (Böhme & Wiedl 1994).

Description of possible threats to the remaining populations as of 2008

Stream 1: The surrounding habitat is regularly treated with insecticides during the growing season. It is assumed that part of the waste found in the stream valley (cans, cigarette packs, empty insecticide bottles, etc.) had been dropped by workers applying the insecticides. Also, plants spreading across the middle of the stream valley were regularly cut. Additionally, trout were found at many sites along the stream; they were restricted to a few pools when the river dried up. It is assumed that they were introduced to the system farther upstream where there is easy access to the river. At several places, rubbish tips were noted. Rubbish is thrown down into the streambed from the road adjacent to the stream. Amongst this rubbish, there were car batteries, old paint cans, electronic devices and fridges. At one site, water is taken from one of the deeper pools to irrigate the fields that lie along the stream. This caused a significantly reduced water level of this pond when compared to neighbouring pools from which no water was taken.

Stream 2: This stream is also regularly polluted with insecticides. Rubbish was present here as at Stream 1. One rubbish tip originating from the road along the stream was noted (Fig. 1). No trout or other fish could be observed. There was no evidence of the removal of water for irrigation.

Dam 1: Grass snakes living in this habitat are highly endangered. There are a number of fish species living in the dam (only koi and pumpkinseeds [*Lepomis* cf. *gibbosus*] were identified with certainty, but more species were present) and consequently, the dam is regularly visited by anglers. Furthermore, the dam was enlarged at its southern end recently (in 2007?). This expansion resulted in the destruction of the vegetation in the area. In 2008, the bed and the shoreline in the southern area of the dam were again worked upon with heavy machines, destroying much of the habitat. At one point at least, water was taken from the dam for irrigation; it is unclear to what extent the water from the dam is used. At least, the dam did not dry up completely during the summer months. Finally, large objects have been discarded along the shoreline and occasionally on the bed of the dam. This dam is probably the only dam currently inhabited by grass snakes in Cyprus and it should consequently be strictly protected since dams have proved to be valuable habitats in the past (Blosat 1998).

Farm ponds: The farm ponds are artificial habitats on private land. It is not known to us whether they are treated with insecticides. Two main factors were identified as likely threats to grass snakes at the only remaining pond with basically suitable conditions. Firstly, the pond (Fig. 3) has a fairly large population of fish (carp, koi, probably further unidentified species). Secondly, there is a pipe with a highly pressurised inflow of water, probably coming from the upper pond. This inflow is so strong that it has destroyed the fragile vegetation in its proximity. Furthermore, this inflow creates a current throughout the pond, which hinders the development of the vegetation cover of the pond.

Miscellaneous observations on the natural history of the Cyprus grass snake

Akinesis: An adult male melanistic specimen (snout-vent length 60.0 cm, tail length 16.2 cm, weight 94 g) showed akinesis as a defensive behaviour when it was caught by hand. The behaviour was probably triggered only because the movement of the hand was targeted directly at the snake. The specimen stopped any movement and turned the first third of its body over, with its underside pointing upwards. The lower jaw was slightly lowered, but the mouth was not widely open. Apart from this, the snake did not show any other behaviour commonly associated with akinesis. After a few seconds, the snake turned back over and tried to flee. Unfortunately, it was not possible to take a photo of this scene due to its short duration.

In the past, the Cyprus grass snake has been specifically noted for lacking any form of akinesis as a defensive behaviour (Blosat 1998) and therefore differentiated from other grass snake subspecies which show well-developed akinesis. Here, however, a mild form of akinesis is described, raising the question of why the Cyprus grass snake (apomorphically?) reduced the degree of expression of akinetic behaviour compared to that in other grass snake subspecies. Akinesis may protect the snake particularly from

Figure 10. A. Markedly shortened tail of a specimen from the farm ponds (tail length 15 mm). B. Markedly shortened tail of a specimen from Stream 1 (tail length 22 mm). C. Tail of a specimen from Stream 2, tail tip reduced by ca. 20 mm. D. Tail with healed wounds of a specimen from Stream 2.

birds and mammals. Cyprus has a particularly impoverished mammalian fauna (the Cyprus fox, *Vulpes vulpes induta*, the long-eared hedgehog, *Hemiechinus auritus dorotheae*, and the black rat, *Rattus rattus frugivorus*, are the only potential predators), which, in addition, may all have been introduced by human settlers (Simmons 1999) and thus might have had only limited influence on the early evolution of the Cyprus grass snake, which may have arrived earlier on the island (Blosat 1998, Baier et al. 2009). Similarly, there is no published information on avian predation on the Cyprus grass snake, although birds are likely to be at least occasional predators. Above all, the occurrence of tail injuries in some specimens is primarily attributed to human impact, see below. Thus, the reduction of akinesis may be correlated to the low number of predators. Secondary loss of defensive traits induced by a lower number of predators is often observed in island faunae (Whittaker 1998: 78).

