OVIDIUS UNIVERSITY OF
CONSTANȚA
DOCTORAL SCHOOL OF
APPLIED SCIENCE
DOMAIN: BIOLOGY

LIFE CYCLE EVOLUTIVE CHARACTERISTICS OF AMPHIBIANS LIVING IN SYNTOPY

Doctoral thesis summary

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Constanța, 2015

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Key words: amphibians, Dobrogea, evolutive characteristics, syntopic poppulations, larvar period, metamorphosis, growth rate, growth efficiency, reproduction, adaptive strategies, trophic offer, *Pelobates fuscus*, *Pelobates syriacus*, *Triturus dobrogicus,* conservation measures

ACKNOWLEDGEMENTS

I am grateful to my PhD coordinator prof. dr. Dan Cogălniceanu, for his trust, guidance, advice and encouragement, suggestions and comments, for the knowledge accumulated over the years of cooperation and especially for the patience offered when building the present paper

I am grateful to conf. dr. Teodora Maria Onciu for precious advice and guidance in carrying out laboratory experiments and field applications. I am grateful for guidance, suggestions and constructive criticism to my referees: conf. dr. Marius Skolka, acad. dr. Dumitru Murariu and prof. dr. Laurențiu Rozylowicz.

I am grateful to my colleagues for a productive collaboration and for their support over the years of research required in building the present paper:

Fieldwork: dr. Székely Paul, Diana Székely, dr. Florina Stănescu, Iosif Ruben, Monica Bogdan, Sebastian Topliceanu,

Laboratory experiments: dr. Székely Paul, dr. Marian Tudor, Diana Székely

Sample processing: Diana Mânzală

Data analysis: dr. Raluca Băncilă

I am grateful for their support and advice to: dr. Daniela Mariana Roșioru, dr. Ciprian Samoilă, dr. Tibor Hartel, dr. Rodica Plăiașu, dr. Cristina Preda, Daniyar Memedemin, Valentina Moraru

The research needed to carry out this thesis was made possible trough financial support provided by Romanian National Authority for Scientific Research by financing yhe project ”Is range limited by life history, environmental conditions or biogeographical barriers? A study case using spadefoot toads (genus *Pelobates*)” from the program PN II IDEI (2011-2016: PN-II-PCE-2011-3-0173), project manager: Prof. Dr. Dan Cogălniceanu. I am grateful to the Danube Delta Biosphere Reserve Administration whose support i have received.

I dedicate this work to my family for all their support and for their efforts that led to the completion of this thesis.

Introduction

Amphibians are a key group of vertebrates for the proper function of the ecosystems to which they belong (Mitchell, Sommer and Speldewinde, 2013) adapted both to terrestrial and aquatic environment, which is why they experienced a diversified adaptive evolution (i.e. physiological, anatomical, morphological and behavioral).

In recent decades there was a decline of amphibian species worldwide (Alford and Richards, 1999; Houlahan et al., 2000), with climate changes representing the main cause of decline (Gibbons et al., 2000; Kiesecker et al., 2001; Carey and Alexander, 2003; Collins and Storfer, 2003; Corn, 2005; Ficetola et al., 2015). Other factors involved in the decline are represented by human direct impact through destruction or fragmentation the amphibian habitats (Cushman, 2006; Becker et al., 2007), impact of harmful chemicals acting directly on their lifecycle producing major physiological imbalances (Berrill, 1998; Mann et al., 2009; Köhler and Triebskorn, 2013), alien invasive species (Johnson et al., 2011; Preston, Henderson and Johnson, 2012; Bleach, 2014), over-exploitation (Wilcove et al., 1998; Natusch and Lyons, 2012). The amphibian species decline will be probably followed by other species decline, species that rely on amphibians' existence or the opposite, the uncontrolled development of the species which represents food for amphibians, even to the point when they become pests. Therefore, amphibian conservation is very important for maintaining the ecosystem equilibrium. In order to apply the most adequate conservation methods for amphibians, a better understanding of their biology and ecology is required. The study of the evolution of their complex adaptations is a very important subject in ecology and conservation.

The present thesis is dedicated to the study of amphibians from the coastal area of the Black-Sea. It is structured in five chapters, covering the most important stages of their life cycle. The studies were focusedon three amphibian species, representatives of two classes Anura and Caudata, (*Pelobates syriacus*, *Pelobates fuscus* and *Triturus dobrogicus*), living in syntopy.

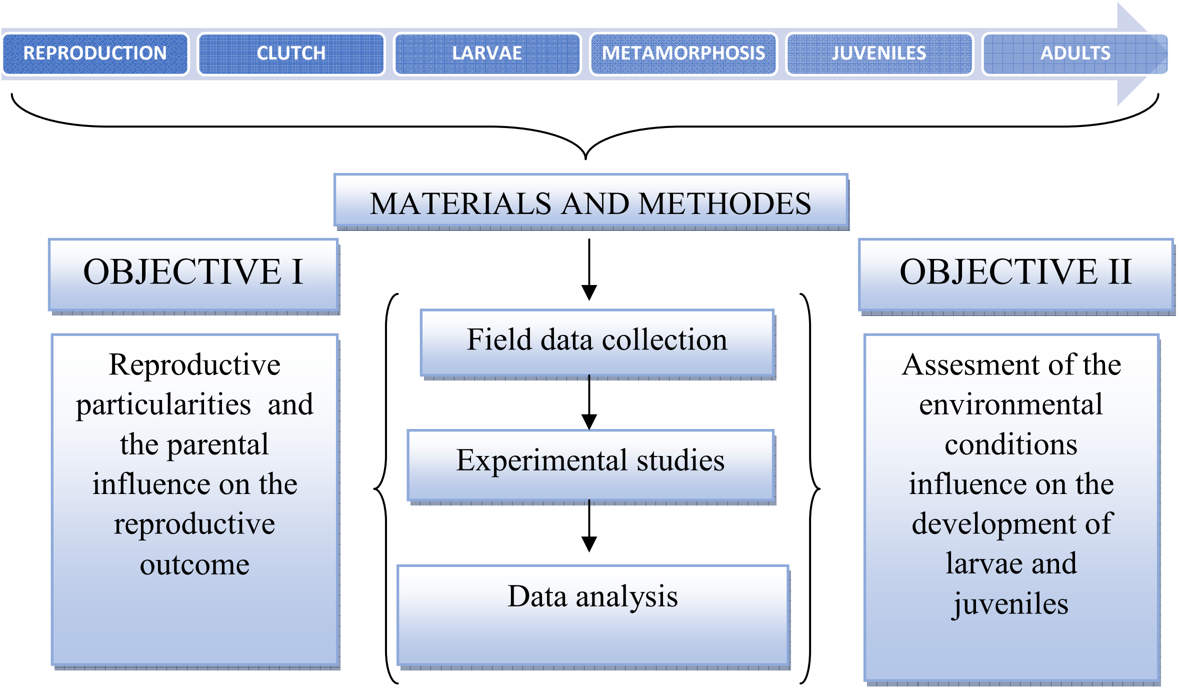

Fig.1. The work plan related to the life cycle of the studied species.

