Migration Behavior of Endangered Dice Snakes (*Natrix tessellata*) at the River Nahe, Germany

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Abstract. The valley of the river Nahe is known to sustain the largest population of dice snakes in Germany. The population is further dispersed into half a dozen subpopulations along the approximately 15 km long Nahe River. A frequented road splits most of the habitat, leading to numerous traffic casualties of snakes every year. Because of its isolation to other populations of dice snakes and its relatively large population size, an active protection of the Nahe population can be considered to be fundamental for the conservation of this nationally endangered species. In 2007, a radiotelemetry study was carried out at one of the subpopulations to analyze the migration behavior of the dice snakes to provide ecological data for future conservation strategies. Five adult female dice snakes were tagged with subcutaneous implanted transmitters to construct movement profiles. The calculated homerange varied depending on the analysis method from 0.22 ha (95% Kernel) to 0.27 ha (MCP). The tagged snakes were mainly active at air temperatures between 20–26 °C and partially cloudy sky. Activity in the water or near the shore was detected every 4–5 days, mostly between 15–18 pm. The daily movement of the tagged snakes was in 80% less than 30 m.

Key words. Activity, Germany, homerange, Kernel density estimation, MCP, mobility, *Natrix tessellata*, radiotelemetry

Zusammenfassung. Die Kernlebensraum der grössten deutschen Würfelnatterpopulation am Fluss Nahe, Rheinland-Pfalz, Deutschland, wird fast entlang des gesamten Flusslaufs von einer teils stark frequentierten Strasse zerschnitten. Hier werden jedes Jahr Dutzende Würfelnattern überfahren. Dies ist eine ernstzunehmende Gefahr für die isolierte Population, die sich entlang des ca. 15 km langen Flussabschnitts auf etwa ein halbes Dutzend Subpopulationen verteilt. Im Jahr 2007 wurde an einem dieser Subpopulationen eine radiotelemetrische Studie durchgeführt, um durch genauere Bewegungskenntnisse effektivere Schutzmassnahmen durchführen zu können. Hierfür wurden fünf adulte Würfelnatterweibchen mit subcutan implantierten Sendern versehen und deren Bewegungsprofile erstellt. Der ermittelte Aktionsraum beträgt je nach Auswertungsmethode zwischen ca. 0.22 ha (95% Kernel) und 0.27 ha (MCP). Die Hauptaktivität der Senderschlangen war bei 20–26 °C Lufttemperatur und wechselhafter Bewölkung. Aktivität im Wasser oder in Ufernähe war etwa alle 4–5 Tage zu verzeichnen, in über 50% der Fälle dann zwischen 15 und 18 Uhr nachmittags. Die täglich zurückgelegte Strecke der Senderschlangen war in 80% der Fälle unter 30 m.

Stichworte. Aktivität, Deutschland, *Natrix tessellata*, Homerange, Kernel-Dichteverteilung, MCP, Mobilität, Radio-telemetrie

Introduction

The Nahe Valley is home of the largest population of dice snakes in Germany (Fig. 1). The valley is not only a suitable habitat for dice snakes and other reptiles, but is also a popular destination for local recreation because of its picturesque landscape and almost mediterranean subclimate. A district road runs parallel to the river, thus dissecting the habitat of the dice snake population. Especially in spring and summer, this road is strongly frequented by several hundred cyclists every day.

In the nineties, the population at the Nahe River was estimated at 250–350 individuals (LENZ & HERZBERG 1996), but a more recent assessment along the entire 15– 20 km long section of the river estimates approximately 800–1400 individuals (LENZ 2007), which are distributed across about half a dozen subpopulations (LENZ 2007). Numerous dice snakes are killed every year by road traffic, mostly during the spring and autumn migrations (NIEHUIS 1996). At one of those migration spots, an increased mortality rate of dice snake has been reported by locals since 2001, despite the installation of a protective guiding fence along the road. Subsequently in 2002 and 2006, this site was the focus of a capture-recapture study and a monitoring of the migratory routes of juvenile dice snakes (LENZ 2007). But these methods provided only limited results, because large parts of this site, especially the shoreline and its preferred basking spots, were difficult to impossible to access. To complement the missing information on this site, a radiotelemetry study was initiated in 2007, incorporated into a MS thesis of the senior author.