Tail injuries: Six specimens (18.2% of all specimens registered individually) had damaged tails. In three specimens, the tail was markedly shortened so that only a stub remained (Fig. 10A, specimen from the farm pond, tail length 15 mm; Fig. 10B, from Stream 1, tail length 22 mm; third specimen from Dam 1, tail length 77 mm). In two specimens, a short part of the tip of the tail was missing (Fig. 10C, from Stream 2, tail length reduced by ca. 20 mm; second specimen from Stream 1, tail length reduced by ca. 30 mm). Finally, one specimen from Stream 2 displayed healed wounds at its tail (Fig. 10D). The reason for this tail damage is not entirely clear. Of the natural predators, only mammals and birds could conceivably attack but not kill the snake, resulting in the survival of the specimen and the healing of its wounds. There is no published information on avian predation on the Cyprus grass snake (see Baier et al. 2009 for a discussion of predation on the Cyprus grass snake), although birds have often been suspected to prey on grass snakes also in Cyprus.

The relatively “neat” structure of the wounds in Figures 10A–10C and of the second specimen with a missing tail tip particularly suggests that the snakes were attacked by humans with a spade or comparable tools. Snake phobia is a common phenomenon in Cyprus and snakes are regularly killed. Although the habitats of the Cyprus grass snake are probably not often visited by humans, there are certainly interactions with farmers taking water for irrigation, workers applying insecticides, etc. The structure of the wound in Figure 10C and of the second specimen with a missing tail tip, and the shortness of the removed part of the tail might even suggest the use of scissors, as if the snakes were scientifically marked in an inappropriate manner.

Mating behaviour: On 19 July 2008, a couple of adult grass snakes were observed at Stream 2 along the edge of a small pool in the afternoon, while they were in the typical mating position of snakes. The male was coiled around the female and had his tail placed in the region of the female cloaca. However, it could not be observed whether the male had inserted a hemipenis into the female cloaca, since the snakes almost immediately became aware that they were being observed and started to flee. It was possible to catch them; the female (melanistic morph) had a snout-vent length of 72.0 cm, a tail length of 17.5 cm, and weighed 264 g. The male (picturata morph) had a snout-vent length of 80.0 cm, a tail length of 20.5 cm, and weighed 285 g.

So far, only two observations (24 May and 9 June 1995) of mating Cyprus grass snakes have been published (Blosat 1998). Mating takes place from May to early June (Baier et al. 2009), while grass snakes from mainland Europe mate from the end of April to May, sometimes with a second mating in September to October (Kabisch 1999). The current observation therefore suggests that the annual mating season of the Cyprus grass snake, at least in some specimens, may be considerably longer than previously thought. Overall, summer mating in grass snakes seems to be unusual. It is also interesting that in the present observation, the male was longer overall and heavier than the female. Grass snakes are well known to display a sexual dimorphism in size and weight, with females being larger and heavier (Kabisch 1999).

Conservation recommendations

Key results of this study (low total number of specimens in the Troodos subpopulations; negative development of age structure and thus also negative development of reproduction activity; high number of threats to the snakes) indicate that the Cyprus grass snake is on the verge of extinction in the Troodos area. Clearly, snakes are difficult to study in the field and positive or negative years for field studies might alter the outcome of the study. The results demonstrated in this report should thus be verified by another field survey as soon as possible. If the situation described above proves to be correct also in the course of the second study, there is the following critical problem: Low or non-reproduction in the field means that the stock of specimens currently alive is the final pool of grass snakes available. This stock does not appear to be reproducing and thus is not being enlarged or rejuvenated; therefore, without external intervention, it will gradually diminish, and the Cyprus grass snake (at least in the Troodos area) will then become extinct with the death of the last of the currently remaining specimens. In this situation, long-term conservation activities (such as habitat improvements or public education) might not help sufficiently, unless steps are taken to ensure the survival of each individual snake. Therefore, the following twofold strategy is recommended to protect the Cyprus grass snake from extinction: (a) Short-term extinction of the remaining grass snake stock can only be prevented by re-initiating reproduction. This can be done reliably by establishing an efficient captive breeding programme, which comprises most of the remaining Troodos specimens available. (b) Long-term extinction of the Troodos grass snakes can only be prevented if the remaining habitats are optimized for grass snakes so that they can support healthy populations. At present, it would not seem reasonable to release captive-bred grass snakes back into the wild – there are simply too many threats. Thus, habitats (Streams 1 and 2, Dam 1, farm ponds) should be strictly protected, maybe even in the framework of a national park. Threats should be removed and the situation prepared for the release of grass snakes. Both of these recommendations should be put in practice simultaneously – this strategy only works if both of its parts are implemented. In order for the Cyprus grass snake to survive, efficient and fast conservation work is both mandatory and urgent.

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