"BABEŞ – BOLYAI" UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY
DEPARTMENT OF TAXONOMY AND ECOLOGY

FAUNISTICAL AND ECOLOGICAL STUDIES ON AMPHIBIANS FROM PODIŞUL DOBROGEI

Doctoral thesis summary

Scientific supervisor: Prof. Nicolae Tomescu, Ph.D.

Ph.D. student: SZÉKELY JÓZSEF-PAUL

Cluj-Napoca 2010

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Key words: amphibians, distribution, Red List, Dobrudja, adaptive phenotypic plasticity, larval period, metamorphosis, spadefoot toads, *Pelobates fuscus*, *Pelobates syriacus*, growth rate, reproduction, sexual dimorphism, body size

Introduction

The present thesis is structured in two main parts, preceded by a general chapter presenting the short history of the herpetological studies from Dobrudja (chapter 1) and the general characterization of the study area (chapter 2). In the first part (chapter 3) we synthesized the available distribution records for the Dobrudjan amphibian species from published papers, museum collections, and our own field data. Based on distribution records we performed a cartographic analysis consisting of three indexes that attempt to indicate shifts in geographic range over time. Using these indexes as a measure of the conservation status we proposed a Red List for the amphibians of Dobrudja based on the distribution data and the changes in species ranges before and after 1990.

The second part (chapters 4-8) is dedicated to the two *Pelobates* species inhabiting this region. Dobrudja is an important region for the study of this spadefoot toad genus because it represents, on one hand, the southern limit of the distribution area of one species (*Pelobates fuscus*), and, on the other hand, the northern limit of the other (*Pelobates syriacus*), by such representing an ideal place for the study of the interaction between the two species. Due to its drier climatic regime, Dobrudja seems to offer better survival conditions to *P. syriacus* (which is a species adapted to semi-arid environments) than to *P. fuscus* (which prefers the colder and wetter climate of Central and Western Europe). In these chapters we analyzed the comparative adaptive response of the two *Pelobates* species to the specific climate regime from Dobrudja, observing the ability of tadpoles to cope with the drying of the breeding habitat and the post-transformational growth rates of the metamorphs in these dry weather conditions. Also, the aim of the present thesis is to bring new data about the biology and ecology (with special emphasis on the reproduction biology) of these amphibians, which due to their nocturnal and fossorial nature, are poorly understood.

The chapters 3-8 are partially independent papers which have been published or are in the process of being published in national or international scientific journals. Any of these chapters can be approached independently because each of them includes an introductory part presenting the theoretical background of the analyzed subject. The main differences between the chapters and the published papers are represented by the amount of details presented, which had to be omitted from the papers due to the limited space available in the scientific journals and the references list format which has been merged into one for the whole thesis.

On the other hand, these chapters can be seen as a whole, as an attempt to provide a comprehensive and accurate account of the present situation regarding the amphibians species from Dobrudja. Also, an important aim of this thesis was to provide additional data about the biology and ecology of the spadefoot toads from this region and so, to contribute to the knowledge of two of the most interesting amphibian species from Romania.

Acknowledgments

First of all I would like to thank my scientific advisor, Prof. Nicolae Tomescu for his guidance, advices and encouragement and especially for the patience he put for in conducting this Ph.D. thesis. I am especially indebted to Prof. Dan Cogălniceanu for his invaluable help. The knowledge gathered during the last few years of working together with him proved to be especially useful and for this I am forever grateful.

I want to thank Prof. Ioan Coroiu for his advices and for his support since the beginning of my studies. My sincere thanks go to Prof. Marius Skolka and Prof. Teodora Onciu for their help in conducting the experiments and also for their kindness. I thank Marian Tudor for his friendship and help since I arrived in Constanţa and for sharing with me his field data.

I also want to thank my friends Sebastian Bugariu, Alida Barbu, Răzvan Zinică, Elena Buhaciuc, Călin Hodor and Daniela Iordache for their precious help on the field. I owe my gratitude to Hartel Tibor for the interesting and challenging discussions and to Nemes Szilard for his valuable help and collaboration. I thank Cristi Domşa and Laurenţiu Rozylowicz for their help with the GIS maps and I am indebted to my colleagues from the Romanian Ornithological Society for their patience and support.

Finally, I wish to thank my family for the help and encouragement given all these years. This thesis is dedicated to Diana, because without her help I could not been able to finalize this work.

I.

Short History of the Studies on the Amphibians from Dobrudja

At the beginning of the 20th century, the works of CONSTANTIN KIRIȚESCU (1876-1965), considered to be the founder of Romanian herpetology, are published. His most important work is published in 1930, "Studies on Romanian herpetological fauna", where he describes in detail a new subspecies of newt, *Triton cristatus* var. *dobrogicus*, which had been reported for the first time in 1903 from the Danube Delta. The first work entirely dedicated to Dobrudja is the paper published by the great GRIGORE ANTIPA (1867-1944) in 1911, in which he describes the biodiversity of the Danube Delta.

In 1954, MIHAI BĂCESCU (1908-1999) described a new anuran species from Dobrudja, the eastern spadefoot toad (*Pelobates syriacus balcanicus*), so enriching the list of amphibian species in our country. During the 60's seven papers referring to Dobrudja are published, the most important of which is by far the grandiose work of ION EDUARD FUHN (1916-1987), fascicle no. 1 from volume XIV of Romanian Fauna: Amphibia, in which all the data available at that time referring to Romanian amphibians was synthesized. In the 70's eleven articles were published, five of which were faunistical studies, the most important being that written by MARIA POPESCU (1977); here the author reconfirmed the presence of *Rana dalmatina* in Dobrudja, in Tulcea county. During the 80's there have been published six papers, the most important are those belonging to ornithologist KISS J. BOTOND, who had described the presence of *Bufo bufo* in Danube Delta.

Starting with 1991 the number of publications steeply rises (over 50 papers published between 1991 and 2009), indicating the growing interest of Romanian herpetologists in this region. For Dobrudja this period is characterized by the publishing of papers that are mainly concerned with the distribution of amphibian species in various areas and with the identification of conservation issues, many works being published by TATIANA KOTENKO, VASILE OȚEL and TÖRÖK ZSOLT. Meanwhile, DAN COGĂLNICEANU published his synthesis work on amphibian distribution in Romania (1991) in which the author updated and completed the data gathered since FUHN's Amphibia in 1960.

II.

General Characterization of the Study Area

The study area is located in Dobrudja, a unique region in Romania due to the presence of the Pontic bioregion but also due to its special weather conditions resulted from the influence of two bioregions: Pontic and Steppic (fig. 2.1). The faunistical and ecological studies were carried out in Podişul Dobrogei (Dobrogea Plateau), ranging on both counties, Constanţa and Tulcea. This morphostructurally complex area is composed from three morphotectonic units: Dobrogea de Nord (Northern Dobrudja), Dobrogea Centrală (Central Dobrudja) and Dobrogea de Sud (Southern Dobrudja; fig. 2.2).

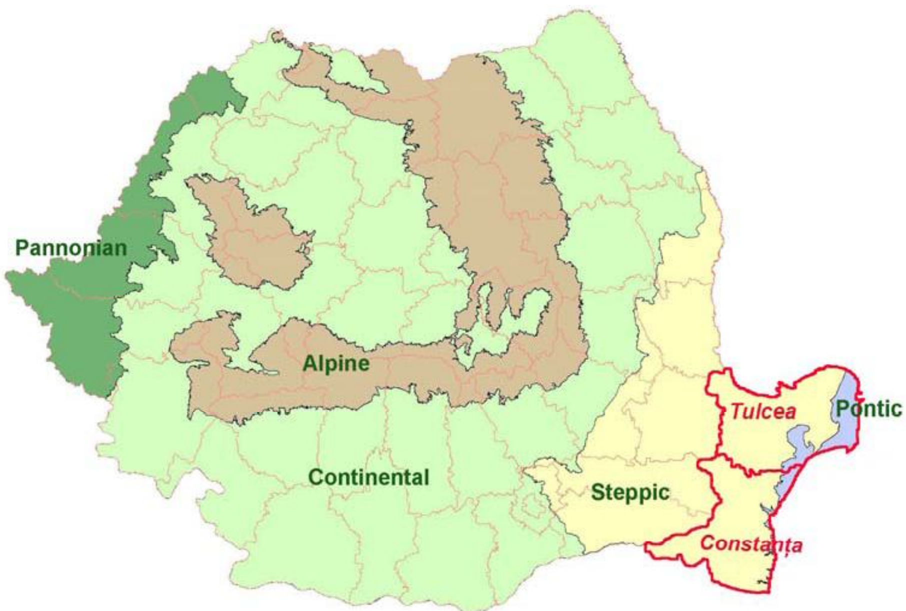


Figure 2.1. Dobrudja (with red) and its bioregions.

From a geographical regionalization point of view Dobrudja is composed from five units (fig. 2.3). The study area was extended to all of them, as follows: Northern Dobrudja, Central Dobrudja, Southern Dobrudja, Southern Dobrudjan seashore and a significant part of the last one (Danube Delta and Razim-Sinoe Lagoon complex), the Razim-Sinoe Lagoon complex.

In other words, **the study area is comprised by Dobrogea Plateau, Southern Dobrudjan seashore and the Razim-Sinoe Lagoon complex**. Thereby, apart from the faunistical studies which were conducted in the whole Dobrudja, no research was done in the main Danube Delta area. We decided to not include Danube Delta because this area is still the most studied part of Dobrudja.

Figure 2.2. The morphostructural units of Dobrudja: Depresiunea Predobrogeană (Pre-Dobrudjan Depression), Dobrogea de Nord (Northern Dobrudja), Dobrogea Centrală (Central Dobrudja) and Dobrogea de Sud (Southern Dobrudja; after Popovici et al., 1984). The studies were carried out in the last three units.

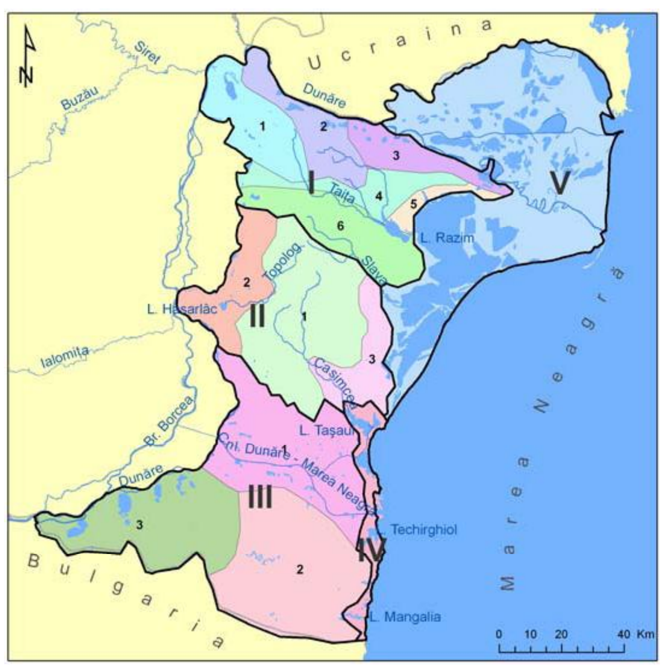


Figure 2.3. Geographical regionalization of Dobrudja (after Popovici et al., 1984): I. Northern Dobrudja: 1 - Măcin Mountains, 2 – Niculițelului Plateau, 3 – Tulcei Hills, 4 - Cerna-Nalbant Couloir, 5 – Razim plain, 6 – Babadag Plateau. II. Central Dobrudja (Casimcei Plateau): 1 – central part, 2 – danubian part, 3 – marine part. III. Southern Dobrudja: 1 – Medgidiei Plateau, 2 - Cobadin-Negru Vodă Plateau, 3 – Oltinei Plateau. IV. Southern Dobrudjan seashore. V. Danube Delta and Razim-Sinoe Lagoon complex.

PART 1

The Distribution of Amphibians in Dobrudja

The main goals of conservation biology are to understand the structure and function of natural ecological systems in the face of a growing human population and then to apply that knowledge to maintain their diversity. Amphibians, like many other groups of organisms, are facing worldwide population declines, range contractions, and even species extinctions. It is imperative that conservation action be taken to reverse this trend and maintain amphibian diversity (Semlitsch, 2003).

The premise of any conservation action is to know in greatest detail the distribution areas of the species in question, which is not possible without having updated and trustworthy distribution records available. In December 1990 the Species Survival Commission of IUCN established the Declining Amphibian Populations Task Force. Its mission statement was to determine the causes of the declines and to promote ways to halt or reverse them (Collins and Crump, 2009). One of the first problems they had to confront was the insufficient and incomplete data regarding the distribution of amphibian species, as much of the information available on the population status of amphibians was anecdotal. In order to determine the real state of the amphibian populations and to detect possible decline tendencies, creation of updated distribution maps became indispensable. Whether we talk about global, European or national conservation initiatives, the evaluation of the conservation status is based almost always on the distribution data.