The results should provide further data to allow for more effective conservation strategies at this accident hotspot. Main objectives were the identification of the migratory routes, the oviposition sites, diurnal shelter and the hibernaculas. The results of the radiotelemetry study are the focus of this paper.



Fig. 1. Dice snake *Natrix tessellata* from the river Nahe. Photo: Benny Trapp.

Research Area

The research area is located in the German Federal Land "Rheinland-Pfalz" (Eng. = Rhineland-Palatinate) and is part of the natural landscape unit "Nordpfälzer Bergland" (Eng. = Northern Palatinate hill-country). The Nahe River meanders through this hilly country and flows into the river Rhine at the town Bingen. The name of the river Nahe is celtic origin and means "wild river". The investigated area is about 120 m a.s.l. The Nahe is relatively shallow and produces temporarily a strongly fluctuating water flow (level at Boos: 3 m³/s to 694 m³/s), rendering it unnavigable (LENZ & HERZBERG 1996). Thus only a few hydro-engineering measures were carried out in the Nahe Valley, enabling the river to remain relatively natural. It contains extensive gravelbanks and the zones of shallow water yield a rich fish fauna (Fig. 2). Especially the run-offs of the weirs offer a perfect habitat for dice snakes (LENZ & HERZBERG 1996, NIEHUIS 1996).

The valley of the river Nahe is predominated by a xerothermic climate with continental characteristics: high temperatures, sparse rainfall and long vegetation periods (UHLIG 1954).

The mean annual temperature in Bad Kreuznach, the largest town in the district, is 9.5 °C (1961–1990; DEUTSCHER WETTERDIENST), which is 4 °C higher than in the adjacent low mountain ranges of the Hunsrück. The average temperatures in July range 18–19 °C (NIE-HUIS 1996). The vegetation period is six weeks longer than in the nearby Hunsrück. It starts on the 30 April and ends on the 10 October (BLAUFUSS & REICHERT 1992). In Bad Kreuznach the total annual precipitation is 517 mm/m², which is one third less than the German average of approximately 800 mm/m² (1961–1990; DEUTSCHER WETTERDIENST). In the vegetation growth



Fig. 2. Habitat of Natrix tessellata at the river Nahe: Photo: BENNY TRAPP.

period from May–July the rainfall in this area accumulates only 160-180 mm (NIEHUIS 1996). Therefore, the Nahe Valley is one of the warmest and driest regions in whole Germany. Today, the Nahe Valley is shaped by viniculture with many steep and rocky slopes used for cultivating vine grapes or left temporary as fallow land, yielding arid grassland and dry slope. An abundance of crevices and dry stone walls provide an ideal habitat for many reptiles, yielding large populations of grass snakes (Natrix natrix), smooth snakes (Coronella austriaca), western green lizards (Lacerta bilineata) and European wall lizards (Podarcis muralis) (LENZ & HER-ZBERG 1996). The Nahe Valley is also home of the largest dice snake population (N. tessellata) in Germany. Three parts of the valley with a total area of approximately 300 ha have been declared as nature protection areas (NSG) and are also part of the European ecological network Natura 2000.

The district road K58 runs parallel to the shore along most of the river course and is also part of the "Nahe-Radwanderweg" (Nahe bike tour road), which is highly frequented by cyclists from spring to autumn. Every year dozens of dice snakes are killed along this route, especially during the spring and autumn migrations, but also while basking on the asphalt road (NIEHUIS 1996). The road's dissection of the snake habitat is the most serious threat for this isolated population at the Nahe River. The nearest population of dice snakes at the rivers Mosel and Lahn are more than 60 km air distance away and thus, an exchange of individuals is not possible (NIEHUIS 1996). Another relevant threat for the local population at the Nahe River is the disturbance through recreation activities. Despite of a ban by the local public authority, certain tributaries of the river are strongly frequented by canoeists and people with rubber boats in the summer months (NIEHUIS 1996). In summary, the river Nahe can be characterized as an ideal habitat for dice snakes due to a close-to-nature river course, containing numerous gravel banks and areas of shallow water for fishing, and a xerothermic microclimate along slopes, vineyards, and dry stone walls, providing many suitable hiding places and sites for oviposition and incubation.

