RESEARCH OF THE HABITAT AND PREY PREFERENCE OF THE DICE SNAKE (*NATRIX TESSELLATA*) AND THE GRASS SNAKE (*NATRIX NATRIX*) IN GLOBAL, REGIONAL AND HABITAT SCALE

Thesis of dissertation

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Background

Importance of river research

Disappearance of several plant and animal species, invasion of exotic species, the breakdown of processes of the controlling system, diminishing value of the landscapes, the loss of undisturbed areas indicate the large-scale transformation of river habitats. Degradation of the natural ecological systems has been recognised not only by ecologists by today but also by the society and governments, which leads to an increasing demand for improvement in the ecological status of regulated waters.

In addition to our incomplete knowledge about aquatic systems, even less is known about the dynamics of their border habitats and the ecology of the species there. This resulted in certain changes in river ecology research in the past few decades as growing attention is paid to the research of processes in ecotones between both aquatic and aquatic-terrestrial systems. The spatial and temporal dynamics of ecotones have significant impact on the diversity of habitats and communities. It is specially true for those species composition which, for some reason, depend on the presence of the ecotones partly or fully

Study species the dice snake (*Natrix tessellata* Lauretti, 1768) and the grass snake (*Natrix natrix* Linnaeus, 1758)

Out of all snake species in Hungary, dice snakes and grass snakes prefer aquatic habitats and riparian zones of rivers in particular, the most because these habitats have their main prey species, fishes and amphibians, in large numbers. In order to understand the ecology of both snake species, it is important to explore the processes in the Hungarian section of River Danube, since the described changes (temporal changes of floods, spreading of invasive species) have their effect on native species, among them on both snake species, though to varying degrees. At the same time, our knowledge is incomplete about the ecology of fish, amphibian and reptile species in the riparian zone of the Danube section and the, which makes it difficult to define the realistic policy of nature conservation in certain situations.

Aims of the study

The two aims of my doctoral thesis are (i) to understand the temporal and the spatial dynamics of the dice snake, the grass snake and their potential prey items in three tributaries of River Danube, in River Ipoly, in Sződrákos and Ilka stream and the estuaries section in

River Danube and (ii) to compare the prey composition of sampled individuals at five areas of Hungary to the data of specialist literature. My study is aimed to give answers to the following questions:

1. How the dice snake and the grass snake adapt to the different prey composition parallel with the habitat preferences in different water level fluctuation in the three river-tributary systems.

2. Which species the dice snake and the grass snake eat in the five different areas of Hungary, and what relationships are shown between the sampled data and the data from the whole distribution area of both *Natrix* species.

I. Research of the relationship between the habitat and the potential fish and amphibian prey species preferences of the dice snake and the grass snake

Material and methods

The potential fish and amphibian prey items as well as the snake species were sampled by electrofishing on the sampling section of the River Danube and the estuary and the upper section of tributaries in 2012-2013. Point- abundant sampling method was used in studying the composition of juvenile and adult fish and larval, juvenile and adult amphibian species, every other week from the beginning of March to the beginning of December. Ten sampling sections were selected for each river-tributary system in my research. Altogether 30-30 point-samples were collected in each sampling session on every occasion, and the sampling sites were characterised by seven abiotic and four biotic environmental variables. The fish too small to be determined properly on the site were preserved in 4% formaldehyde and taken to the laboratory for microscopic examination and measurements while all individuals of amphibian species were determined in the field and set free.

Single- and multivariate statistical methods were used to analyse the collecting data of the dice snake, the grass snake and their prey items:

Sampled-based rarefaction curve analysis was used to examine the relationship between the number of prey species and the samples, and individual-based rarefaction curve was used to examine the relationship between the number of prey individuals and the samples in all the three tributaries.

Cluster analysis and non-metric multidimensional scaling (NMDS) were used to analyse the temporal and spatial distribution pattern of the snakes and their prey species in three research areas.

Correspondence analysis (CA) was used to analyse which prey species could connect to the dice snake and the grass snake at the time of sampling in the three river-tributary systems.

Redundancy analysis (RDA) was used to analyse the effect of the determinative environmental variables for the spatial distribution and habitat preferences of the dice snake and the grass snake as well as their potential fish and amphibian species, and that of the dice snake and the grass snake and the juvenile fish classified by size and amphibian prey species classified by age.

Results and discussion

All in all, 208 individuals of dice snake and 209 individuals of grass snake were observed under fielded research and 11782 individuals of 34 fish species and 5625 individuals of 11 amphibian species were caught and identified. Large floods did not occur either in River Danube or in the tributaries during the first year of the research (2012), while during the second year (2013) several record floods took place in River Danube and its tributaries. Two years of sampling with different water-level fluctuation allowed a comparative analysis of the effect of water level on sampling.