The growing pressure that economical development has put on the natural habitats along with climate change has determined habitat fragmentation and local biodiversity loss in Dobrogea as well. In the next chapter we have synthesized all the available data, both historical and recent distribution records, regarding the amphibian species from this region. By analyzing the existing distribution data and performing a cartographic analysis, we were able to make an evaluation of the conservation status and, in the end, to propose a Red List for the amphibians of Dobrudja.

III.

The Distribution and Red List of Amphibians in Dobrudja

In this chapter we synthesized the available distribution records from published papers, museum collections, and our own field data. These distribution data were treated separately as historical data (before 1990), continuous data (records from both before and after 1990) and recent ones (after 1990). Based on distribution records we performed a cartographic analysis consisting of three indexes that attempt to indicate shifts in geographic range over time. Using these indexes as a measure of the conservation status we proposed a Red List for the amphibians of Dobrudja based on the distribution data and the changes in species ranges before and after 1990.

We used distribution data from published articles or books, museum collections, and our own field data. From the total of 1130 recordings, 807 represent previously published data, 11 come from museum collections (Iaşi, Deva and Oradea Museums) and 313 are unpublished field data. From these unpublished data, 260 are our own (representing 23%) and the rest were provided by Ion Fuhn, Gheorghe Sin, Martin Kyek, Jan (Pim) Arntzen, Török Zsolt, Mathieu Denöel, Tatiana Kotenko and Ioan Coroiu.

For the cartographic representation we have used UTM grid maps system with 5 X 5 km squares (Lehrer and Lehrer, 1990). This was preferred to geographical coordinates since most literature records refer mostly to localities and fewer to toponyms, and offer no information on the area covered within a location. The data analysis used for compiling the Red List was based on surface data (UTM 25 km^2 squares) and not the actual number of records. Based on the distribution records we computed three indexes for the cartographic analysis that attempt to capture shifts in geographic range and measure our degree of knowledge of their distribution. Distribution records were separated as records before 1990 (P), continuous records i.e. both before and after 1990 (C), records after 1990 (N) and total number of records (T). The three indexes proposed are the relative change in species distribution [$Rc = P / (C + N)$], the continuity index [$Ci = C \times 100 / (P + C)$], and the relative degree of knowledge [$K = (C + N) \times 100 / T$]. The relative change in species distribution ratio when greater than unit indicates a reduction in area occupied from a larger historical range to a smaller present range. The continuity index is a measure of the constant sighting at a particular site, the higher the value the higher the continuity. Finally, the relative degree of knowledge is a ratio between the recent number of records (both new and re-sights) and the total number. A high value indicates that most records are after 1990, while a lower value indicates that more records were previous to 1990, i.e. historical records. In the studied area 12 amphibian species (nine species and a species complex) are present, some with a restricted distribution while others widely distributed (fig. 3.1). Of the total number of records 66% are after 1990, 14% before 1990 and 20% are continuous. According to the administrative units, 40% of the records are located in Constanța county and 60% in Tulcea county.

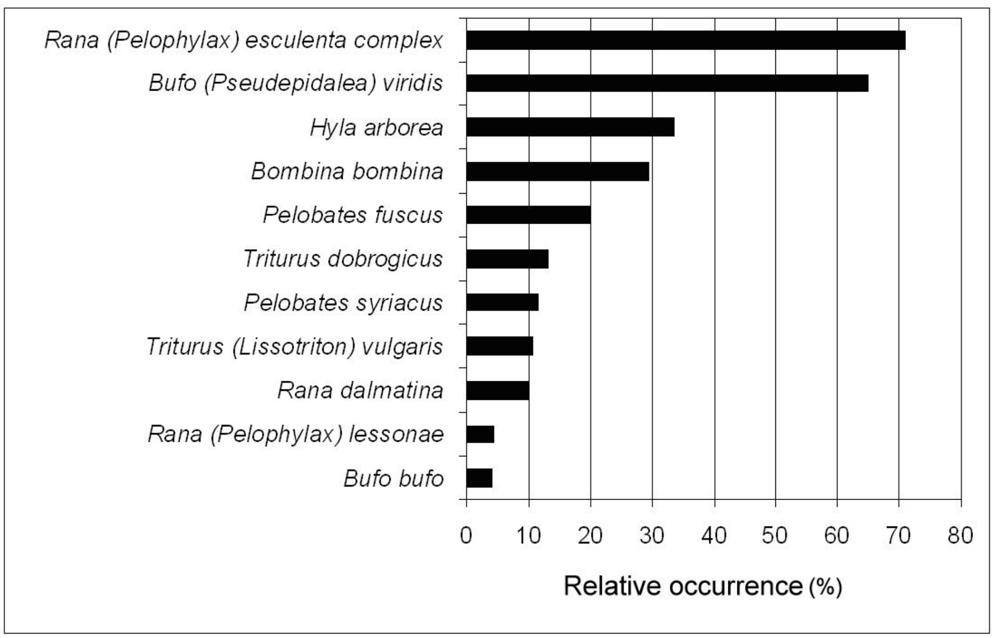
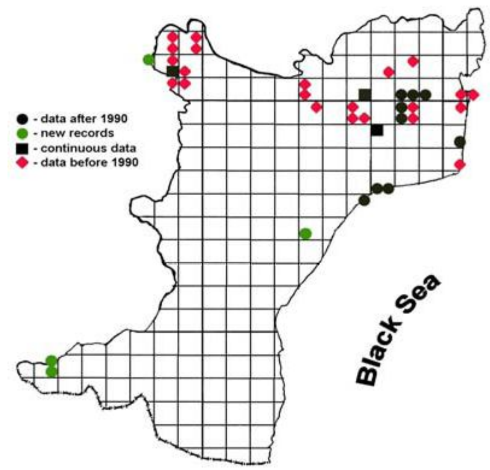


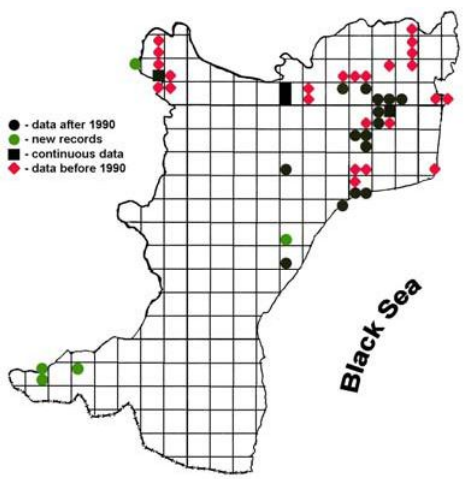
Figure 3.1. The relative occurrence of amphibians based on the number of quadrates occupied by a species as a percentage of the total number of records (i.e. 340 squares of 25 km^2).

The structure of the distribution records according to the species investigated, date of observation, and the indexes calculated are presented in Table 3.1. Thus, the two newt species have suffered the highest reduction in area, as suggested by the values of the Rc and Ci indexes. The two most common species, the green toad (*Bufo* (*Pseudepidalea) viridis*) and water frogs (*Rana (Pelophylax) esculenta* complex) show the highest values for the Ci index and also of the K index. While proposing the Red List for Dobrudja we did not refer to previously proposed statuses at global, national or local levels, but attempted to base them on the cartographic analysis alone (Table 3.2). Including five species in the "Endangered" category is based on their limited distribution or decreased re-sighting after 1990.

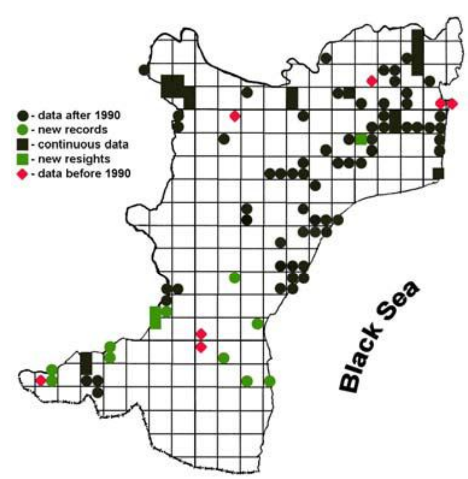
Total number of 5x5 km² with distribution records



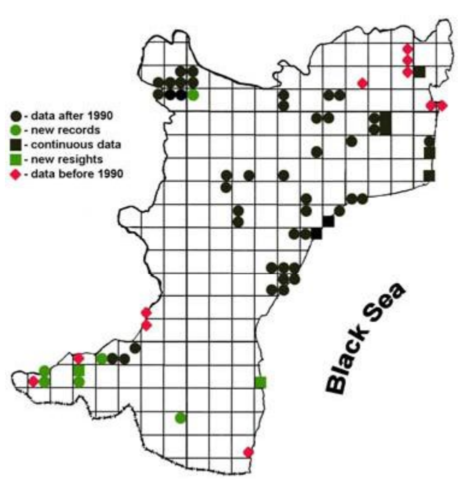
Triturus (Lissotriton) vulgaris



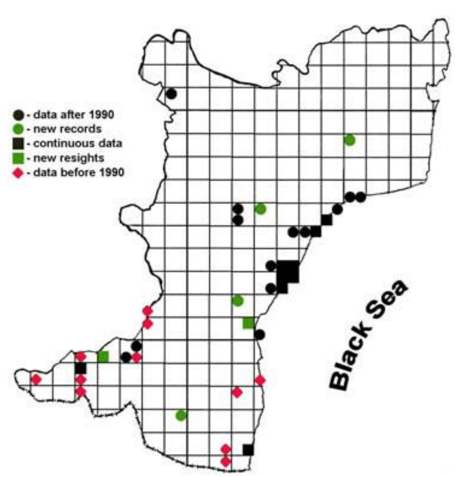
Triturus dobrogicus



Bombina bombina



Pelobates fuscus



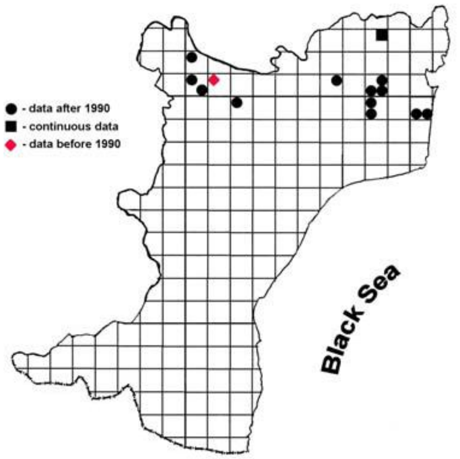
Pelobates syriacus

Table 3.1. The distribution records of the amphibian species from Dobrudja and their three indexes calculated.

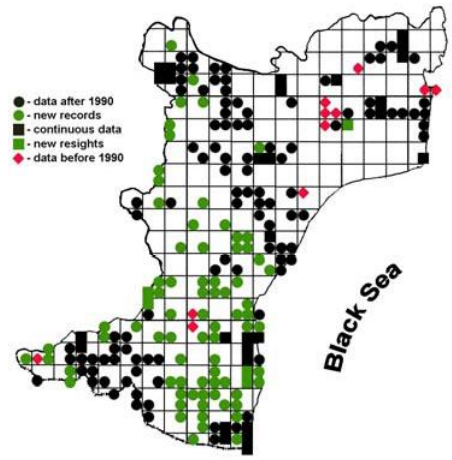
Species	Records before 1990 (P)	Continuous records (C)	Records after 1990 (N)	Total number of records (T)	Relative change in species distribution P / (C + N)	Continuity index C × 100 / (P + C)	Relative degree of knowledge (C + N) × 100 / T
Triturus (Lissotriton) vulgaris	22	3	13	**38**	1,38	12,0	42,1
Triturus dobrogicus	24	4	20	**48**	1,00	14,3	50,0
Bombina bombina	7	21	73	**101**	0,07	75,0	93,1
Pelobates fuscus	11	9	49	**69**	0,19	45,0	84,1
Pelobates syriacus	11	11	17	**39**	0,39	50,0	71,8
Bufo bufo	1	1	12	**14**	0,08	50,0	92,9
Bufo (Pseudepidalea) viridis	11	33	179	**223**	0,05	75,0	95,1
Hyla arborea	9	15	91	**115**	0,09	62,5	92,1
Rana dalmatina	5	4	25	**34**	0,17	44,4	85,3
Rana (Pelophylax) lessonae	2	0	14	**16**	0,14	0,0	87,5
Rana (Pelophylax) esculenta complex	24	90	129	**243**	0,11	78,9	90,1

Table 3.2. The conservation statuses assessed for the amphibian species in Dobrudja by the different Red Lists at global, national and local levels. The last column represents our proposal for the Red List of Dobrudja ([1]Baillie et al., 2004; [2]Botnariuc and Tatole, 2005; [3]Măcin Mountains National Park Red List - Török, 2006 and [4]Danube Delta Biosphere Reserve Red List - Oțel, 2000).