The research was done in two sites, located at the distribution limit were the studied species coexist (i.e. northern limit for *P. syriacus,* southern limit for *P. fuscus* and eastern limit for *T. dobrogicus*). The two study sites are located in the Danube Delta Biosphere Reserve (i.e. Grindul Lupilor: 44° 37.256'N; 28° 48.729'E and Histria: 44°32'56" N; 28°45'56"), located in the Razelm-Sinoe lagoon complex.

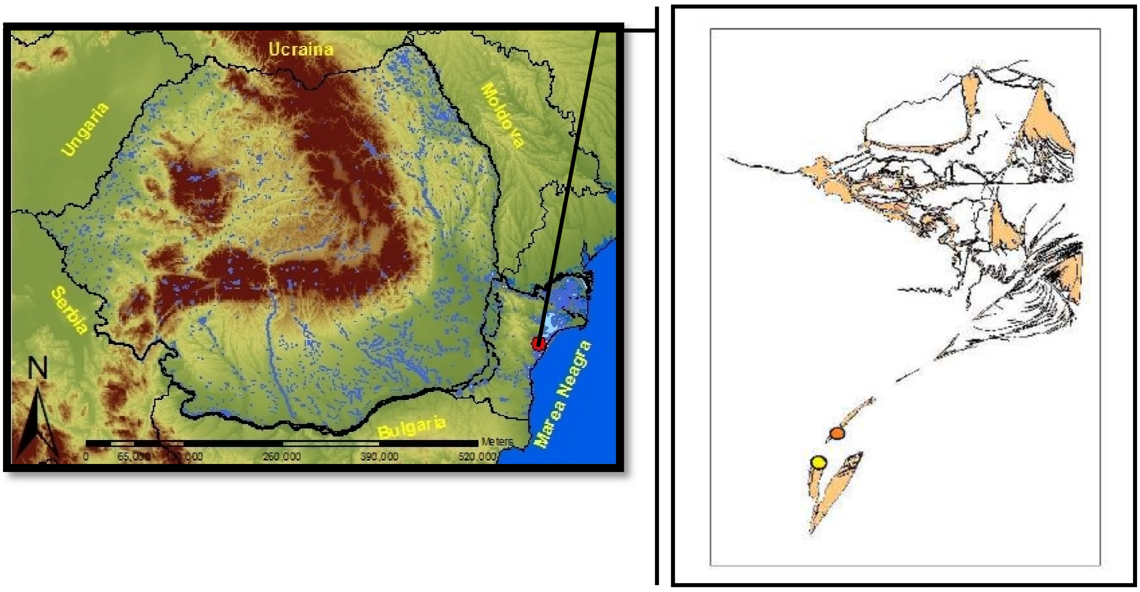

Fig. 2. The two studied areas position in Romania (A) and their location in the Danube Delta Biosphere Reserve (B) Grindul Lupilor (orange circle), Histria (yellow circle).

The methodology used in performing the present study was in agreement with the applicable regulations and has been approved by the Ethics Committee of Ovidius University, Constanța. The field activity was conducted based on the annual permits issued by the Danube Delta Biosphere Reserve Administration. For data analysis I used parametric and non-parametric statistical methods for the intra and interspecific comparisons and linear mixed effects models (LMM), for the quantification of the relations between variables.

Chapter I – Population parameters characterization

The study of the population structure parameters is necessary for the population characterization and dynamics (Good, 1953; Kimura, 1958).

I performed a comparative evaluation of the population structure parameters for the studied species (*P. fuscus*, *P. syriacus* and *T. dobrogicus*):

(i) body size distribution (body mass and length)
(ii) age distribution (adults/juveniles)
(iii) sex distribution (females/males)
(iv) the number and the spatial distribution of clutches

Biometric data collection

In 2010 and 2011 spring, I performed field applications on the studied area (Grindul Lupilor), where I collected 18 *Pelobates fuscus* adults (11 females and 7 males), 76 *Pelobates syriacus* adults (39 females and 37 males) and 27 *Triturus dobrogicus adults* (15 females and 12 males). In 2013 spring I collected 26 *T. dobrogicus* adults (16 females and 10 males). Biometric data was collected *in situ* and in the laboratory, depending on the experiment conducted.

Reproductive dynamics characterization

In order to estimate the number and density of clutches and the spatial distribution of adults, in early April 2013 and late March 2014, I delimited 10 m length segments, over a distance of 100 m, along the shore of the reproduction site.

I estimated the clutch number and the number, sex and body size of individuals for the studied species. The observations have been made on a lateral distance of 2 m length from the shore, outwards and inwards of the reproduction pond.

Biometric data of the studied species are presented in Table I.1. In *P. syriacus* there is no sexual dimorphism (ANOVA: $F_{2,76}$= 0.99, P>0.05), meanwhile in *P. fuscus* (ANOVA: $F_{2,18}$= 6.38, P=0.02) and *T. dobrogicus* (ANOVA: $F_{2,53}$= 4.60, P=0.03) females are significantly larger than males. During the reproductive period the percentage of females observed in water related to the total number of females differ significantly from the percentage of males observed in water related to the total number of males (ANOVA: $F_{2,172}$ = 7.12, P= 0.029). Sex ratio is 7.5:1 male biased. The body size of the *P. syriacus* *s*olitary and in amplex adults is similar (ANOVA: $F_{2,898}$ = 0.21, P= 0.921 for males and ANOVA: $F_{2,172}$= 0.48, P = 0.628 for females).

Table I.1. Biometrics of the amphibian individuals collected from syntopic populations from Grindul Lupilor (Danube Delta Biosphere Reserve). The presented data represents mean and standard deviation and the domain of values (minimum-maximum) in brackets.