The rarefaction analyses demonstrated that the individuals of the dice snake and the grass in three partly independent river-tributary systems occur in those habitats where the number of potential fish and amphibian prey species is not below 8-10 and the number of individuals of prey items is 15-40 in a section of 100-120 meters. Different trends were not found between the species during number-based rarefaction analyses, but they were found in the individual based rarefaction analyses in the three sampling areas in both years.

Priority effect of seasonal water level fluctuation was proved for the spatial and temporal dynamics of prey species and through them for the dice species in the three river-tributary systems. Most adult and young individuals of dice snake and grass snake were found in those sections of River Ipoly and Stream Sződrákos where the most diverse fish and amphibian species composition were measured, because River Danube and the tributary effect not only the fauna composition but also the hydrological connectivity of the section. Such relationships could not be found in Stream Ilka which is regulated with a sluice. Results of the analyses of seasonal dynamics proved that the species composition were similar in River

Danube - Stream Ilka and River Danube - Stream Sződrákos systems both in the years with low water level and with several floods. My results show that the presence or absences of floods have no effect on the prey species composition in the autumn samples. Effect of the spring and summer floods on prey species composition can be detected until end of summer, early autumn. By this time the most amphibian species were developed and left the aquatic habitats, so the prey composition of the dice snake and the grass snake are composed primarily of fish and those amphibian species which connect strongly to the aquatic habitats. By contrast, the species composition in the autumn seasons of both years were different in River Ipoly which includes a higher number of prey species..

Redundancy analysis (RDA) was used to analyze the effect of determinative environmental variables on habitat preferences of the dice snake, the grass snake and their potential fish and amphibian species and the size-classed juvenile fish and age-classed amphibian prey species. Due to the high abundance of taxons measured during the analyses, close relationship was indicated between the dice snake and grass snake and the distribution patterns of their prey species in all river-tributary systems. Analyzes show that species preferring lentic water habitats (common spadefoot, European bitterling, green frogs) and species which like such habitats (monkey goby, perch, pumpkinseed) and the dice snake and grass snake are determined by the same environmental variables. RDA for dice snake, grass snake and classed juvenile fish and amphibian prey items showed they favoured slow-flowing or still sections with aquatic macrophytes and a gravelly, sandy or muddy bottom. Moreover, fish 30 mm and longer positively influenced the presence of the dice snake, as it occurred more frequently at sections where such optimal prey size fish was present. It is related to the sit and wait hunting strategy in the aquatic macrophytes. As for the grass snake, the strongest relationship was related to the terrestrial macrophytes which are inundated by the floods along the banks of rivers or on water surfaces. Several amphibian species and a lot of juvenile and adult fish individuals prefer these shady habitats. My research showed that the occurrences of both snake species are determined collectively by the presence of prey species and environmental variables in River Danube and at the three tributaries. In my view, so as to establish more accurate relationships between the age groups of the dice snake and the grass snake more sampling is required.

II. Research of the relationship between the distribution and the feeding spectrum of the dice snake and the grass snake

Material and methods

In order to examine the feeding spectrum of the Hungarian population of the dice snake and grass snake, individuals killed on normal and on bicycle roads in five regions of the country were collected between 2013-2016 and, after removing the remnants of the prey items by dissection where it was possible, identified and the prey database of the regions concerned was compiled.

Scientific and other documentary sources presenting the feeding ecology of the dice snake and grass snake in their distribution area in details were collected for comparative analyses of the literature sources. When summarizing the scientific results, I also used digitalized photos and films by professionals and laymen from webpage on nature photos and filming eg. www.dght.de, www.herpetozoa.at, www.shdmr.org, www.varangy.hu.

The number of prey items of the dice snake and grass snake, their habitat preferences (marine, freshwater, terrestrial) and frequency (quantitative data, rare, frequent, common) were collected into higher taxonomical groups and the prey species were defined for the countries of the distribution area. Using the presence-absence data of the prey taxa of the dice snake and the grass snake, association analysis was carried out by way of non-metric cluster analysis to elucidate zoogeographical differences among the countries. Additional association analyses with the presence-absence data of the prey fish species of the dice snake and the grass snake were used to define the degree effect of non-native fish species for zoogeographical relations.

Results and discussion

All in all, 259 prey items were explored by dissection and identified partially from 256 individuals of dice snake and 357 individuals of grass snake from five areas of Hungary. During my research 26 fish and amphibian prey species were identified for dice snakes, whereas 23 fish, amphibian and reptile prey species were identified for grass snakes. My research proved that the dice snake consume amphibians as well as fishes in the habitats of Lake Balaton and River Danube, and the grass snake consume reptiles as well as fishes and amphibians in the habitats of River Drava and Stream Koppány.