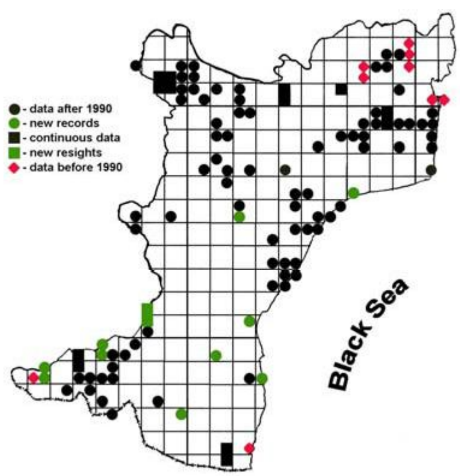
Species	IUCN 2004[1] categories	Romanian Red List[2] categories	MMNP Red List[3] categories	DDBR Red List[4] categories	**Proposed Red List categories**
Triturus (Lissotriton) vulgaris	LC	NT	-	DD	**EN**
Triturus dobrogicus	NT	EN	-	LC	**EN**
Bombina bombina	LC	NT	-	LC	**NT**
Pelobates fuscus	LC	VU	-	LC	**VU**
Pelobates syriacus	LC	EN	-	LC	**EN**
Bufo bufo	LC	NT	EN	LC	**EN**
Bufo (Pseudepidalea) viridis	LC	NT	VU	LC	**LC**
Hyla arborea	LC	VU	VU	LC	**NT**
Rana dalmatina	LC	VU	VU	-	**EN**
Rana (Pelophylax) lessonae	LC	-	-	DD	**EN**
Rana (Pelophylax) ridibunda	LC	-	VU	LC	**LC**
Rana (Pelophylax) esculenta	LC	-	-	-	**LC**



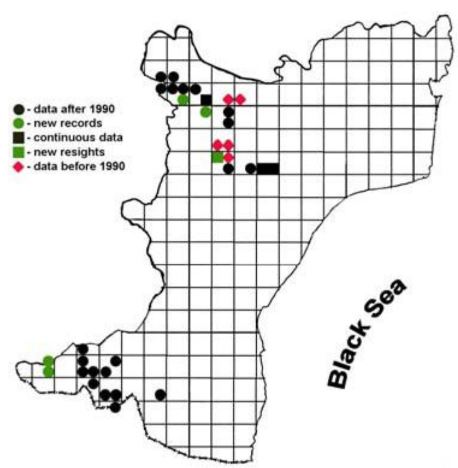
Bufo bufo



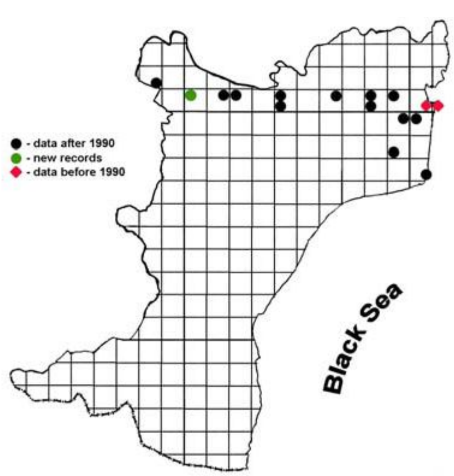
Bufo (Pseudepidalea) viridis



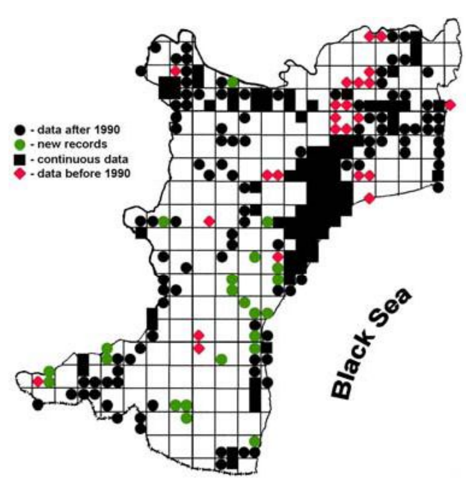
Hyla arborea



Rana dalmatina



Rana (Pelophylax) lessonae



Rana (Pelophylax) esculenta complex

PART 2

Aspects of the Biology and Ecology of the Genus *Pelobates* in Dobrudja

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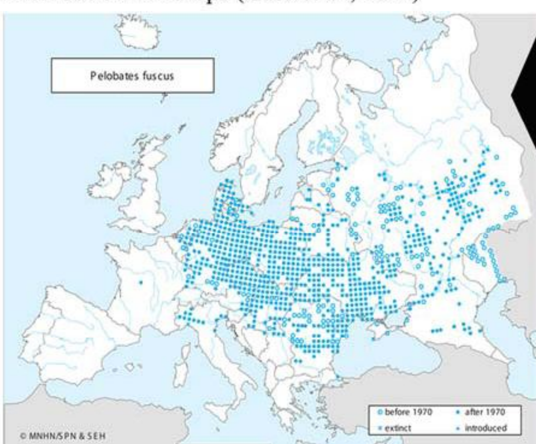
The Pelobatidae family is a morphologically conservative group of ancient primitive frogs that have obscure relationships to the remaining clades of Anura (Ford and Cannatella, 1993; García-París et al., 2003; Frost et al., 2006). Until recently, due to their similar life style and general morphological appearance, in this family were included also the seven species of North American spadefoot toads, but recent studies have placed them into a separate family, Scaphiopodidae, with two genus, *Scaphiopus* and *Spea* (García-París et al., 2003; Frost et al., 2006). Now, the Pelobatidae family contains a single genus, *Pelobates* with four species: one from Morocco, *P. varaldii*, one from Western Europe, *P. cultripes*, one from Central and Eastern Europe, *P. fuscus* and one in south-east Balkans, east to south-eastern Transcaucasia, northern Iran, and south to the Levant, *P. syriacus*. In Romania only the last two species are present.

The species of this genus are strictly nocturnal outside the breeding season and hide during daylight in deep burrows. Not surprisingly, due to their secretive life-style, the spadefoot toads were described quite late in many countries from the Balkans. Thus, *P. syriacus* was described from Macedonia only in 1928 (Karaman, 1928), in Bulgaria in 1932 (Müller, 1932), in Romania in 1954 (Băcescu, 1954) and in Greece in 1975 (Böhme, 1975). The Balkan Peninsula is of crucial importance for an understanding of the many poor known attributes, such as historical and current chorology, phylogeography, taxonomical diversity etc., of spadefoot toads from Europe (Džukić et al., 2005). Dobrudja is particularly important because it represents one of the southernmost portions of the range of *P. fuscus* and at the same time the northernmost portion of the range of *P. syriacus*.

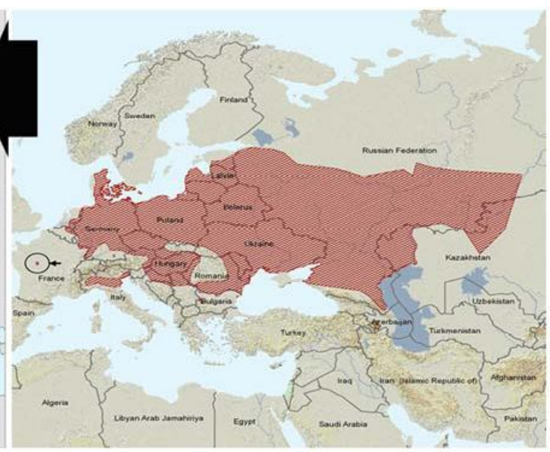
In the next five chapters we presented the results of our studies regarding the biology and ecology of reproduction, adaptive phenotypic plasticity of the tadpoles in response to the breeding habitat desiccation, post-metamorphic growth rates of the juveniles and comparative morphometry of these two fascinating spadefoot toad species.

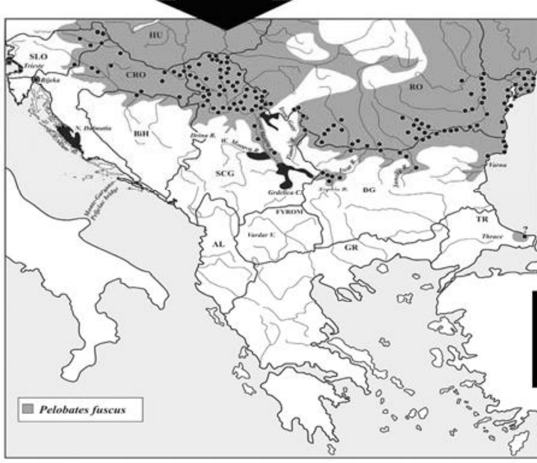
Pelobates fuscus
(Laurenti, 1768)

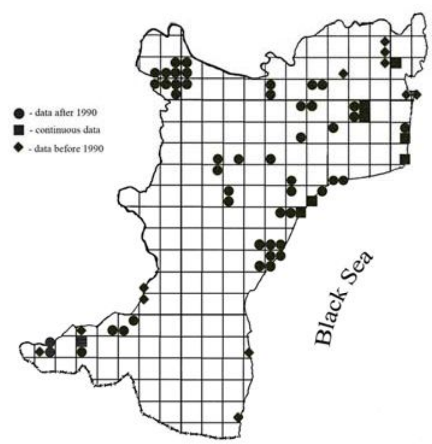
Distribution in Europe (Gasc et al., 1997)



Distribution in Eurasia (IUCN, 2010)



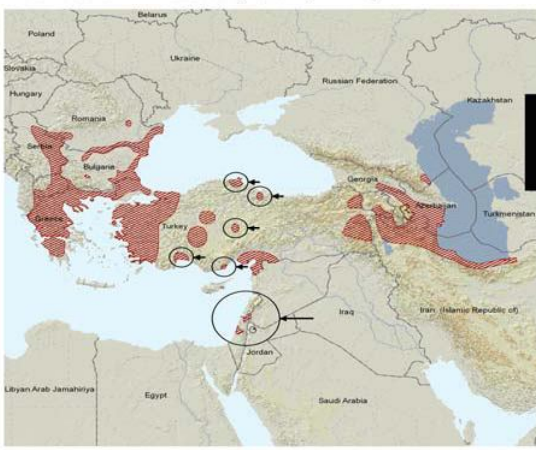




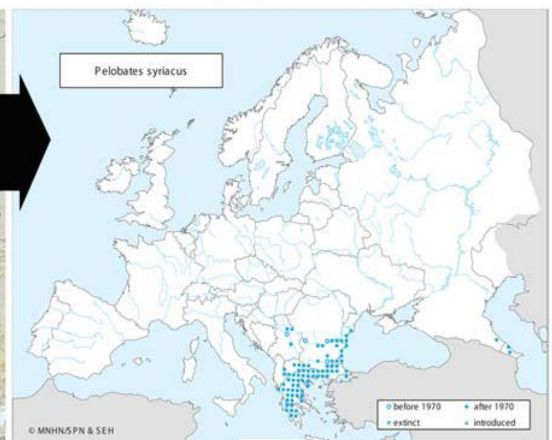
Distribution in Balkan Peninsula (Džukić et al., 2005) Distribution in Dobrudja (Székely et al., 2009)

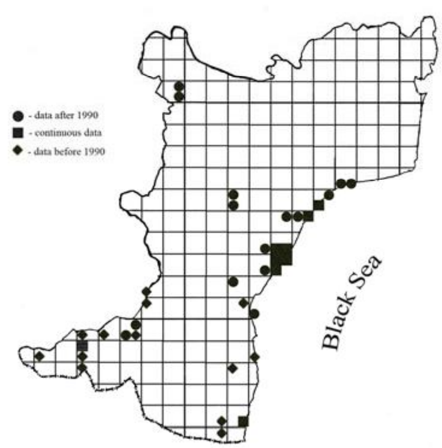
***Pelobates syriacus* (Boettger, 1889)**

Distribution in Eurasia (IUCN, 2010)

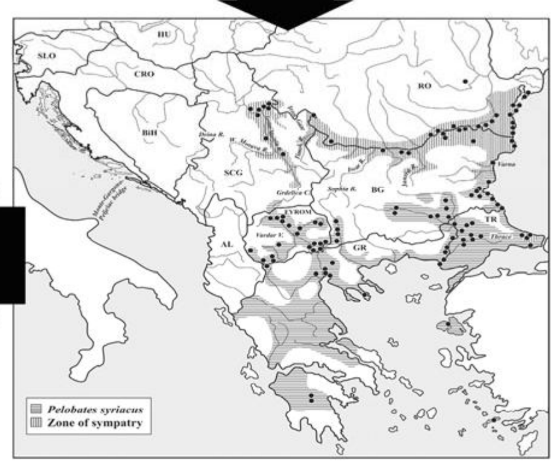


Distribution in Europe (Gasc et al., 1997)





Distribution in Dobrudja (Székely et al., 2009)



Distribution in Balkan Peninsula (Džukić et al., 2005)

IV.