Species	**Parameters**			
	SVL (mm)	**Body mass (g)**	**SVL (mm)**	**Body mass (g)**
Pelobates syriacus	♀ (n=39)		♂ (n=37)	
	73.76±11.50	56.82±25.03	76.12±10.59	51.53±20.90
	(55.4-99.5)	(19.24-118.18)	(59.3-93.7)	(21.28-91.9)
Pelobates fuscus	♀ (n=11)		♂ (n=7)	
	46.05±4.83	10.4±3.62	41.52±2.49	7.64±1.46
	(37.5-51.8)	(5.2-17.5)	(39.4-46.2)	(5,9-9,8)
Triturus dobrogicus	♀ (n=31)		♂ (n=22)	
	67.92±4.66	6.81±1.04	59.78±6.26	4.7±0.97
	(56.73-76.4)	(5.26-9.21)	(38.7-71.0)	(3.44-7.09)

Body size of the males and females in amplex is similar (ANOVA: $F_{2,34}$= 0.33, P = 0.491) (Fig. I. 1), result which indicates the absence of sexual selection.

Clutch distribution in the studied area indicates that in *P. syriacus* the reproductive activity occurs localized, certain areas being favored (Fig. I.2). The reproductive dynamics analysis shows the explosive breeding characteristic of *P. syriacus* (Fig.I.3).

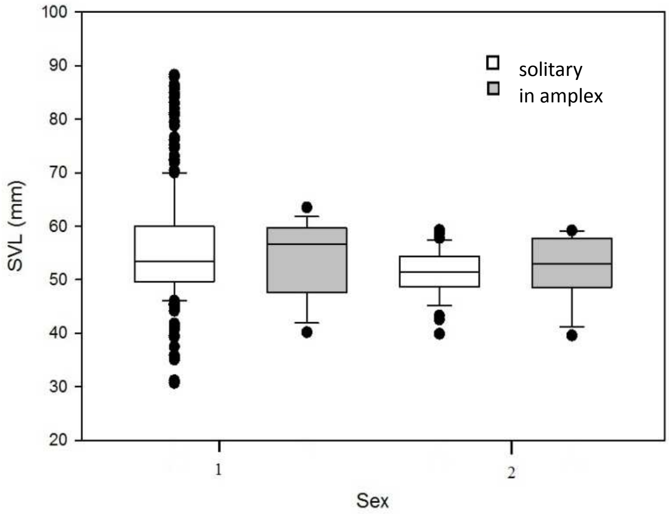

Fig.I.1. *Pelobates syriacus* adults body size (1=males, 2=females), solitary or in amplex. The graph shows the first and the third quartile around mean values (continuous line), minimum and maximum values (terminal line) and the extrem values (black dots).

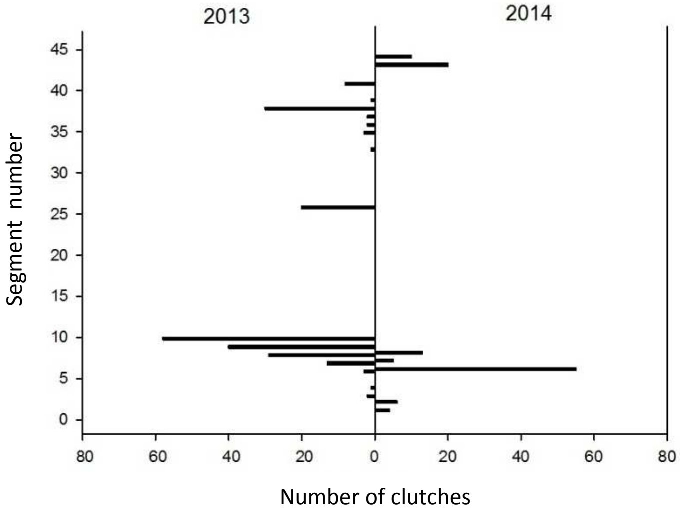

Fig. I.2. Clutch distribution along the track. April 2013 (A) and March 2014 (B).

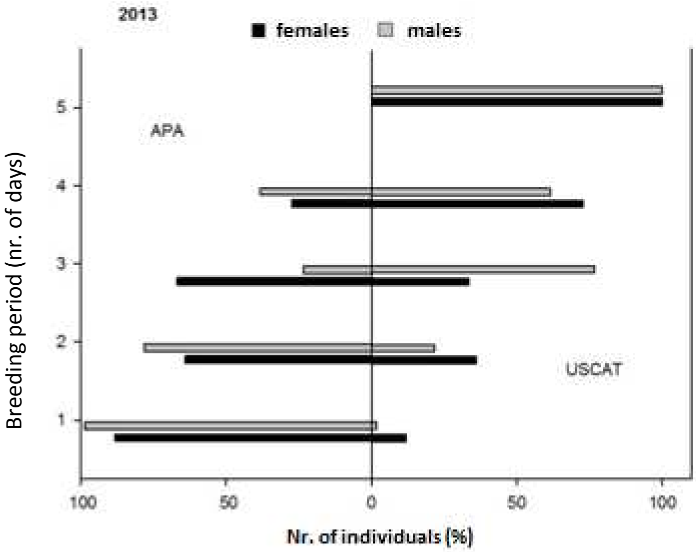

Fig. I.3. Reproductive dynamics during the reproductive period in *P. syriacus*.

Sexual dimorphism is present in *P. fuscus* and *T. dobrogicus,* where females are significantly larger than males, while in *P. syriacus* body size of both sexes are similar. The results of the present study, exclude the hypothesis of assortative mating in *P. syriacus* trough the explosive breeding characteristic (e.g. 4-6 days) and the almost complete absence of vocalization which causes forming of adults clumps and unselective pairs. The *P. syriacus* reproductive population consists mainly from male adults (88.23%). This ratio can induce a physiological stress on females altering their behavior (Grayson et al., 2012). The uneven clutch distribution along the segments, grouped into distinct areas, in both observation periods, shows a high fidelity for the egg deposition location in *P. syriacus*.

Chapter II – Reproductive success. Comparative study between *Pelobates syriacus* (Anura) and *Triturus dobrogicus* (Caudata)

The way energy resources are allocated to reproduction represents an important factor which influences the reproductive characteristics (Kaplan and King, 1997). During the evolution process, two major reproductive strategies diverged (i) the *r* reproductive strategy, characterized by numerous descendants without parental care, (ii) the *K* reproductive strategy, characterized by few descendants, based on the high competitiveness between them, descendants which benefits of parental care (Nichols et al., 1977). All the amphibian species from Romania, as the majority amphibian species from the temperate zone, adopted an *r* reproductive strategy.

I compared the reproductive strategy for *Pelobates syriacus* (Anura) and *Triturus dobrogicus* (Caudata). *T. dobrogicus* shows a more complex form of the reproductive strategy, with the appearance of an egg protection behavior. I compared the influence of the different reproductive strategies on the reproductive success, for the studied species.