The survey took place on one of the population hotspots, an approximately 800 m long section along the river Nahe. The center of this area extends over approximately 400 m along the district road. This is the section where the highest number of dice snakes is killed each year. For reasons of protection, I abstain from a more detailed description as well as supplemental map material.

Materials and Methods

General methods followed recommendations by AMR-HEIN (2006). Transmitters (Biotrack Ltd., UK: http:// www.biotrack.co.uk) were spheres with a length and



Fig. 3. Transmitters from different views; the button cell (battery) is visible in the bottom view.

width of 12 mm and a height of 10 mm (see Fig. 3). The weight was 4.5 g with a pulse rate of 40 per minute and a pulse length of 15 ms. The transmitters were designed for a minimum runtime of four months according to Biotrack. A maximum range of 150 m was determined via two blind tests on a plain without obstacles. Measuring inaccuracy at a distance of 75 m was approximately 5 m, at a distance of mare than 120 m up to 10 m. The receiver "TRX-16S" from Wildlifematerials International Inc, USA (http://www.wildlifematerials.com) in combination with a Yagi-antenna was used to detect the transmitter signals.

One main focus of this study was the identification of the local oviposition sites, so only adult females were considered for the implantation of transmitters, which were implanted subcutaneously (Figs. 4 and 5). Isofluran was used for the anaesthetization. After the surgery, the dice snakes were kept in quarantine for 4–5 days before being released at the site of their capture. The implantations of the transmitters were carried out by the veterinarian J. WIECHERT (Mainz, Germany).



Fig. 4. Subcutaneous implanting of the transmitter.



Fig. 5. A dice snake directly after surgery, still numb from the anaesthetization; the red arrow shows the location of the subcutaneous transmitter.

To record the movement pattern of tagged dice snakes, I applied crossing-bearing and homing-in methods. Most of the research area consists of steep slopes with gradients of 60–70% and was difficult or impossible to access. Consequently, the tagged snakes had to be located usually indirectly by crossing-bearing.

The first radiotelemetric localizations were made about one hour after releasing the tagged snakes into their natural habitat. The acquisition of telemetric data started on 15 May 2007 and ended on 13 July 2007. The recordings were usually conducted for six days in a row, followed by a pause of 1–2 days. Daily localizations begun around 9:30 am and ended duirng the sunset between 20 and 21 pm. The tagged dice snakes were located once per hour and crossing-bearing data measured within 1–3 minutes. Along with each localization the air temperature and the cloudiness were recorded.

Data collected from the release site until the last registered movement were used to calculate the home range, which was analysed with the Multi-Convex-Polygon-method (MCP) (MOHR 1947) and the Kernel-density-estimation (HALLER 1996, WORTON 1989). For the construction of the Multi-Convex-Polygon the software "Convex_Hulls v.1.23" (JENNESS 2007) was applied. The more significant Fixed-Kernel-density-estimation was carried out with Hawth's Analysis Tools for ArcGIS (BEYER 2004). This study was authorized by the public authority "Struktur- und Genehmigungsdirektion Nord – Rheinland Pfalz", file number: 425-104.133.0701.

Results and Discussion Behavior Results

Five mature female dice snakes were tagged with transmitters, whereof only three individuals provided sufficient data over an extended duration for further analysis (Tab. 1). The first localizations of any snake yielded no data, because the signals of the transmitters were missing for 2–4 days following the release of the snake. The snakes presumably continued to remain hidden deep in a crevice, where transmitter signals were absorbed by the rock, in order to completely heal their wounds after surgery.

The calculated home range varies depending on the analysis method from 0.23 ha (95%-Kernel-density estimation) to 0.27 ha (Multi-Convex-Polygon-method). These calculated home ranges cover only the summer habitat without spring- and autumn migrations. The re-

Tab. 2: Calculated Home range of adult female dice snakes; Multi-Convex-Polygon (MCP) and Fixed Kernel estimation.

	МСР	95% Kernel	90% Kernel	50% Kernel
Snake No. 1	3549 m²	2389 m²	1405 m ²	281 m ²
Snake No. 2	1746 m ²	1830 m ²	1259 m ²	321 m ²
Snake No. 3	2798 m ²	2669 m ²	1530 m²	278 m ²
Average	2698 m²	2296 m ²	1398 m²	293 m²

Tab. 1: Basic data on dice snakes tagged with transmitters; "No. of days recording/location points" is the time span between date of release and date of last reported activity.