Adaptive Phenotypic Plasticity in Timing of Metamorphosis in *Pelobates fuscus* and *P. syriacus* Tadpoles

Tadpoles of several anuran species accelerated metamorphosis in response to habitat desiccation (e.g. Crump, 1989; Loman, 1999; Richter-Boix, Llorente and Montori, 2006). In the present study we investigated the phenotypic plasticity of *Pelobates syriacus* tadpoles from a temporary pond and *P. fuscus* tadpoles from a permanent pond exposed to different water volume reduction rates to test (i) if tadpoles will express a faster development rate when facing decreasing water levels; and (ii) if the accelerated development affects size at metamorphosis.

Eastern spadefoot toad (*P. syriacus*) tadpoles, in stage 25 (Gosner, 1960), were collected from a temporary pond complex near Vadu, Constanța county (44°26’12’’N, 28°44’13’’E) and common spadefoot toad (*P. fuscus*) tadpoles, in stage 25, from a permanent pond near Murighiol, Tulcea county (45°01’37’’N, 29°09’40’’E) and reared in 8 litre opaque plastic containers (27 x 27 x 12 cm). The tadpoles were exposed to the following water reduction treatments: 1 – constant high, control treatment, with 6000 ml water/container (90 mm depth), 2 - slow decreasing treatment, from 6000 to 800 ml, the water level being reduced by 200 ml every four days for the first 20 days and every third day afterwards (from 90 mm to 16 mm depth), 3 - fast decreasing treatment, from 6000 to 800 ml, the water level being reduced by 300 ml every four days for the first 20 days and every three days afterwards (from 90 mm to 16 mm depth) and 4 - constant low volume with 1500 ml water/container (27 mm depth). Each of these four treatments was replicated six times for *P. syriacus* (using 96 tadpoles) and three times for *P. fuscus* (using 48 tadpoles).

The tadpole wet mass was recorded with an electronic balance (Kern model ABJ) to the nearest 0.01 g, while snout-vent length (SVL) was measured to the nearest 0.1 mm with a digital calliper at both forelimb protrusion (Gosner stage 42) and metamorphosis (Gosner stage 45). We measured time to forelimb protrusion (the beginning of metamorphic climax, stage 42), as the time (days) elapsed since the start of the experiment until the emergence of the first forelimb; and time to metamorphosis (end of the metamorphic climax, stage 45), as the number of days elapsed since the start of the experiment until the metamorphs have a tail which was shorter than body length.

Time of metamorphosis

The *P. syriacus* tadpoles from the fast decrease treatment (3) accelerated their metamorphosis in response to water reduction when compared with the control (treatment 1) (t = 3.174, P = 0.010, Student's t test; fig. 4.1). They had a reduction of two days, or 4.4% of the 45 days larval period in constant water. A similar effect was observed at forelimb emergence but it was not statistically significant (t = 2.205, P = 0.052). The response of tadpoles from the constant low treatment (4) did not differ from the control (t = 0.700, P = 0.500).

The *P. fuscus* tadpoles were not able to accelerate their metamorphosis in response to the water reduction (fig. 4.1). The tadpoles from the fast decrease treatment (3) accelerated their metamorphosis compared with the control (treatment 1) but the difference were not statistically significant (t = 0.550, P = 0.612, Student's t test). Those from the slow decreasing treatment (t = -0.0261, P = 0.980) and constant low treatment (t = -0.0904, P = 0.932) completed their metamorphosis in the same time as the tadpoles from the control.

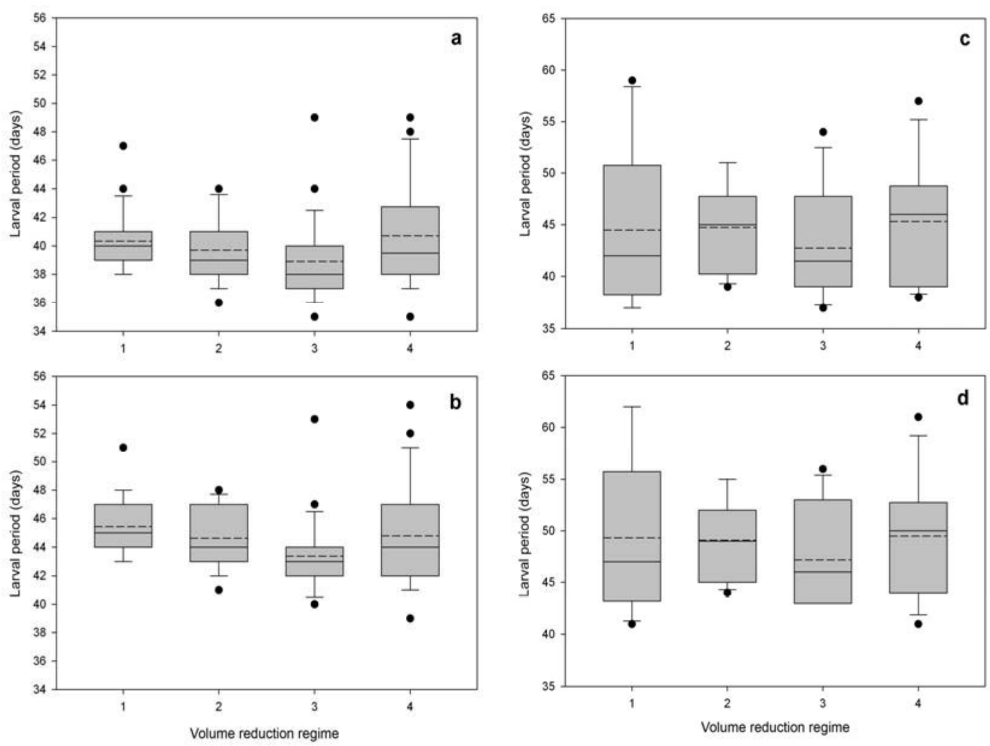


Figure 4.1. Developmental time for the four desiccation levels of the *Pelobates syriacus* (left) and *P. fuscus* (right) tadpoles at Gosner (1960) stage 42 (a and c) and stage 45 (b and d). The mean for each treatment is represented with dashed line.

Size at metamorphosis

Water level had a significant effect on the body mass and SVL of tadpoles at metamorphosis for both *Pelobates* species and both at Gosner stage 42 and Gosner stage 45. The *P. syriacus* metamorphs were significantly smaller in the fast decrease treatment than in the constant high and slow decrease treatments both at forelimb emergence (body mass $P < 0.001$, SVL $P < 0.008$, Holm-Sidak test) and end of metamorphosis (body mass $P = 0.022$, SVL $P < 0.005$; fig. 4.2). However, the smallest size was observed in the tadpoles reared in constant low water levels (treatment 4) both at forelimb emergence (body mass $P < 0.001$, SVL $P < 0.001$) and end of metamorphosis (body mass $P < 0.001$, SVL $P < 0.001$; fig. 4.2), even if the length of their larval period was not significantly different from the constant high one.

The *P. fuscus* metamorphs from treatment 3 were also significantly smaller than those from the constant high (1) and slow decrease (2) treatments at forelimb emergence (body mass $P = 0.001$, SVL $P = 0.014$, Holm-Sidak test; fig. 4.2). However, this effect was not observed at the end of metamorphosis (body mass $P = 0.009$). For this species too the smallest size was observed in the tadpoles reared in constant low water levels (treatment 4) both at forelimb emergence (body mass $P < 0.001$, SVL $P < 0.003$) and end of metamorphosis (body mass $P < 0.009$; fig. 4.2)

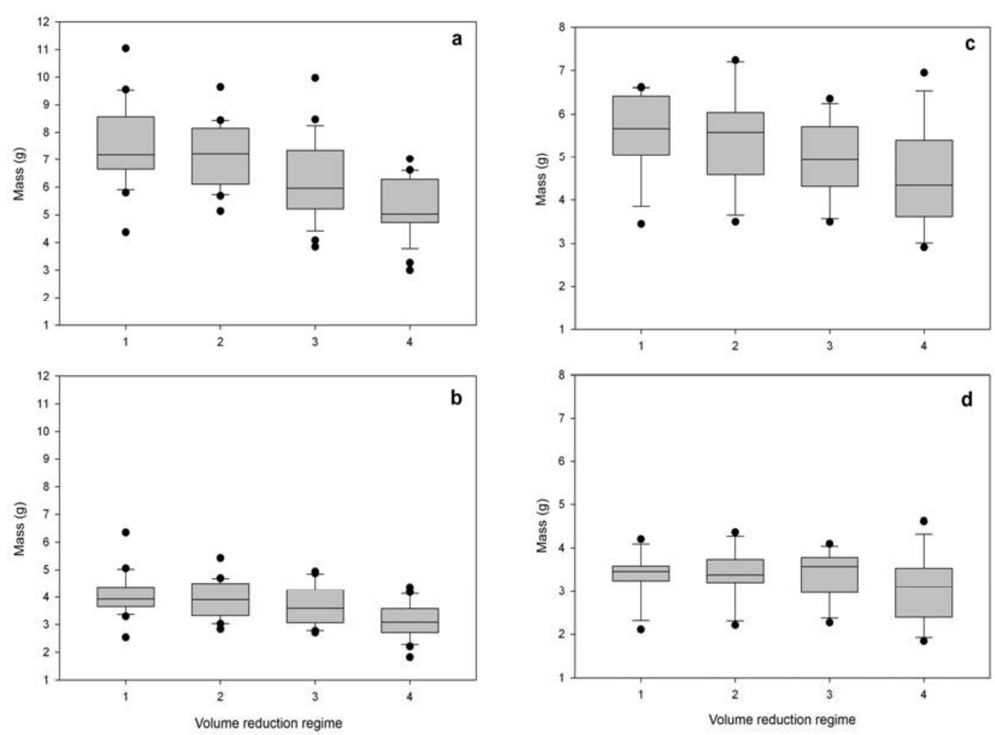


Figure 4.2. Body mass of the *Pelobates syriacus* (left) and *P. fuscus* (right) tadpoles at Gosner (1960) stage 42 (a and c) and 45 (b and d) for the four desiccation levels.

Growth rate

There was a significant difference between the four treatments of *P. syriacus* in growth rates (ANOVA, $F = 38.060$, $P < 0.001$), the tadpoles from both the fast decrease ($P < 0.012$) and constant low ($P < 0.001$) treatments having the smallest average maximum body weights/treatment compared with the control. For *P. fuscus* tadpoles a similar difference was recorded between the treatments (ANOVA, $F = 5.180$, $P = 0.028$), the tadpoles from the constant low treatment (4) having the smallest average maximum body weights/ treatment compared with the control. However, the biggest average maximum body weights/treatment were observed at the tadpoles from treatments 2 (slow decrease) and 3 (fast decrease), indicating that the effect of water reduction regime was not similar to that observed in the *P. syriacus* tadpoles case.

Partial tadpole growth rates for both species displayed a two-stage pattern: a period of intensive growth followed by a phase of decrease in the tadpoles' body mass (fig. 4.3). The maximum mass of the metamorphosing tadpoles was reached a few days before the forelimb protrusion (fig. 4.4). The *P. syriacus* tadpoles from the constant low treatment showed significant differences in body mass after day 27 ($P < 0.001$, Holm-Sidak test), whereas in the case of tadpoles reared in the fast decrease treatment, these differences appeared after day 33, at a 3300 ml water volume ($P = 0.017$). Also, we recorded a significant correlation between the increase of tadpole mass and water conductivity, the containers with the constant low water level having the highest values. Conductivity increased as the water level decreased and the tadpole body mass in each container grew.

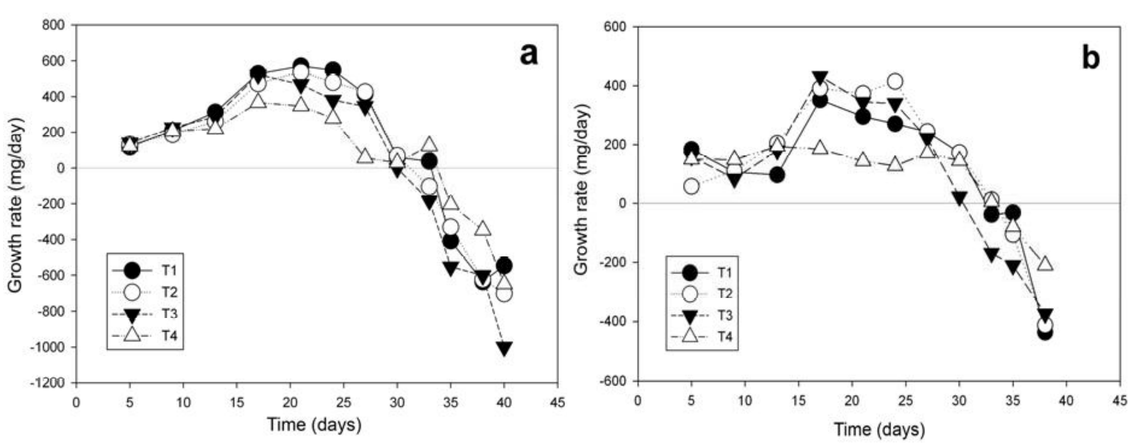


Figure 4.3. Dynamics of average daily growth rates of the *Pelobates syriacus* (a) and *P. fuscus* (b) tadpoles in the four experimental treatments.