Processing the scientific and other sources, I managed to identify 140 prey species from 25 countries from the distribution area of the dice snake, 103 prey species from 36 countries from the distribution area of the grass snake. The species belong to seven zootaxonomic classes (Gastropoda, Insecta, Pisces-Actinopterygii, Amphibia, Reptilia-Diapsida, Aves, Mammalia). The largest number of prey groups was fish for the dice snake, and the same number of fish and amphibian prey species for the grass snake. The importance of non-fish species in the diet of the dice snake was especially pronounced in deserts, high mountains and in dry Mediterranean areas. In the feeding spectrum of the grass snake, fish and amphibians are important for populations living near large water bodies, while the importance of other prey taxa (reptiles, birds, small mammals) grows for those living in dry habitats, deserts, in the Mediterranean or in the mountains, like in the case of the dice snake.

During the analyses of the feeding spectrum of the dice snake, the non-metric cluster analysis divided the 25 countries into four main groups, and in the case of the analyses of the feeding spectrum of the grass snake the 36 countries were divided into eight main groups by the non-metric cluster analysis. Analyses with prey lists without non-native fish species show that the non-native fish species affect the feeding spectrum on the whole distribution area for both the dice snake and the grass snake. The effect is more significant for the dice snake as in the, analysis without non-native fish species the countries are in biogeographical groups, which are modified by the effect of the non-native species. Certain countries may belong to groups which are not only located at big distances but also belong to different biogeographical groups. Considerable rearrangement is seen for the grass snake, too, as the analysis without the non-native fish species put the countries concerned into two larger groups instead of the eight groups based on the total prey list. One group has several Mediterranean countries near the Ponto-Caspian region while the other group includes several European countries on basis of the biogeographical region. Analyses for the drainage area of River Danube showed the importance of non-native fish species in the feeding spectrum of the dice snake and the grass snake in all the 13 countries because not only Ponto-Caspian gobiid but several non-native species which were introduced for economic purposes spread and appeared in the diet of both species.

New scientific results of the dissertation

1. I made the first complex habitat and prey preference analyses of the dice snake and the grass snake in the habitats of River Danube and some tributaries.
2. I showed what number of potential prey species and individuals are necessary for the dice snake and the grass snake to occur in three partly independent river-tributary systems.
3. I described the effect of seasonal water level fluctuation of the co-occurrence for the prey species and the dice snake and the grass snake composition.
4. I showed that the water level have important role for the prey composition and the habitat preferences of the dice snake and the grass in all three river-tributary system.

5. I managed to sample quantitative data on the diet of the two snake species from Hungary and thereby the feeding of fish and amphibians by the dice snake and feeding of the fish, amphibian and reptiles by the grass snake in five regions of Hungary for the first time.
6. I compiled the prey list for nearly the whole distribution area of the dice snake and the grass snake by processing the scientific and other sources.
7. I showed that the non-native fish species have a role in the prey composition for both the dice snake and the grass snake.

Publications and manuscripts

Articles included in the dissertation

- WEIPERTH A. (2014): **Analysis of structure, composition, spatial and temporal changes of juvenile fish community in a Danube-tributary system in the Middle Danube River Basin**. *Acta Zoologica Bulgarica Supplement 7: 45-50.*

- WEIPERTH A., GAEBELE T., POTYÓ I., PUKY M. (2014): **A global overview on the diet of the dice snake (*Natrix tessellata*) from a geographical perspective: foraging in atypical habitats and feeding spectrum widening helps colonisation and survival under suboptimal conditions for a piscivorous snake**. *Zoological Studies 53:(1): 42, 9 p.*

- WEIPERTH A., POTYÓ I., PUKY M. (2014): **Diet composition of the dice snake (Natrix tessellata Laurenti, 1768) (Reptilia: Colubridae) in the Danube River Catchment Area**. *Acta Zoologica Bulgarica Supplement 7: 51-56.*

- WEIPERTH A., STASZNY Á., FERINCZ Á. (2013): **Idegenhonos halfajok megjelenése és terjedése a Duna magyarországi szakaszán – Történeti áttekintés** [**Occurrence and spread of nonnative fish species in the Hungarian section of River Danube - A historical review**]. *Pisces Hungarici 7: 103-112.*

Other publications

- BÓDIS E., BORZA P., POTYÓ I., WEIPERTH A., PUKY M., GUTI G. (2012): **Invasive mollusc, macrocrustacea, fish and reptile species along the Hungarian Danube section and some connected waters.** *Acta Zoologica Academiae Scientiarum Hungaricae 58 (Supplement 1): 29-45.*

- GAEBELE T., POTYÓ I., WEIPERTH A., GUTI G., PUKY M. (2013): **Abundant prey or optimal microhabitat? *Natrix tessellata* stays hidden in safe areas in a diverse floodplain along the Danube at Göd, Hungary**. *North-Western Journal of Zoology, 9(2): 374-382.*