Tagged snake	No. 1	No. 2	No. 3
Date of capture	10 May 2007	10 May 2007	30 May 2007
Date of release	15 May 2007	15 May 2007	5 June 2007
Sex	Ŷ	P	Ŷ
Total-length [cm]	93	94	96
Weight [g]	302	256	306
Reproductive status at capture	None	None	Gravid
Later recapture/sighting	No	No	Recapture
Duration of telemetric recording	15 May – 13 July	15 May – 13 July	5 June – 21 June
No. of days recording	40	15	13
No. of location points	257	88	97
Weight ratio transmitter/body (%)	1.49	1.76	1.47

sults also indicate that females dice snakes were rather loyal to their site (Tab. 2; 50% Kernel). Their home ranges stretched maximally 100 m parallel to the shoreline and up to 15 m deep on land. In central and southern Switzerland (BENDEL 1997, CONELLI & NEMBRINI 2007, CONELLI et al. 2011) and in Prague, Czech Republic (VE-LENSKÝ et al. 2011), researchers observed similar to more distant movements of dice snakes in the summer, usually along a stretch parallel to the shoreline of 100–500 m, and max. up to ~ 1000 m. But their spring and autumn data show increased distances migrated to and from the hibernation sites.

The radiotelemetered data revealed that female dice snakes stayed mostly on land during the day. The hiding places of the snakes were in the crevices of a steep slope with a south western exposition, approximately 5 m above mean water level. The maximum distance to the shoreline was less than 10 m. The entrance of the crevices and its proximity was often used as a spot to thermoregulate. In the case of an imminent threat such as an approaching person, the snakes were able to retreat quickly to their hiding place within approximately 3 seconds. Even though there was sufficient areas with trees and srcub, radio tracked dice snakes on land were always located in semi-open to fully open, rocky segments of the shore environment. The principal terrestrial habitat that was used for thermoregulation and as nocturnal shelter consisted of a road-supporting wall made of large block stones (Fig. 6). Registered movements were normally short migrations from their shelter to other basking spots with better expositions or less vegetation, usually not farther away than 5–15 m. All tagged dice snakes were located in their terrestrial habitat within 15 m from the shoreline. The average daily covered distance was less than 30 m in about 80% of all localizations. The longest registered daily movement by a dice snake was 250–260 m, from which ~200 m were covered in water.

Every 4–5 days, the female dice snakes descended from their basking spots or shelter high up on the sloped wall, passed a semi-open area, containing a tree, a few bushes, and plenty of herbaceous plants (Fig. 7) to finally enter the water for 1–2 hours of fishing. The successful snakes landed with the fish at the semi-open area to swallow it and returned right after consummation to their shelter to digest (Fig. 6). Foraging activity



Fig. 6. Radio tracked dice snakes were predominantly located in this open, road-supporting wall made of large block stones that were used for thermoregulation and nocturnal sheltering.



Fig. 7. This semi-open area was passed by the dice snakes to enter the water, and used again to consume a fish that they brought on land. Shortly after complete ingestion, they returned to their basking site to digest the prey (see Fig. 6).

(entering the water) was rarely before 12 am (less than 10%), but was recorded mostly in the afternoon between 15-18 pm (~55.9%) (Fig. 8). These results resemble the observations by LENZ & GRUSCHWITZ (1993) from another German population of dice snakes, where 10% of active individuals were observed up to 12 am and ~80% between 13 and 17 pm. Observation on Czech dice snakes point towards a similar behavior (LANKA 1978). On days with registered movement in the river, the daily distance of radiotelemetred snakes covered was usually between 50-100 m. The active outdoor residence times were short, as most snakes remained in the water or in close proximity during one or two hourly localisations (Fig. 8). Average residence time was ~90 minutes; longest residence time was ~150 minutes. This contrasts the statement that dice snakes in a proximate population at the river Lahn spend most of the afternoon in the water (LENZ & GRUSCHWITZ 1993). However, those data were not acquired through independent radiotelemetry nor constant observation of individuals, hence, terrestrial stay in the afternoon may have been missed when the snakes remained invisibly hidden on land.