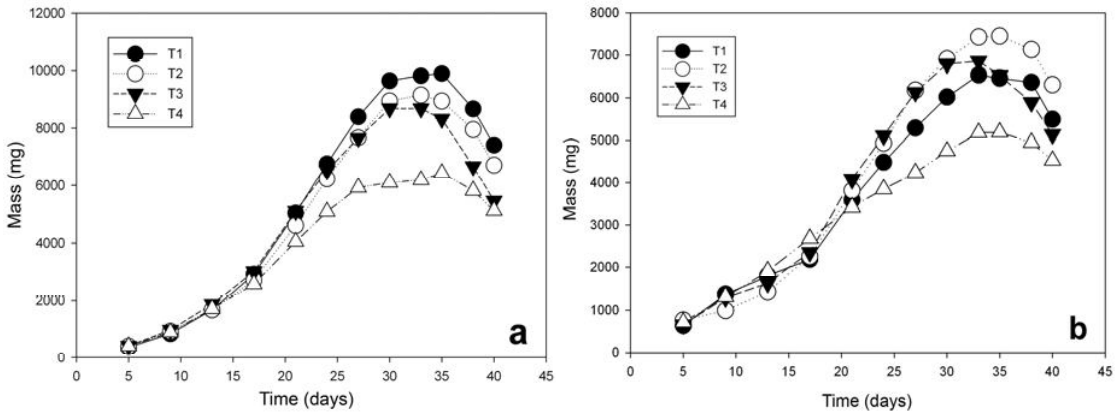


Figure 4.4. Dynamics of average tadpole mass of the *Pelobates syriacus* (a) and *P. fuscus* (b) tadpoles in the four experimental treatments.

Our results support the hypothesis that phenotypic plasticity in response to pond drying is higher in species breeding in ephemeral desert ponds than those using other types of temporary ponds (Wells, 2007). Also, our results indicate that *P. syriacus* tadpoles are able to respond to pond drying by speeding up their development and thus increase their survival rate. It is interesting that the tadpoles from the constant low treatment did not complete their development sooner (e.g. Denver et al., 1998; Spieler, 2000) or later (e.g. Crump, 1989) than the control treatment, but at almost the same time; the constant low water level affected only the size, but not the time of metamorphosis. On the other hand, the *P. fuscus* tadpoles from our experiment were not able to respond to the reduction of water level, probably because these tadpoles were collected from a permanent pond.

Since a faster development in amphibians is usually associated with reduced size at metamorphosis (Wilbur and Collins, 1973; Newman, 1992), we expected to find differences in sizes between the different treatments. Indeed, the reduction of the developmental time in the fast decreasing treatment (3), of the *P. syriacus* tadpoles, was accompanied by a smaller metamorphic size, representing a significant reduction with 9% of mass and 4% of SVL at metamorphosis as compared to the control. The smallest size was observed in the tadpoles reared in constant low water levels (treatment 4) that suffered a reduction of 23% of mass and 8% of SVL at metamorphosis even if the length of their larval period was not significantly different from the control (treatment 1).

In our experiment, the comparison of the two metamorphic climax moments revealed that while the effect of decreasing water level on the time of metamorphosis was evident only at the end (stage 45, when metamorphosis is almost completed), the effect on the size of metamorphs was similar in both moments.

V.

Early Post-Metamorphic Growth in two Species of Spadefoot Toads (*Pelobates fuscus* and *P. syriacus*)

The two spadefoot toad species, *P. fuscus* and *P. syriacus* are clearly differentiated species, belonging to two different phylogenetic lineages within the genus *Pelobates* (Maglia, 1998; García-París et al., 2003). Dobrudja is one of the several places where these two species coexist (Džukić et al., 2005; Crottini et al., 2007; Džukić et al., 2008; Tarkhnishvili et al., 2009), *P. syriacus* being the dominant species with significantly larger populations than *P. fuscus* (personal observation). The two species differ significantly in body size, adult individuals of *P. syriacus* being on average much larger than those of *P. fuscus*, within both the area of their allopatry and sympatry (Rot-Nikčević et al., 2001). Interestingly, these two spadefoot species start terrestrial life at approximately the same body size, but diverge soon during the first year of their juvenile phase (Rot-Nikčević et al., 2001).

The aim of the present study was to determine the comparative growth rate of the juveniles from both species, in the first four months after the metamorphosis. We were particularly interested if (i) the *P. syriacus* juveniles are able to grow faster than those of *P. fuscus* when reared in controlled laboratory conditions and if (ii) the food availability influences significantly the growth rate of the two species.

For the experiment we used 40 juveniles (20 of *P. syriacus* and 20 of *P. fuscus*) from Vadu, Constanţa county and Murighiol, Tulcea county, which had resulted from the phenotypic plasticity experiment. The juveniles were reared in individual plastic containers and fed with crickets (*Gryllus assimilis* and *Acheta domestica*) during the experiment. In order to provide a relatively similar food quantity for each individual, the juveniles were fed with approximately 10% of the experimental group average mass. We used two experimental treatments for both species (10 juveniles per treatment), the individuals from treatment I being fed twice a week and those from treatment II, only once.

The mass of every individual was recorded once at every nine days, with an electronic balance (Kern model ABJ) to the nearest 0.01 g. Prior to weighing, each juvenile was blotted dry and pubic pressure was applied to void bladder urine. Snout-vent length (SVL) was measured to the nearest 0.1 mm with a digital calliper. For both species the partial, average and general growth rates as well as the food conversion ratio were computed.

The mass and SVL of the juveniles differed significantly between the two species and the two treatments (table 5.1). As expected, the juveniles from treatment I, of both species, became significantly larger than those from treatment II (fig. 5.1 and fig. 5.2), the final mass and SVL of the juveniles differing significantly for *P. syriacus* and *P. fuscus*. Additionally, the final masses of the *P. syriacus* juveniles were significantly bigger than those of *P. fuscus* for both treatments. The final SVL was also bigger in the case of *P. syriacus*, but the differences were not statistically significant, proving that the accentuated growth of the *P. syriacus* juveniles was more explicit at mass level than at SVL.

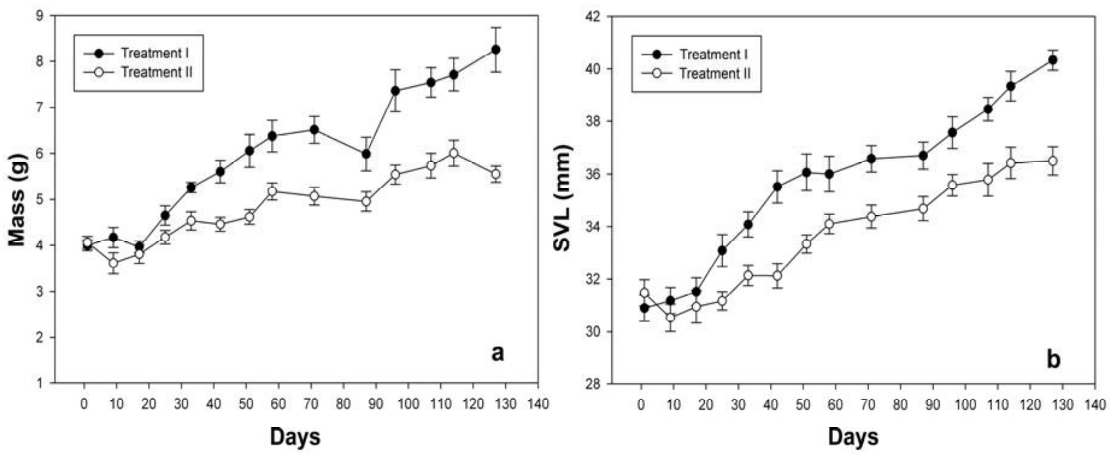


Figure 5.1. Growth of juveniles *Pelobates syriacus* from the two experimental treatments as expressed by mean mass (a) and SVL (b) changes. Data are shown as mean ± SE (Treatment I – fed twice a week, Treatment II – fed once a week).

In our experiment, the only variable, for both species and treatments, was the food quantity, the temperature, humidity and food quality being similar for all individuals. The partial growth rate for both species fluctuated during the experiment but the average and general growth rate of the *P. syriacus* individuals was higher than those of *P. fuscus*. The *P. syriacus* juveniles proved to be more efficient, having a general mass growth rate with 21% (treatment I) and 17% (treatment II) higher than those of *P. fuscus*. Likewise, the general SVL growth rate was higher, with 4% (treatment I) and 2% (treatment II) than those of *P. fuscus*. These differences were gained in spite of the fact that the individuals from both species were fed with (proportionally) same amount of food. Also, the *P. syriacus* juveniles proved to be more efficient in converting the ingested food mass into body mass, by having the food conversion ratio with 33% lower, in treatment I, and with 15% lower, in treatment II, than those of *P. fuscus* (table 5.1).

Table 5.1. Initial and final mass and SVL values, average and general growth rates, amount of ingested food and the food conversion ratio for the *P. syriacus* and *P. fuscus* juveniles from the experiment.

Parameter	Treatment I	Treatment II
Pelobates syriacus		
n	10	10
Initial average mass (g) ± SD	3,99 ± 0,35	4,06 ± 0,42
Range (initial mass)	3,55-4,53	3,54-4,85
Final average mass (g) ± SD	8,26 ± 1,53	5,55 ± 0,57
Range (final mass)	5,63-10,94	4,73-6,58
Average mass growth rate (g/day)	0,034	0,012
General mass growth rate (%)	93,95	50,75
Initial average SVL (mm) ± SD	30,9 ± 1,59	31,5 ± 1,57
Range (initial SVL)	28,6-33,7	29,8-35,1
Final average SVL (mm) ± SD	40,3 ± 1,22	36,5 ± 1,72
Range (final SVL)	37,9-41,9	34,4-38,8
Average SVL growth rate (mm/day)	0,074	0,039
General SVL growth rate (%)	26,21	16,93
Average ingested food mass (g/day) ± SD	0,077 ± 0,008	0,044 ± 0,003
Food conversion ratio ± SD	2,68 ± 0,533	3,13 ± 1,019
Pelobates fuscus		
n	10	9
Initial average mass (g) ± SD	3,36 ± 0,43	3,36 ± 0,43
Range (initial mass)	2,78-4,21	2,74-4,15
Final average mass (g) ± SD	6,45 ± 1,11	4,67 ± 0,48
Range (final mass)	4,46-7,95	4,12-5,69
Average mass growth rate (g/day)	0,024	0,010
General mass growth rate (%)	72,40	35,49
Initial average SVL (mm) ± SD	30,4 ± 1,67	30,7 ± 1,60
Range (initial SVL)	28,1-33,1	28,1-33,3
Final average SVL (mm) ± SD	38,8 ± 2,09	35,6 ± 0,90
Range (final SVL)	34,5-41,2	34,2-37,1
Average SVL growth rate (mm/day)	0,066	0,038
General SVL growth rate (%)	21,89	14,46
Average ingested food mass (g/day) ± SD	0,067 ± 0,005	0,038 ± 0,001
Food conversion ratio ± SD	3,58 ± 1,138	3,62 ± 0,688

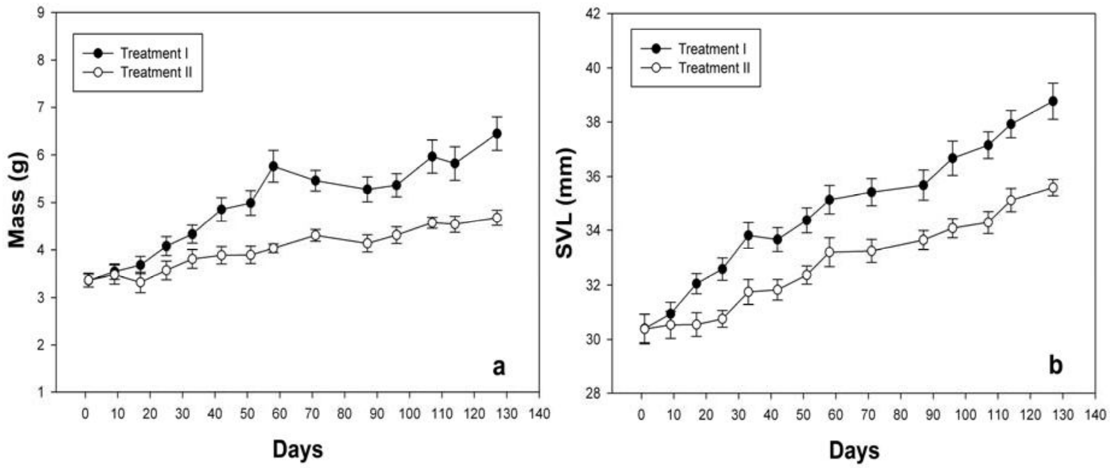


Figure 5.2. Growth of *Pelobates fuscus* juveniles from the two experimental treatments as expressed by mean mass (a) and SVL (b) changes. Data are shown as mean ± SE (Treatment I – fed twice a week, Treatment II – fed once a week).