During March-April 2013, at the beginning of the reproductive period, field applications were conducted. 22 *Pelobates syriacus* adults were collected (11 pairs) at the end of March 2013 and 26 *Triturus dobrogicus* adults (14 females and 12 males) were collected in early April 2010. Adult SVL (snout-vent length) and body mass were collected before and after egg deposition, and the total number of eggs deposited by each female was recorded. I measured 20 eggs from the clutch of each female for both studied species. The adults and the clutches were released at the end of the experiment at the collection site.

The majority of the parameters which characterizes the reproductive activity in *P. syriacus* and *T. dobrogicus* females differ significantly between the two studied species (e.g. body size, egg deposition period, eggs number), but the egg volume was similar (Tab.II.1). The comparative analysis of *P. syriacus* female body size during the reproductive period, in two different years (i.e. 2013, 2014), shows a reproductive behavior size dependent. Small females were present at the breeding ponds at the beginning of the breeding period, followed by the large females after two-three days. But, the large females are usually older (Cogălniceanu et al., 2013a). Therefore, *P. syriacus* females are probably starting their annually reproductive period age dependent. This fact can explain the small body sizes of the *P. syriacus* females studied in 2013. (Tab. II.2.).

Table II.1. Parameters of reproductive activity in *T. dobrogicus* and *P. syriacus* females. The data represents mean and standard deviation and the domain of values (minimum and maximum) in brackets.

Parameters	***T. dobrogicus***	***P. syriacus***
	n=15	n=11
SVL (mm)	70.86±3.19 (65.10-76,40)	52.20±8.96 (44.40-76.20)
Body mass (g)	6.64±1.13 (5.20-9.21)	21.20±19.13 (10.14-77.66)
Egg deposition period (days)	13-31	1
Total number of eggs deposited	286.57±136.99 (29-490)	724.63±541.20 (350-2260)
Egg volume (mm^3)	3.50±0.68 (2.27-4.51)	3.19±1.12 (2.22-6.03)

Body mass of *T. dobrogicus* females does not influence total number of eggs deposited (n=15; R^2=0.00631; $P > 0.05$) but, between the body mass of *P. syriacus* females and total number of eggs deposited there is a significant positive correlation (n=11; R^2= 0.928; $P < 0.05$). Egg size is not influenced by neither body mass nor length (SVL) of females in *T. dobrogicus* (n=15; R^2= 0.00683; $P > 0.05$). For the *P. syriacus* females there is a weak positive correlation between this two variables (n=11; R^2= 0.118; $P < 0.05$). SVL of *T. dobrogicus* females is positive correlated with the egg jelly volume (n=15; R^2= 0.113; $P < 0.05$). The eggs volume between the two studied species is similar (ANOVA: $F_{2, 500}$= 0.64, P=0.065).

There is a negative correlation between the egg jelly volume and total number of eggs in *T. dobrogicus* females (n=15; R^2= 0.173; P<0.05), most likely due to energy resource depletion, the females being unable to produce thicker gelatinous capsules. In *P. syriacus*, eggs volume decreases with the increase in numbers (n=19; R^2= 0.201; P<0.05). The body mass before and after eggs deposition differ significantly in *P. syriacus* females (ANOVA: $F_{2,\ 22}$=1.08, P<0.05) (Fig. II.1.) but in *T. dobrogicus* the body mass before and after eggs deposition is similar (ANOVA: $F_{2,\ 30}$ = 0.23, P = 0.818).

Table II.2. Dynamics in time of reproductive activity parameters for *P. syriacus.* Based on the results of a previous study (i.e. 2010, 2011) (Cogălniceanu et al. 2013b) and the results of the present study. The data represents mean and standard deviation and the domain of values (minimum and maximum) in the brackets.

Parameters	***P. syriacus***		
	2010 **n=22**	**2011** **n=16**	**2013** **n=11**
SVL (mm)	69.24±9.71 (55.40-87.90)	78.36±10.31 (55.60-89.60)	52.20±8.96 (44.40-76.20)
Body mass (g)	50.57±21.59 (19.24-92.55)	61.58±24.53 (20.91-102.62)	21.20±19.13 (10.14-77.66)
Total number of eggs deposited	2474.05±1470.14 (865-5812)	3225.93±1591.71 (927-5266)	724.63±541.20 (350-2260)

P. syriacus females experienced a higher reproductive effort compared to *T. dobrogicus,* reflected in *P. syriacus* females decrease in body mass during the breeding period with an average of 20% of their body mass. At first, SVL of *T. dobrogicus* females influence eggs volume. When correlating the results of the present study with the results of a previous one (Cogălniceanu et al., 2013a), I can suggest that egg volume is influenced by females age.

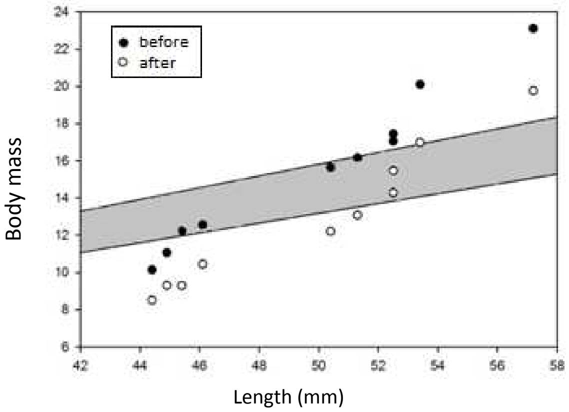

Fig. II.1. The differences in body body mass before and after eggs deposition in *Pelobates syriacus* females. The shad corresponds to the regression line indicating the loss in body mass.

T. dobrogicus females' body size is influencing the egg jelly volume, which has a protective purpose for the egg yolk. The egg jelly has the origin in the oviduct, different parts of the oviduct generating different layers of the egg jelly (Duellman and Trueb, 1994). A female with large body size, has most likely, a large oviduct, leading to the formation of an egg jelly with large volume. Therefore, the eggs are better protected from environmental factors (e.g. temperature, radiation, different fungus).

The number of eggs deposited by *T. dobrogicus* females can be influenced by internal factors (e.g. hormonal control) being demonstrated the fact that the females stop from eggs deposition even if the eggs stock is not depleted (Cogălniceanu et al., 2013a). The maternal care granted by the newts females to their eggs represents a reproductive behavior strategy which contributes to increasing reproductive success, compensating for the smalll number of eggs deposited and for the increased embryos mortality caused by a chromosomal mutation (Sessions et al., 1988).