The rare and short residence times in the river are also contrary to the information of MERTENS (1947) and BLAB & VOGEL (1996) who noted that dice snakes stay for several hours in the water. But the short stay in the water in this study might relate to a behavior strategy of gravid females in which they prevent cooling their body to promote embryogenesis. In this context, they may search for a terrestrial site with better conditions for thermoregulation and ultimately oviposit away from the water or simply stay on land to thermoregulate and accelerate embryogenesis (DUSEJ 2007, MEBERT 2011). Observed time spans of up to two weeks in which the gravid snakes did not visit the river suggest such a particular behavior.

The different distances moved by each of three snakes are shown in Figure 9. Snake No. 1 provided the largest amount of data (40 days, 257 location points, cf. Tab. 1). Snake No. 2 seemed to be the most active one with frequent short movements. However snake No. 3, which remained most of the time in hiding, covered the longest single daily distance of about 250 m. But on the day of the long distance movement, the Nahe River expe-



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Average 60 50 40 n = 68 [%] 30 20 10 0 no activity 10-30 30-50 50-75 75-100 100+ (<10) daily movement in [m] dice snake No. 1 70 60 50 n = 40 40 [%] 30 20 10 0 no activity 10-30 30-50 100+ 50-75 75-100 (<10) daily movement in [m] dice snake No. 2 60 50 40 30 [%] 20 10 0 10-30 30-50 50-75 75-100 100+ no activity daily movement in [m] (<10) dice snake No. 3 90 80 70 60 50 [%] n = 13 40 30 20 10 0 10-30 30-50 75-100 100+ no 50-75 activity daily movement in [m] (<10)

Fig. 9. Daily movement of tagged snakes; n = number of recorded days (cf. Tab. 2); less than 10 m movement is categorized as "no activity", because of possible measuring inaccuracy.

Fig. 8. Daytime activity of tagged snakes in water or on land at a distance of approx. 5 m from the shoreline; n = number of hourly localizations in water or near shore; d = number of days with localizations in the river or on land near the shore; x = n/d

daytime

rienced a high tide from heavy rainfall of the previous days. Hence, this distance may be a result of a drift and a remigration (LENZ & GRUSCHWITZ 1993).

This study has further confirmed the observations made by TRUTNAU (1981) and LENZ & GRUSCHWITZ (1993) concerning the relation of air temperature and main activity. The activity peak of snakes No. 1 and No. 2 could be registered at a temperature range of 20–25 °C and partially cloudy sky, which is in accordance to the hitherto assumed range of preferred air temperatures between 20–26 °C, and 19–26 °C respectively.

Problems with Radiotelemetry

Originally, it was intended to collect data from the mating season in May (for the identification of the mating places) until the start of hibernation in September/October (for the identification of the hibernation sites). Therefore, transmitters were chosen with a minimum operating time of 4 months. Due to the planed operating time, relatively large transmitters were selected, which may be able to have biased the snake's behavior. Yet, the weight ratio transmitter/body was substantially below the recommended (KENWARD 2001) maximum of 5% (cf. Tab. 1). Another issue that might had an effect on the snake's behavior concerns a possible postsurgery trauma. The transmitters greatly stretched the skin around the implantation area (Fig. 5), which may have caused the snake to behave unnaturally for a couple of days by remain hidden for a longer period after its release than usual.

Movements of snake No. 1 have been recorded nearly up to the end of this study. Apparently, this snake had the least problems with the implanted transmitter. Snake No. 2 appeared to produce problems with the transmitter, because signals indicated a termination of its movements three weeks after its release. The transmitter signal was located directly under the asphalt of the district road at a site which is used by the dice snakes as a resting place. The signal accuracy was determined to half a meter using homing-in-method. Either dice snake No. 2 was able to successfully stripe off the transmitter or it died in its resting place. No verification was possible due to the inaccessible location under the asphalt. After the last registered movement of this snake, an additional 166 localisations were taken over a time span of about 3 weeks until the end of the radiotelemetric survey.