Amphibian growth and adult body size are characteristically highly plastic and indeterminate, implying that environmental factors substantially affect the genetically fixed growth pattern (Jørgensen, 1995). The most important environmental factors which can affect the development and growth of amphibians are temperature, water, and food availability, which may vary seasonally depending upon latitude and altitude of the geographical location of the habitat.

In Dobrudja the size differences between the two species are substantial most of the captured *P. syriacus* individuals being much bigger than those of *P. fuscus* (see Chapter VII). A similar situation was described by Rot-Nikčević and her colleagues (2001) from Serbia, were in a sympatry area of the two species, both the females and males of *P. syriacus* were significantly larger than the *P. fuscus* individuals. Additionally, the authors of the above mentioned paper, managed to estimate the time of sexual maturation (by skeletochronology, Castanet et al., 1993) for the two species, showing that *P. syriacus* reaches the sexual maturity, on average, one year earlier than *P. fuscus*. The authors presume that this feature of the individuals from the cited populations is due to a substantial difference in the two species juvenile growth rates. Thereby, our experiment confirms that the *P. syriacus* juveniles have a significantly higher growth rate in the first four months after the metamorphosis. Furthermore, the *P. syriacus* juveniles proved to be more efficient than those of *P. fuscus* in both treatments, demonstrating a higher capability to grow in adverse conditions with scarcer food resources. These data contribute to elucidate the reasons for the higher success of eastern spadefoot toads in sustaining viable populations in the dry and harsh environment of Dobrudja.

VI.

Aspects of Breeding Activity in a Population of *Pelobates syriacus*

At the present time there are relatively few data available about the reproductive biology of the eastern spadefoot toad in the literature. The main goal of this study was to detail various aspects of the breeding activity, such as breeding phenology, reproductive strategy, sexual dimorphism and female fecundity of a population from Dobrudja (fig. 6.1).

Figure 6.1. A *Pelobates syriacus* pair in amplexus (left) and next day, after ovipozition (right).

The study was conducted in the spring of 2010, on Grindul Lupilor, (44°37'20''N, 28°48'46''E), Constanța county, in a mosaic of permanent and temporary ponds used for breeding by both spadefoot toad species. 22 *Pelobates syriacus* pairs in amplexus were collected and transported in the laboratory where every pair was assigned to an individual plastic container filled with water, suitable for oviposition. All individuals were measured before and after oviposition, the wet mass being recorded with an electronic balance (Kern model ABJ) to the nearest 0.01 g and the snout-vent length (SVL) was measured to the nearest 0.1 mm with a digital calliper. In order to analyze and compare fecundity, the females were assigned into two size categories, with small females having a SVL $\leq$ 63.5 mm (n = 8) and large ones with SVL $\geq$ 65 mm (n = 11). After oviposition the egg clutches were individually photographed, analyzed and the number of eggs from each clutch counted and the egg diameter was measured.

Breeding phenology

The breeding period was very short, lasting only a few days. The breeding started on 21st March and ended on 25th, being strongly correlated with an abrupt increase of the average temperature, from 2-5˚C to 14-16˚C.

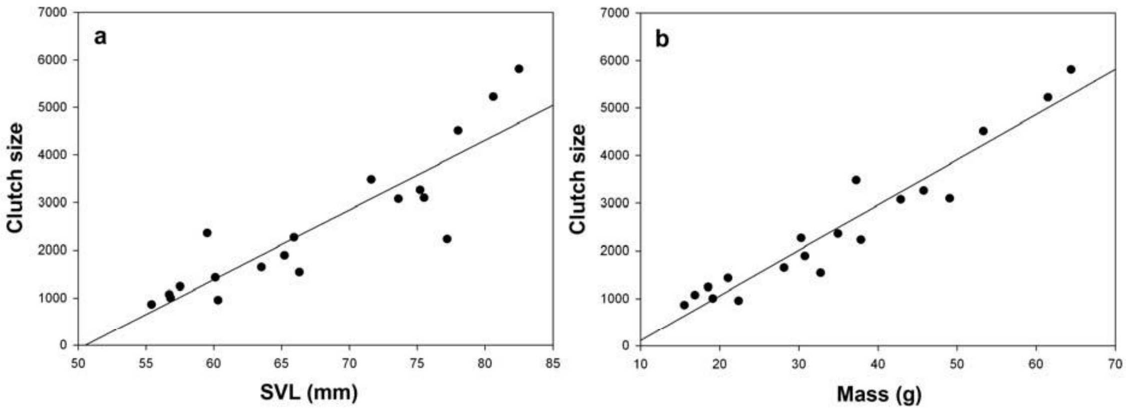


Figure 6.2. Correlation between the females SVL (a) and final mass (b) and the clutch size.

Fecundity

With the exception of two pairs, which had delayed the spawning for two days, all pairs finalized the oviposition in the first 12 hours. The average clutch size was 2474 eggs (SD = 1470.14; range: 865-5812; n = 19). The clutch size was strongly correlated with both the females SVL (fig. 6.2.a) and final mass (fig. 6.2.b).

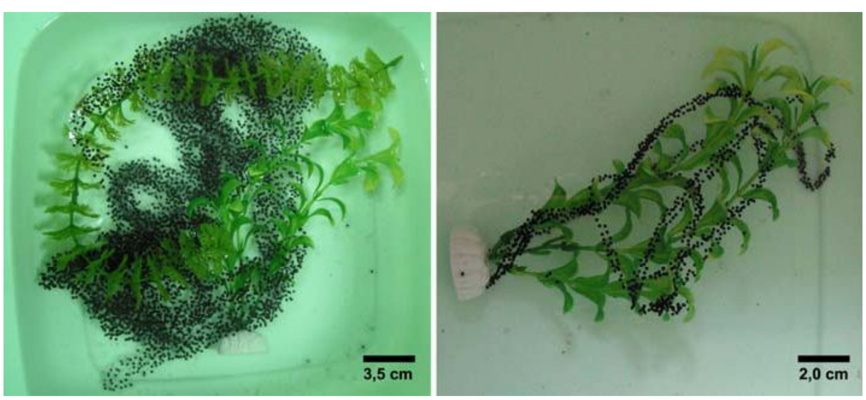


Figure 6.3. Size difference between a large female clutch (left) and a small one (right).

The large females clutch size was significantly larger than that of small ones (t = -3.883, P = 0.001, Student's t test), being with approximately 60% larger. The small females had a clutch size of 323.63 ± 492.44 eggs and the large females of 3310.73 ± 1375.43 (mean

± SD; fig. 6.3). Also, the clutch volume was significantly correlated with the females SVL (fig. 6.4.a) and especially with their final mass (fig. 6.4.b). On the other hand, no significant correlation was found between egg size and clutch size and nor between the egg size and the SVL and mass of the females.

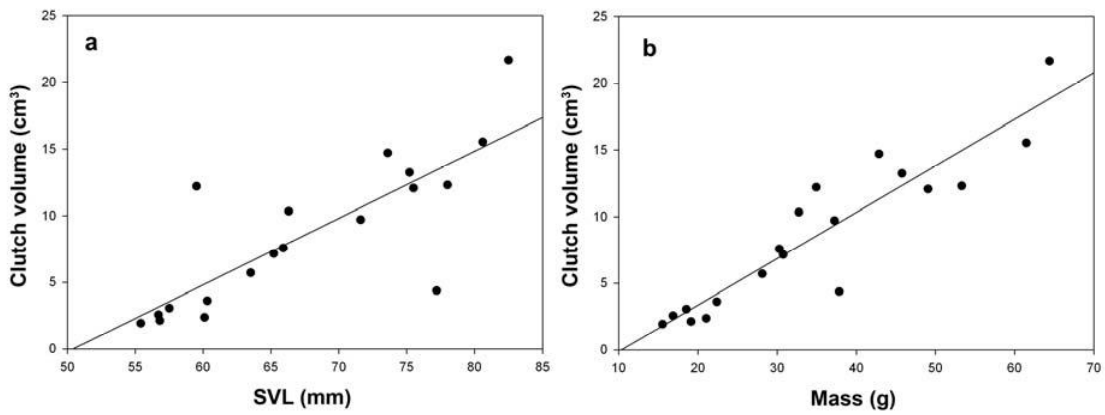


Figure 6.4. Correlation between females SVL (a) and final mass (b) and the clutch volume.

The females mass before and after oviposition differed significantly (t = 8.908, P < 0.001, paired t test), the final mass could be with up to 40% (in average with 27%) smaller after oviposition than the initial mass (fig. 6.5). Because the oviposition took a very short time, this mass difference mostly represents the clutch mass.

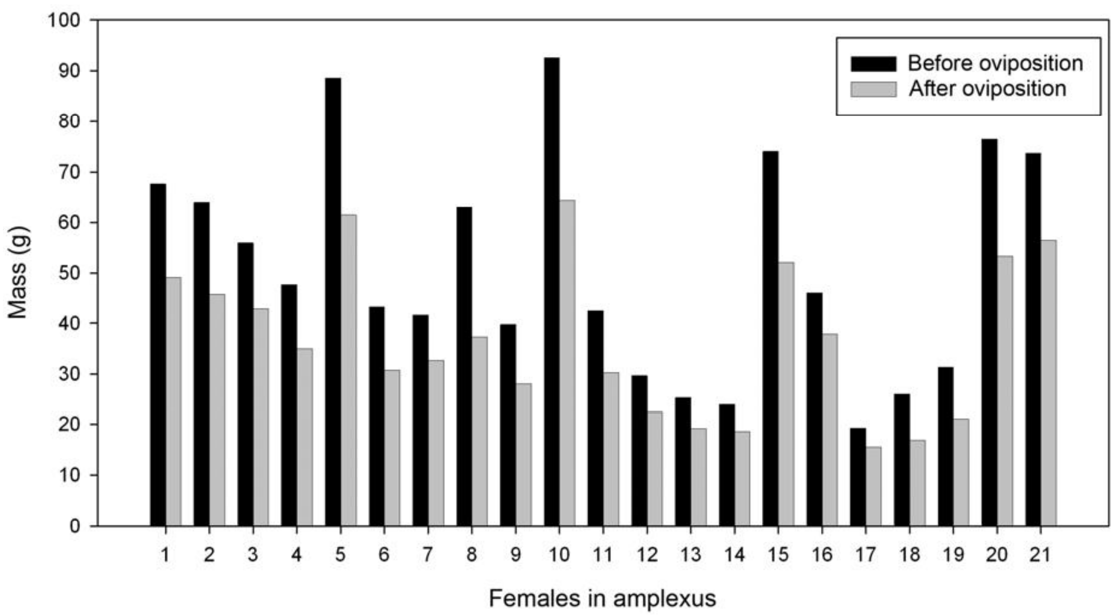


Figure 6.5. Females mass before and after oviposition.

Interestingly, the males mass also differed significantly, before and after oviposition (t = -6.778, P < 0.001, paired t test) the mass difference could represent even up to 10% (in average 4%) from the initial mass (fig. 6.6).

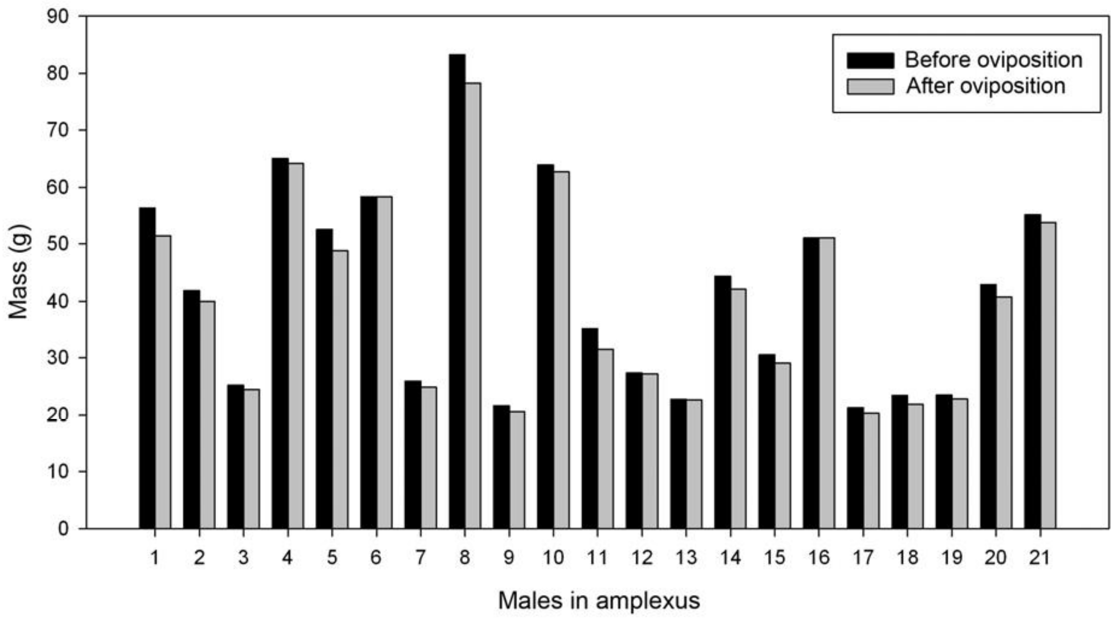


Figure 6.6. Males mass before and after oviposition.