Chapter III - Parental influence and the crowding effect on the *Pelobates syriacus* larvae development

Parental contribution effects are important factors in larval development (Mousseau and Fox, 1998; Michimae et al., 2009). They can be correlated with phenotype plasticity (Royle et al., 2001; Mitchell and Read, 2005; Nussey et al., 2005; Bernardo, 1996b). A previous study tested the *P. syriacus* tadpoles' response to water level decrease (Székely et al., 2010). One of the conclusions of this study was that larval development and size at metamorphosis are not influenced by water level but by water level decrease rate. The small body sizes of metamorphs raised in constant low water level can be caused by high density level (i.e. crowding effect). I aimed to test the influence of two factors on larval development, using *P. syriacus* tadpoles:

(i) parental body size effect on larval development hatched from similar sized eggs;

(ii) crowding effect on survival rate and metamorphosis in constant environmental conditions;

Parental body size effect

During reproduction in March 2013, two *P. syriacus* pairs were selected: large body size (g♀=77.66 g; g♂=68.58 g) and small body size (g♀=20.11 g; g♂=14.13 g). The pairs deposited the eggs in laboratory conditions. From each pair, we selected similar sized eggs. Whene the tadpoles hached we selected randomly 40 individuals. The tadpoles were then assigned in two different experimental treatments (treatment 1: tadpoles from the large body size pair; treatment 2: tadpoles from small body size pair). Once every two weeks the tadpoles were photographed on plotting paper in order to measure their dimensions (i.e. body length: snout tip-caudal artery crutch; tail length: caudal artery crutch-tail tip; total length: sum of body and tail length), using Image Tool 2.0. The experiment lasted for 464 days (2013-2014). At the end of the experiment metamoprhs body length (i.e. SVL: snout-vent length).

Laval development parameters are presented in Table III. 1. Tadpoles growth rate differ significantly between the two treatments (Kruskal-Wallis: H=24.94, P <0.001). Final tadpoles length and metamorphs length was similar between the two treatments: treatment 1 (Kruskal-Wallis: H = 2.55, P=0.11) and treatment 2 (Kruskal-Wallis: H=1.39, P=0.23) but tadpoles from treatment 1 reached metamorphosis faster than tadpoles from treatment 2 (Kruskal-Wallis: H=12.76, P=0.02; Tukey: q=2.98, P<0.05).

Final larval length is positively correlated with growth rate for both treatments (Pearson, R^2=0.33, P<0.05) (Fig. III. 1). *P. syriacus* tadpoels from both treatments differ significantly in length and length growth rate depending on parental body size. During the initial stage of development and immediatley before metamorphosis tadpoles from treatment 1 grow faster than tadpoles from treatment 2. However, the differentiation trend did not continue until metamorphosis. Tadpoles from treatment 1 reached metamorphosis faster than tadpoles from treatment 2, in constant environmental conditios, suggesting that age at metamorphosis is influenced by genetic parental contribution.

Table III.1. Initial (Li) and final larval length (Lf), metamorphs length (Lm), length growth rate and age at metamorphosis of *P. syriacus* tadpoles. The data represents mean and standard deviation and the domain of values (minimum and maximum) (n=40 for each experimental treatment).

Parameters	**♀♂ small body size**	**♀♂ large body size**	**Comparison between treatments**
Li (mm)	5.27±0.537 (4.30-6.89)	5.81±0.51 (4.19-6.70)	ANOVA: $F_{2.80}=20.77$ $P<0.001$
Lf (mm)	104.34± 8.28 (88.68-117.81)	107.72±10.47 (87.78-126.35)	Kruskal-Wallis: H=2.55 P=0.11
Lm (mm)	30.65±4.21 (20.03-44.74)	31.31±3.68 (24.88-41.86)	Kruskal-Wallis: H=1.39 P=0.23
Growth rate (mm/day)	0.24±0.016 (0.20-0.27)	0.26±0.02 (0.20-0.31)	Kruskal-Wallis: H=24.94 $P<0.001$
Age at metamorphosis (no. of days)	415.17±26.06 (366-464)	404.41±17.50 (380-450)	Kruskal-Wallis: H=4.74 P=0.02

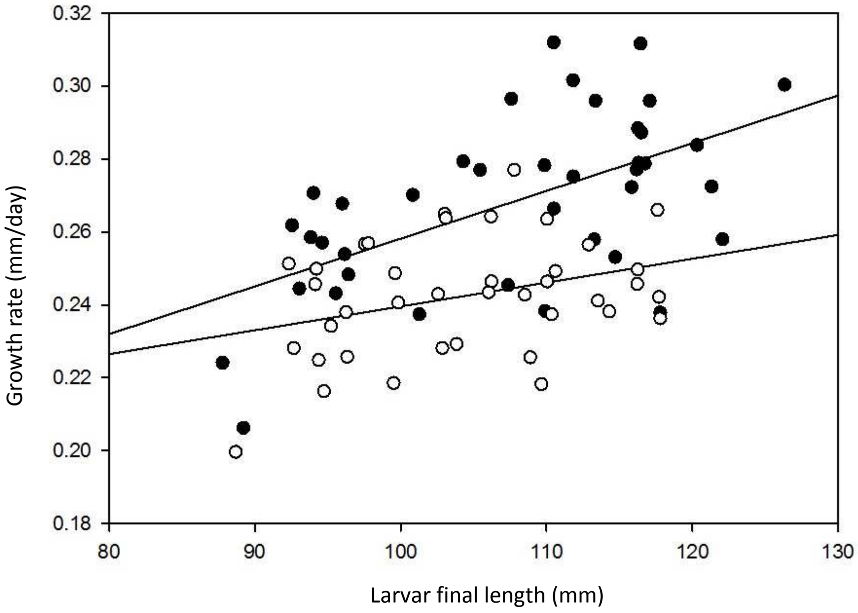

Fig. III.1. Length growth rate and final larval length relation for each *P. syriacus* tadpoles for both clutches (black circle - large body size pair, white circle - small body size pair).

Crowding effect

I used 4 experimental treatments with 10 tanks for each treatment: treatment 1 – a tadpole for each tank (10 tadpoles), treatment 2 – two tadpoles for each tank (20 tadpoles), treatment 3 – three tadpoles for each tank (30 tadpoles), and treatment 4 – four tadpoles for each tank (40 tadpoles). The tadpoles were selected from a single *P. syriacus* pair, in order to avoid possible genetic influences on further development. Tadpoles' body length was measured once a month using digital calipers with a precision of 0.01 mm. At the end of the experiment metamorphs body length was measured (SVL). Laraval development parameters depending on experimental treatment are presented in table III. 2. High density values determines a decrease in tadpoles length (Kruskal-Wallis: H=29.29, P<0.001), survival rate (Kruskal-Wallis: H=20.83, P=0.01) and length growth rate (Kruskal-Wallis: H=22.24 P<0.01) (Fig. III. 2).