Snake No. 3, which was recaptured during the survey, had shedding problems along the suture of the implantation area and the transmitter was visible through an open wound in the skin (Fig. 10). The snake excoriated its skin, presumably to get rid off the implanted transmitter. Fortunately the wound was dry and not inflamed. After the recapture, snake No.3 was brought back to the veterinary clinic and the transmitter was removed. The snake was then kept for ten days in a terrarium for recovery, and subsequently released into its natural habitat (Fig. 11).

While recovering in the veterinary clinic snake No. 3 laid 16 eggs in the night from 2 to 3 July. The eggs were incubated at 27 °C and hatched after 37-41 days and a last egg was laid after 47 days. One ovoposition site was later found in autumn by digging suspected substrates. The site contained 70 open eggs in a compost pile in about 20 cm depth. The pile was located ~50 m away from the shoreline and about 1.5-2 m above the waterline. On noon of 13 July an exhausted, emaciated adult female dice snake was found only 3-4 m away from the compost pile. This observation suggests that this individual has just laid eggs on this day or the night before and that the clutch is indeed from at least one dice snake. The time span for oviposition is nearly the same as VELENSKÝ et al. (2011) observed in a Czech population of dice snake (5–15 July).



Fig. 10. Tagged snake with an open, dry wound. The transmitter is visible (red arrow). Apparently the snake had some shedding problems because of the surgery seam.



Fig. 11. The same snake approx. two weeks after removal of the transmitter on its release day. For disinfection and a better healing, Dentisept*-salve was applied on the wound.

The radiotelemetric survey ended abruptly on the 13 July, a defective contact in the receiver disabled the continuing of data collection. It was not feasible to acquire a substitute receiver within 2 weeks. At this time, snake No. 4 had been tagged only for 11 days with 47 localisations and snake No. 5 for less than a week, therefore the collected data were insufficient to be included into the analysis (less than the recommended 30 localisations by KENWARD, 2001). Without a receiver it was impossible to track the tagged snakes in the following weeks, a period in which the visible activity (or outdoor presence) of adult dice snakes decreases drastically about 90% at this site. Such a decrease of dice snake activity in the summer has been observed by several authors (HECHT 1928/1929, LANKA 1978, LENZ & GRUSCHWITZ 1993, ME-BERT 2001, 2007) and is presumed to relate to an aestivation (summer rest) period.

Conclusions

It was clear at the start of this project that only some of the goals could be accomplished due to the tight schedule allowed for a M.S. study (usually only three months are permitted by the university for data acquisition until finished draft). So it came to no surprise that not all goals of this survey could be achieved. For example, the identification of the migratory routes of dice snakes failed, because of the insufficient movement data of the tagged snakes and the short duration of the radiotelemetric survey in the spring/summer (May-July), which was outside the migration period. Such migrations routes of dice snakes in early spring and autumn have been detected in radio-tracked dice snakes from Ticino, southern Switzerland (CONELLI & NEMBRINI 2007, CONELLI et al. 2011) and in Prague, Czech Republic (VELENSKÝ et al. 2011). The relatively late start at 9:30 am for the daily radiotelemetric localizations was presumably the reason for the failed identification of the oviposition sites. LENZ & GRUSCHWITZ (1993) suggested that the egg laying takes place at night or in the early morning, which was confirmed by the overnight oviposition of snake No. 3. Luckily, information about oviposition sites was acquired after finding such a site in autumn by digging suspected substrates.

Due to the breakdown of the receiver, it was not possible to locate the hibernation sites in late autumn. Despite of the scarce data, this study provided some interesting information about the behavior of local dice snakes, such as the reduction of movements in gravid females. Because of the presumably greater mobility of male than female dice snakes (see refs. in CONELLI & NEMBRINI 2007), tagging of male dice snakes may be more appropriate for future identification of migratory routes, but the issues with their lower weight, and thus the requirement of smaller transmitters, should be resolved first. According to A. CONELLI (pers. comm.), the telemetry method should be improved in order to i) reduce the size of transmitters while enhancing their life span, ii) simplify the implantation procedure, thus minimizing the impact on the animals' physiology and health, and iii) reduce the effort by operators in the field (e.g. apply automatic GPS localization and data analysis). Finally, collecting data over more than one year may produce more reliable information than a short survey over only a couple of months.

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