In the spring of 2010 the breeding season of the studied *P. syriacus* population was a typical explosive breeding season, lasting only a few days. Generally, the other *Pelobates* species are considered prolonged breeders and not explosive ones (Nöllert, 1990; Eggert et al., 2006). This observed breeding pattern, with such a short period of time, was more similar with that observed in North American spadefoot toads (*Scaphiopus* sp. and *Spea* sp.) from the desert areas than the breeding pattern described for the other species of this genus.

The observed mating system was a typical scramble competition (Wells, 2007), in which the males tend to arrive at breeding ponds somewhat earlier than females, they gather in very dense clumps and they typically search for females in the water or on land around the breeding site (Davies and Halliday, 1979; Forester and Thompson, 1998). Within three days we observed a few thousand specimens gathering together to participate at the breeding.

One of the most interesting observations was related to the males vocalization during the breeding period. To our surprise, in the studied population the *P. syriacus* males did not emitted the characteristic courtship calls or territorial calls; the only observed vocalization being the release call, the call emitted by males and unreceptive females.

VII.

Sexual Dimorphism in the *Pelobates* Genus

In contrast to many birds and mammals, females of most anuran species (aprox. 90%) are considerably larger than males (Shine, 1979; Monnet and Cherry, 2002). Natural selection probably favors large body size in females because larger females produce larger clutches of eggs (Salthe and Mecham, 1974). This pattern was found to be true in *Pelobates fuscus*, *P. cultripes*, and *P. varaldii* but in the case of *P. syriacus* there were described populations with males having similar sizes or being even bigger than the females (Rot-Nikčević et al., 2001; Ugurtaş et al., 2002).

In the present study we compared the sexual size dimorphism of a *Pelobates syriacus* population in Grindul Lupilor (44°37'20''N, 28°48'46''E), from Dobrudja, a syntopic *P. fuscus* population from the same place and a *P. fuscus* population from Transylvania, near Sălicea locality, Cluj county (46°40'58''N, 23°32'38''E). The wet mass and snout-vent length were measured for 221 *P. syriacus* individuals (99 females and 122 males), 36 *P. fuscus* individuals from Grindul Lupilor (16 females and 20 males) and 297 from Sălicea (132 females and 165 males).

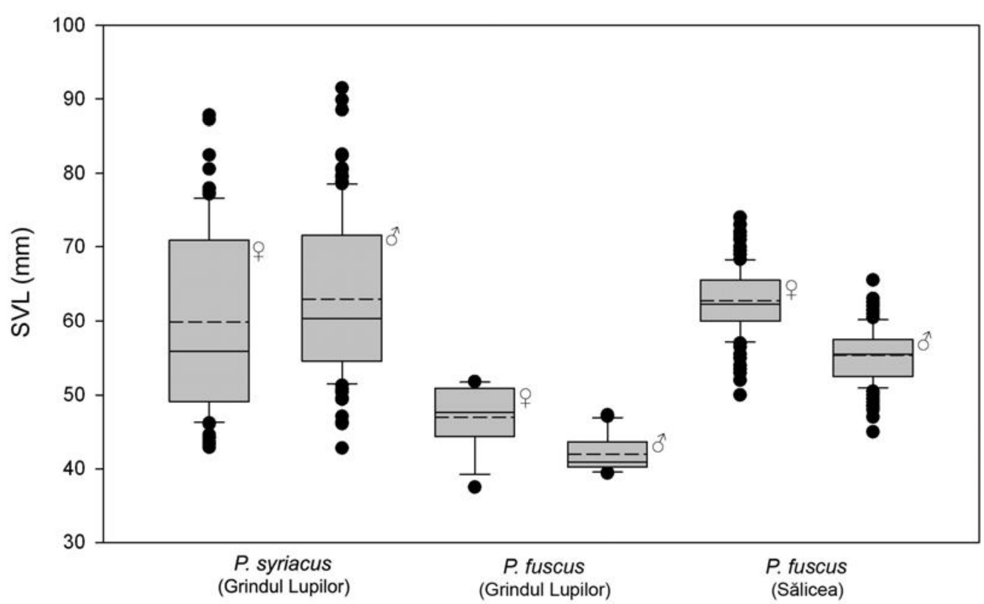


Figure 7.1. The snout-vent length (SVL) of the two *Pelobates* species from Grindul Lupilor, Dobrudja and Sălicea, Transylvania. The mean is represented with dashed line.

Sexual size dimorphism (SSD) was calculated as the mean female – male ratio, with values larger than 1 when the females were larger than the males and with values smaller than 1 when the males were the larger ones.

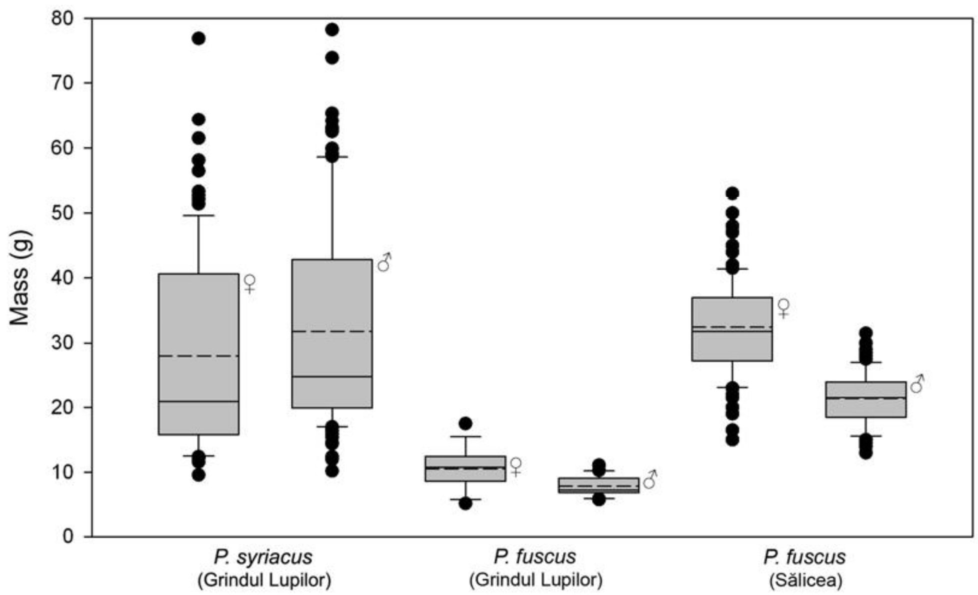


Figure 7.2. The mass of the two *Pelobates* species from Grindul Lupilor, Dobrudja and Sălicea, Transylvania. The mean is represented with dashed line.

The largest size was observed at the *P. syriacus* individuals, followed by the *P. fuscus* individuals from the Sălicea population and finally the *P. fuscus* individuals from Grindul Lupilor (fig. 7.1 and fig. 7.2). The mass and SVL of the males and females differed significantly for both species and study areas. Also, the mass and SVL differed significantly between the two species and the two *P. fuscus* populations in both females and males.

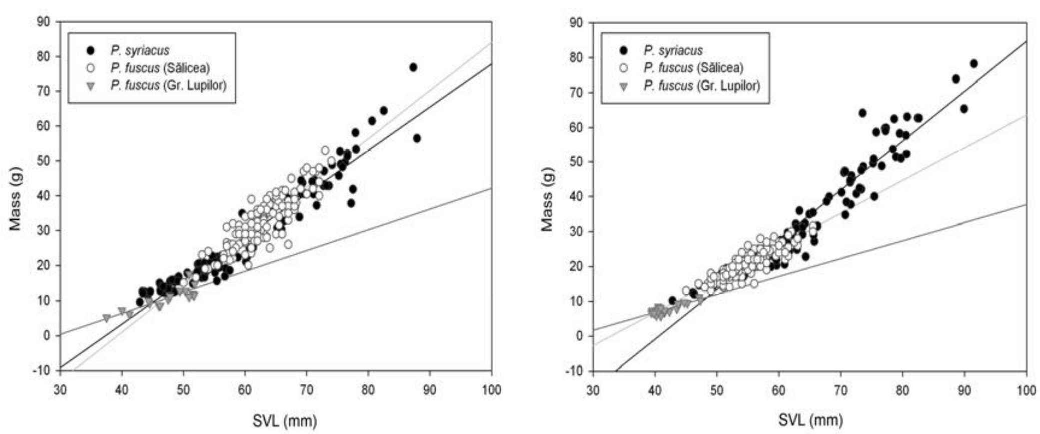


Figure 7.3. Correlation between mass and SVL of the females (left) and males (right) of the two *Pelobates* species from Grindul Lupilor, Dobrudja and Sălicea, Transylvania.

For both species the SVL was significantly correlated with the mass, in the case of females as well as males (fig. 7.3). For *Pelobates syriacus* the males proved to be significantly larger than the females, having the mass SSD value of 0.95 (fig. 7.2) and that of SVL 0.88 (fig. 7.1). In the case of *P. fuscus*, the males were significantly smaller than the females both in the Grindul Lupilor (mass SSD value 1.34 and SVL 1.12) and Sălicea (mass SSD value 1.52 and SVL 1.13) populations (fig. 7.1, fig. 7.2).

In the case of *Pelobates fuscus* the majority of the field data confirms that the females are larger than males (e.g. Nöllert and Günther, 1996; Rot-Nikčević et al., 2001). The situation is mostly similar in the other two western species, *P. cultripes* and *P. varaldii*. In the *P. fuscus* populations studied by us the females proved to be significantly larger than the males, having a bigger SVL and mass. The high SSD value of the mass in the case of the females belonging to the Sălicea population is due the fact that most individuals were captured before oviposition and so the mass includes also the mass of the eggs.

The reason why we analyzed such small *P. fuscus* sample from Dobrudja (compared to the *P. syriacus* sample) is that the population living in sympatry with *P. syriacus* in Grindul Lupilor is appreciably smaller. Even so, we consider that the results obtained and implicitly the analyzed sample reflects the reality from the field. We observed this situation in almost all *Pelobates* populations from Dobrudja in which the common spadefoot toad is living in sympatry with the eastern spadefoot toad. The gathered data seems to point out that *P. syriacus* is much better adapted to the special dry environment from Dobrudja, managing to sustain significantly larger populations than *P. fuscus*. Additionally, the *P. fuscus* individuals from the Sălicea population are significantly larger than those from Grindul Lupilor, the differences are most likely due to the different environmental conditions and available food sources.

In the *Pelobates syriacus* case, some data point out that the males have similar sizes with the females (Rot-Nikčević et al., 2001; Ugurtaş et al., 2002), or that they are larger than the females (Zaloğlu, 1964; Rot-Nikčević et al., 2001; Ugurtaş et al., 2002). In our case the males were significantly larger than the females and this observation proved to be true both in the case of the individuals analyzed in the laboratory (see chapter VI) and the individuals measured after the breeding period. The SSD values recorded for the eastern spadefoot toads for both sexes and parameters are relevant because the sample size was large enough and the females mass was recorded after oviposition.

VIII.

Variation in Body Size in two Distinct *Pelobates fuscus* Populations

For many anuran species, analyses of intraspecific geographical variability in morphology have often revealed extensive variation in body size (Lee, 1993; Mendelson, 1998; Castellano et al., 2000; Schäuble, 2004). The aim of the present study was to describe geographical variation in body size of the common spadefoot toad (*Pelobates fuscus*), by comparing morphometric data of individuals belonging to two different populations from different elevations and different bioregions, one Continental (Sălicea from Transylvania) and one Pontic (Grindul Lupilor in Dobrudja).

Figure 8.1. Grindul Lupilor, with temporary ponds (left) and the permanent pond situated near Sălicea (right) used for breeding by the common spadefoot toad populations.