Table III.2. Initial (Li) and final (Lf) length, metamorphs length (Lm) and length growth rate for *P. syriacus tadpoles* depending on density treatment (experimental treatment from 1 to 4). The data represents mean and standard deviation and domain of values (minimum and maximum) in the brackets, **n_i=initial tadpoles number, n_f= final tadpoles number**

Parameters	**1**	**2**	**3**	**4**
	(n_i=10; n_f=10)	**(n_i=20; n_f=7)**	**(n_i=30; n_f=5)**	**(n_i=40; n_f=2)**
Li (mm)	14.99±1.33	14.00±1.33	14.13±1.12	14.16±0.84
	(12.33-16.72)	(12.54-16.35)	(12.04-17.23)	(12.33-16.41)
Lf (mm)	102.48±6.73	90.41±11.94	86.69±9.16	81.31±10.76
	(95.54-113.28)	(66.12-120.57)	(67.90-116.77)	(55.43-115.26)
Lm (mm)	30.33±2.59	29.50±1.21	29.40±1.51	26.99±2.51
	(26.92-35.63)	(27.45-30.77)	(27.11-31.23)	(25.21-28.77)
Length growth rate (mm/day)	0.55±0.03	0.54±0.04	0.53±0.04	0.49±0.07
	(0.50-0.62)	(0.46-0.63)	(0.41-0.62)	(0.32-0.63)

Length growth rate and age at metamorphosis of *P. syriacus* tadpoles decreased directly proportional with density level. Increase of density levels has a significantly negative effect on survival rate which decrease from 100% in treatment 1 reaching until 5% in treatment 4. Metamorphs body size from all 4 treatments differs significantly, with metamorphs body length decreasing from treatment1 towards treatment 4.

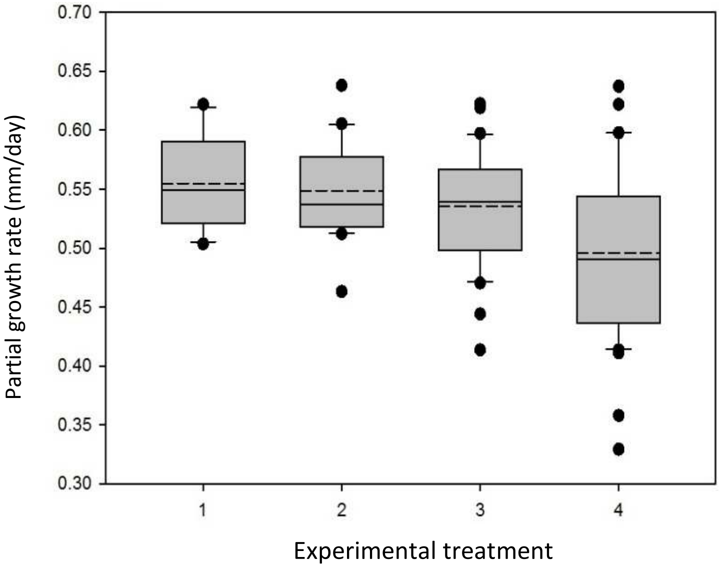

Fig. III.2. Length growth rate of *P. syriacus* tadpoles from each experimental treatment (1=1 tadpole, 2=2 tadpoles, 3=3 tadpoles, 4=4 tadpoles). In the graph are presented first and third quartile around median values (continuous line), mean values (dotted line), minimum and maximum values (terminal line) and extreme values (black dots).

Chapter IV- A comparative study on postmetamorphic growth rates in *Pelobates fuscus* and *Pelobates syriacus*

Growth rate is influenced by trophic resource availability and quality (Blankenhorn, 1999) and fluctuations of growth rate and body size values conditioned by food availability are widespread and generally predicted by life history models (Berrigan and Charnov, 1994; Atkinson and Sibly, 1997). Postmetamorphic growth studies may help to identify the elements that control or influence the growth rate and the body size at sexual maturity

I tested the hypothesis that food availability influence growth rate of postmetamorphic juveniles and their growth efficiency using *P. syriacus* and *P. fuscus* juveniles in three different feeding intensity experimental treatments.

The main objectives were (1) estimation of food availability on growth rate in constant environmental conditions and (2) intra and interspecific differences evaluation regarding growth rate and growth efficiency depending on feeding intensity.

The present study lasted for three months, September-November 2013. 72 postmetamorphic juveniles were collected (36 *P. fuscus* and 36 *P. syriacus* juveniles, from Histria). The juveniles from each species were divided in three experimental treatments: intensive feeding (once every other day), moderate feeding (once every three days) scarce feeding (once every six days).

At the beginning of the experiment the juveniles were photographed individually in order to identifiy them from the experimental tanks, after the dorsal pattern (Gamble, Ravela and McGarigal, 2008). They were fed alternatively with five invertebrate species: *Tenebrio molitor* larvae, *Blaptica dubia*, *Nauphoeta cinerea*, *Acheta domesticus*, *Gryllus assimilis*, from personal stock farm. Each juvenile was weighed before and after the feeding period with an electronic scale My Body mass, Triton T2 400, with a precision of 0.01 g. Juvenile snout-vent length (SVL) was measured at the beginning of the experiment and every two weeks, using digital calipers with a precision of 0.01 mm.

Juvenile growth parameters are presented in table IV.1. Using linear mix effect models, significant differences were found between final growth and SVL, length and body mass growth rate of the juveniles between the two studied species. Food availability had a significant effect on total food consumption ($F_{2,64}$=176.861, P<0.001), final body mass ($F_{2,64}$=8.011, P=0.001), body mass growth rate ($F_{2.64}$=5.747, P=0.005) and growth efficiency ($F_{2,64}$=12.967, P<0.001). *P. syriacus* juveniles final SVL from the intensive feeding treatment was significantly higher than those from moderate feeding treatment (z=-4.754, P=0.001,) and scarce feeding treatment (z=-4.754, P=0.001) (Fig. IV.1).

The results of the present study show that food availability influences growth rate and growth efficiency. *P. fuscus* juveniles from intensive feedin treatement grew up faster but with decreased growth efficiency than juveniles from the other two experimental treatments. The reaction of the juveniles of the two studied species differs significantly, with *P. syriacus* juveniles having higher growth rates and growth efficiency than *P. fuscus* juveniles. *P. syriacus* juveniles increased their growth efficiency when food availability is decreasing. This adaptive strategy allows *P. syriacus* juveniles to have a constant growth reaching similar body sizes, independent of the variation in food availability. *P. fuscus* juveniles havea similar growth efficiency for all three experimental treatments, showing sensitivity to food availability variations.