The data for the Dobrudjan population were collected from an area (0 m elevation) in Grindul Lupilor, (fig. 8.1) were *P. fuscus* lives in sympatry with *P. syriacus*. This area is characterized by a mosaic of permanent and temporary ponds used for breeding by both spadefoot toad species. For the *P. fuscus* from Transylvania we analyzed the data gathered during five years of study (2000-2004), in a population located near Cluj, Sălicea locality (fig. 8.1). This is a hilly area, at 723 m elevation, with a couple of permanent ponds and many temporary ones, the number of which varies according to the rainy season and the quantity of snow accumulated during the winter. Most of these temporary ponds usually dry out by middle of June. The spadefoot toad population from this area used for breeding, in all study years, only one permanent pond, the largest and deepest one (fig. 8.1).

Wet mass of the captured individuals was measured with a Pesola balance to the nearest 0.2 g (Sălicea) and with a field digital scale (MyWeigh, model 400-Z) to the nearest 0.1 g (Grindul Lupilor). In order to examine morphological variation seven variables were measured with a digital calliper to the nearest 0.1 mm in a standardized manner: body snout-vent length (*SVL*), head width (*Ltc*), femur length (*F*), tibia length (*T*), tarsus length (*Ltars*), first toe length (*Dp*) and forearm length (*Lma*). In total, we measured 36 *P. fuscus* individuals from Grindul Lupilor (16 females and 20 males) and 297 individuals from Sălicea (132 females and 165 males).

The *Pelobates fuscus* individuals from Sălicea proved to be significantly larger than the ones from Grindul Lupilor (fig. 8.2), for both sexes. For females, excepting the femur length (*F*), all the variables were significantly different between the two populations, the females from Sălicea being notably larger than the ones from Grindul Lupilor. Also, the males from Transylvania were much larger than those from Dobrudja, all eight morphometric variables being significantly different between the populations. For both studied populations, the sexes differed significantly in terms of body sizes, with the females being larger than the males. Each of the eight variables was significantly different and the sexual size dimorphism index, SSD, was bigger than 1 for each measured feature.

The results of this study confirm the existence of significant geographic variation in body size between the two *Pelobates fuscus* populations. There are many factors which may explain differences in body size among populations, such as climatic conditions, trophic resources, metabolic proprieties, interspecific competition or predator – prey interactions. Since these causes are not mutually exclusive, it is difficult to determine their individual influence on the optimal size for species or for particular location (Kozlowski, 1992).

However, in our case, the most probable explanation for the observed body size differences of the spadefoot toads from the two populations is that these are due to the radical climatic differences of the study areas. Dobrudja represents one of the southern limits of *P. fuscus* distribution area (Džukić et al., 2005; Crottini et al., 2007; Džukić et al., 2008), and it is probably that the characteristic, dry environment is the most important limitative factor of the attainable body size for the spadefoots in this region. Also, the same environmental conditions are probably responsible for the relative small size of the common spadefoot toad populations inhabiting Dobrudja. And finally, another possible explanation for the size differences could be the competition, in larval or adult stages, with *P. syriacus* because in Dobrudja both species occupy the same ecological niche.

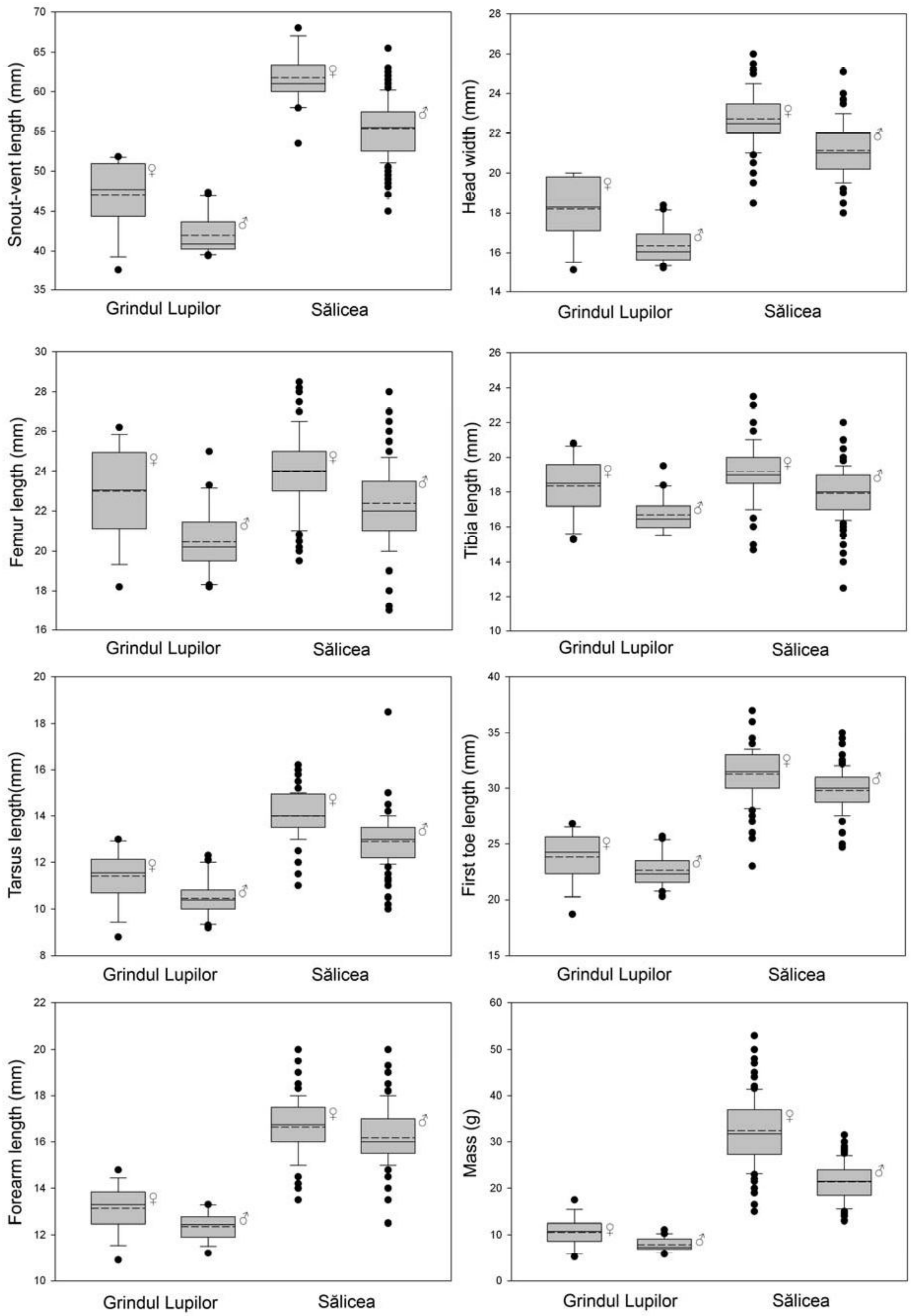


Figure 8.2. The eight morphometric variables analyzed in the *Pelobates fuscus* individuals from Grindul Lupilor, Dobrudja and Sălicea, Transylvania. The mean for each variable is represented with dashed line.

Conclusions

The Distribution of Amphibians in Dobrudja

In Dobrudja there can be found 12 amphibian species (nine species and a species complex), some with limited distribution and others widespread: *Triturus* (*Lissotriton*) *vulgaris* (23 locations), *Triturus dobrogicus* (30 locations), *Bombina bombina* (75 locations), *Pelobates fuscus* (51 locations), *Pelobates syriacus* (33 locations), *Bufo bufo* (12 locations), *Bufo* (*Pseudepidalea*) *viridis* (195 locations), *Hyla arborea* (98 locations), *Rana dalmatina* (32 locations), *Rana* (*Pelophylax*) *lessonae* (14 locations) and *Rana* (*Pelophylax*) *esculenta* complex (211 locations). From the total number of records, 66% are after 1990, 14% are before 1990 and 20% are continuous data. From the records after 1990, 45% are our own data, 22.5% being new locations. According to the administrative units, 40% of the records are located in Constanța county and 60% in Tulcea.

Based on the cartographic analysis we proposed the following conservation statuses for the amphibian species in Dobrudja: six species are considered **Endangered** - *Triturus (Lissotriton) vulgaris*, *Triturus dobrogicus*, *Pelobates syriacus*, *Bufo bufo*, *Rana dalmatina* and *Rana (Pelophylax) lessonae*; one species is **Vulnerable** - *Pelobates fuscus*; two species are **Near Threatened** - *Bombina bombina* and *Hyla arborea*; three species are listed as **Least Concern** - *Bufo* (*Pseudepidalea) viridis*, *Rana (Pelophylax) ridibunda* and *Rana (Pelophylax) esculenta*.

Aspects of the Biology and Ecology of the genus *Pelobates* in Dobrudja

The results of our phenotypic plasticity experiment showed that *Pelobates syriacus* tadpoles were able to respond to pond drying by speeding up their metamorphosis and that metamorphosis was not influenced by water level, but by water level decrease rate. This response represented a reduction with 4% of the larval period compared to the control group. The reduction of the developmental time in the fast decreasing treatment was accompanied by a smaller metamorphic size, representing a significant reduction with 9% of mass and 4% of SVL at metamorphosis as compared to the control.

Regarding the post-metamorphic growth, the *P. syriacus* juveniles proved to be more efficient, having a general mass growth rate with up to 21% higher than those of *P. fuscus*. Likewise, the general SVL growth rate was higher, with up to 4% than those of *P. fuscus*. These differences were gained in spite of the fact that the individuals from both species were fed with (proportionally) same amount of food. Also, the *P. syriacus* juveniles proved to be more efficient in converting the ingested food mass into body mass, by having the food conversion ratio with up to 33% lower than those of *P. fuscus*.

Pelobates syriacus can be an explosive breeder, similarly to other species from semi-arid environments, in the spring of 2010 the breeding season of the studied population lasting only a few days. The observed mating system was a typical scramble competition and the males did not emit the characteristic courtship calls or territorial calls; the only observed vocalization being the release call, the call emitted by males and unreceptive females.

The *P. syriacus* clutch size was strongly correlated with both the females SVL and final mass. Also, the clutch volume was significantly correlated with the females' SVL and especially with their final mass. On the other hand, no significant correlation was found between egg size and clutch size nor between the egg size and the SVL and mass of the females. The females mass before and after oviposition differed significantly; the final mass could be with up to 40% smaller than the initial mass. Interestingly, the males mass also differed significantly, before and after oviposition, the mass difference could represent even up to 10% from the initial mass. The large females ($\geq$ 65 mm) clutch size was significantly larger than that of small ones ($\leq$ 63.5 mm), being approximately 60% larger.

In contrast to the most anuran species, the *Pelobates syriacus* males from Grindul Lupilor proved to be significantly larger than the females. As for the *P. fuscus*, the males were significantly smaller than the females in both Grindul Lupilor and Sălicea populations

The results of our study confirm the existence of significant geographic variation in body size between the two studied *Pelobates fuscus* populations. The individuals from Sălicea proved to be significantly larger than the ones from Grindul Lupilor, all eight measured morphometric variables being significantly bigger in both females and males.

List of papers

This thesis is based on the following papers:

Published papers

Székely, P., Tudor, M., Cogălniceanu, D. 2010. Effect of habitat drying on the development of the Eastern spadefoot toad (*Pelobates syriacus*) tadpoles. *Amphibia-Reptilia* 31: 425-434.

Székely, P., Plăiaşu, R., Tudor, M., Cogălniceanu, D. 2009. The distribution and conservation status of amphibians in Dobrudja (Romania). *Turkish Journal of Zoology* 33: 147-156.

Székely, P., Plăiaşu, R., Tudor, M., Cogălniceanu, D. 2009. A preliminary record list of amphibians in Dobrudja (Romania). *Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, s. Biologie Animală* Tom LV: 145-153

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Tudor, M., **Székely, P.** 2008. Date privind herpetofauna Parcului Naţional Munţii Măcinului. *Lucrările Conferinţei Naţionale de Ecologie, „Protecţia şi restaurarea bio şi ecodiversităţii", 11-14 Octombrie 2007 Mamaia.* Edit. Ars Docendi 59-60.

Manuscripts

Székely, P., Buhaciuc, E., Cogălniceanu, D. Adaptive phenotypic plasticity in timing of metamorphosis in two species of *Pelobates* (Anura: Pelobatidae), *P. fuscus* and *P. syriacus*.

Székely, P., Buhaciuc, E., Butănescu, D. Cogălniceanu, D. Variation in body size and sexual dimorphism in two distinct *Pelobates fuscus* populations

Székely, P., Buhaciuc, E., Butănescu, D. Cogălniceanu, D. Aspects of breeding activity in a population of *Pelobates syriacus* from Dobrudja, Romania.

Székely, P., Cogălniceanu, D. Early post-metamorphic growth in two species of *Pelobates* (Anura: Pelobatidae): a comparative study.

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