Table IV.1. Final body mass (g) final length (mm), body mass and length growth rate, growth efficiency (%), initial (Ki) and final (Kf) body condition index and total food consumption (CTH) for *P. fuscus* and *P. syriacus* metamorphs collected from syntopic populations in Histria (Danube Delta Biosphere Reserve). The data represents mean values and standard deviation and confidence interval with minimum and maximum, n=12 for all 6 experimental treatments.

Parameters	***Pelobates syriacus***			***Pelobates fuscus***		
	Intensive feeding treatment	**Moderate feeding treatment**	**Scarce feeding treatment**	**Intensive feeding treatment**	**Moderate feeding treatment**	**Scarce feeding treatment**
Final body mass(g)	8.14 ± 1.24 5.91-9.80	794 ± 0.75 6.50-9.29	7.51 ± 0.78 6.02-8.87	6.56 ± 1.11 5.25-8.60	5.72 ± 0.23 5.30-6.24	5.21 ± 0.92 4.05-7.00
Final length (mm)	40.90 ± 2.09 37.03-43.75	39.27 ± 1.98 36.03-43.54	39.58 ± 1.90 36.21–42.31	36.91 ± 1.80 34.17-40.35	36.37 ± 0.68 35.17-37.43	36.50 ± 1.13 34.91-39.09
Growth rate (g/day)	0.02 ± 0.01 0.01-0.04	0.02±0.00 0.01-0.03	0.02 ± 0.00 0.01–0.03	0.02 ± 0.01 0.01-0.04	0.01 ± 0.00 0.00–0.02	0.01 ± 0.00 0.00–0.02
Growth rate (mm/day)	0.05 ± 0.01 0.02-0.09	0.04 ± 0.01 0.02-0.05	0.05 ± 0.02 0.00-0.09	0.03 ± 0.01 0.00-0.06	0.03 ± 0.01 0.01-0.06	0.03 ± 0.01 0.01-0.06
GE	28.28 ± 7.29 15.52-42.14	45.40 ± 8.75 30.42-57.51	58.20 ± 12.48 39.68-79.87	31.03 ± 10.21 15.84–47.46	33.87 ± 9.51 17.19-48.87	38.42 ± 13.81 7.69-61.02
Ki	1.16 ± 0.08 0.97-1.29	1.15 ± 0.06 1.06-1.24	1.20 ± 0.15 1.00-1.59	0.99 ± 0.11 0.82-1.25	1.15 ± 0.15 0.89-1.50	1.02 ± 0.16 0.80-1.32
Kf	1.18 ± 0.12 1.05-1.45	1.31 ± 0.10 1.12-1.54	1.21 ± 0.12 0.96-1.46	1.29 ± 0.13 1.08-1.55	1.19 ± 0.09 1.06-1.41	1.06 ± 0.11 0.86-1.21
CTH (g)	9.20 ± 2.11 (6.25-13.60)	5.93 ± 0.76 (5.00-7.45)	4.33 ± 0.66 (3.11-5.13)	8.25 ± 1.33 (6.70-10.87)	4.62 ± 0.53 (3.84-5.55)	3.08 ± 0.53 (2.45-4.18)

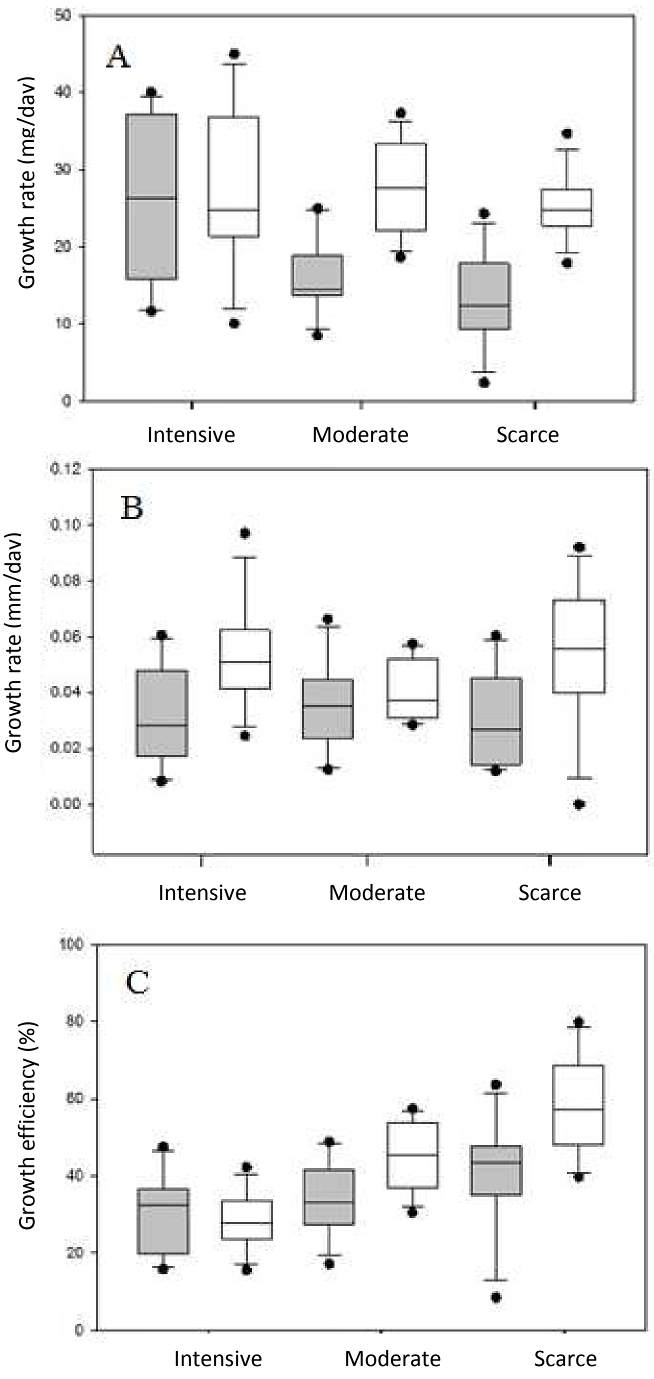

Fig. IV.1 Food availability effect on growth of the spadefoot toads juveniles (gray- *P. fuscus*, white- *P. syriacus*). (A) Growth rate (mg/day); (B) Growth rate (mm/day); (C) growth efficiency (%). The data represents the first and the third quartile around the median values (continuous line), minimum and maximum values (terminal line) and extrem values (black dots).

Chapter V – Trophic resource exploitation by a *Pelobates syriacus* population

Pelobates syriacus has a narrow ecological niche (Sofianidou, 1977), and the study of the trophic spectrum and trophic offer is required for knowing how they exploits the trophic resource, their selectivity and feeding strategy, in order to apply the best conservation methods (Anderson et al., 1999; Gunzburger, 1999).

In this study I present the way *P. syriacus* studied population from Grindul Lupilor exploits the trophic resource to elucidate whether it is or not an opportunistic species and the main objectives of this study are:

(i) trophic resource characterization and dynamics during one year;
(ii) trophic niche characterization and dynamics during one year;
(iii) exploitation method of the trophic resource of *P. syriacus* and variation in time of feeding selectivity;

Data collection

This study was conducted during September 2013 and March-July 2014. The trophic resource determination was possible using pitfall traps for terrestrial invertebrates. (Paulson, 2005). The pitfalls were placed in 5 different collection sites, along a linear track across the studied area depending on the vegetation type, starting from the pond shore towards the interior of the levee. The stomach content analysis was made on recently road kills of which stomach content could be collected. Because of the narrow interval of body sizes of the road kills, I chose to complete the evaluation of the trophic offer exploitation method collecting stomach content through stomach flushing from six *P. syriacus* individuals with large body sizes. Sorting and taxonomic identification of the invertebrates from the pitfalls and from the stomach content was conducted in the laboratory using a binocular and specific invertebrate identification guides. Dimensions of the invertebrates from the stomach content were determined using a gradual ocular in order to further determination of stomach content volume.

The stomach content of 415 *P. syriacus* individuals was analyzed. A total number of 2046 prey individuals were found in their stomach content. In the Barber pitfalls 23.052 invertebrates individuals were found (Table V.1.).

The stomach content analysis from the stomachs lavage showed the presence of two *P. syriacus* juveniles. Therefore, tthe cannibalism seems to represent an important food resource allowing the adults to exploit the small sizes trophic offer. The results of the PCA analysis of the identified invertebrates showed a high variation in September. The difference between September (post-hybernation period) and April (breeding period) being obvious (Fig. V.1). The study of the feeding dynamics of *Pelobates syriacus* studied population from Grindul Lupilor revealed seasonal high dynamics, for the trophic offer and its exploitation.

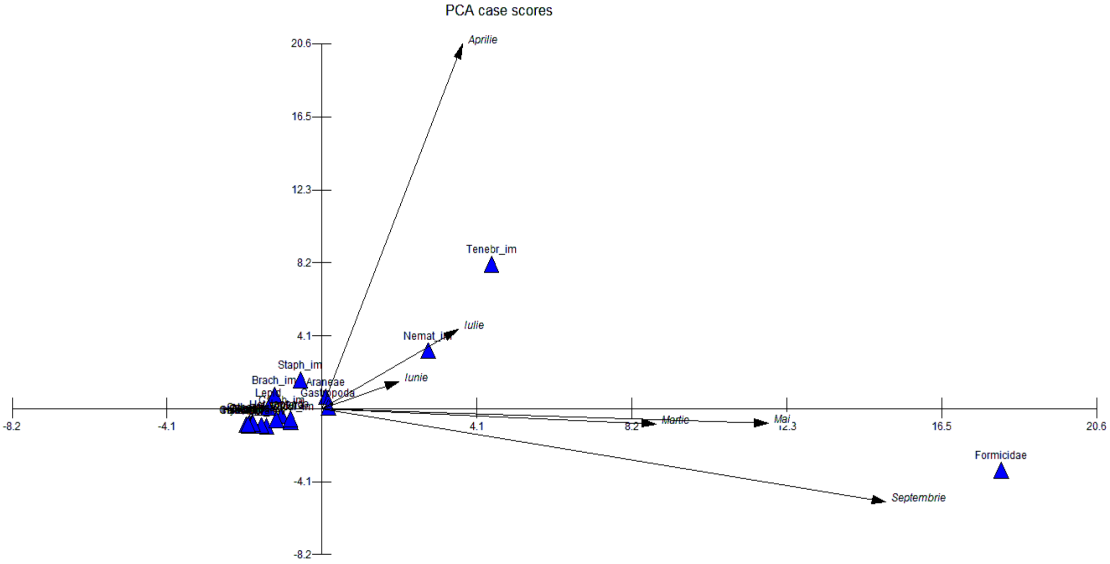

Fig. V.1. PCA analysis of the invertebrates taxa from the stomach content and trophic offer

For evaluating the feeding selectivity I used Ivlev index because it allows pointing the preferred and the avoided taxa. The results of the analysis of Ivlev index outlines that *P. syriacus* showed feeding specialization and a high selectivity towards certain invertebrates' taxa (Table V.2.).

Table V.1. Summary results of *P. syriacus* stomach content and trophic offer analysis from the studied area. The results are divided on months, starting with September 2013 until July 2014. TO = trophic offer SC = stomach content

Month Parameter	IX	III	IV	V	VI	VII	Total
Nr. of studied *P. syriacus* individuals	109	68	63	76	48	51	415
Nr. of prey items in SC	861	279	21	589	155	141	2046
Nr. of prey items in TO	2959	1406	2522	6125	5032	5008	23052
Nr. of stomachs with content	102	54	10	30	36	23	257
Percentage of stomachs with content	93.58	77.9	15.87	39.5	75	45.1	61.93
Mean nr. of prey items/ stomach	8.44	5.26	2.1	19.6	4.30	6.13	8.24
Maximum nr. of prey items/stomach	36	71	4	66	15	20	71
Nr. of identified taxa in SC	19	16	7	18	10	11	24
Nr. of identified taxa in TO	18	17	18	21	20	19	23

Table V.2. Characteristics of *P. syriacus* stomach content and trophic offer analysis from the studied area. The results are divided on months, starting with September 2013 until July 2014. TO = trophic offer SC = stomach content.

Month Parameter	IX	III	IV	V	VI	VII	Total
Berger Parker dominance index in SC	0,.63	0.39	0.38	0.48	0.26	0.21	0.47
Berger Parker dominance index in TO	0.36	0.27	0.53	0.61	0.40	0.47	0.41
Dominant taxa in SC (An>10%)	Formicidae (63%) Coleoptera varia (15%)	Formicidae (39%) Coleoptera varia (12%)	Coleoptera Tenebrionidae (38%) Nematocera (23%) Coleoptera larvae (14%)	Formicidae (48%) Nematocera (26%)	Gastropoda (26%) Coleoptera Carabidae (20%) Coleoptera varia (14%)	Araneae (21%) Coleoptera varia (18%) Gastropoda (12%)	Formicidae (47%) Coleoptera varia (13%)
Dominant taxa in TO (e.g. An>10%)	Acarina (36%) Collembola (27%)	Collembola (27%) Formicidae (19%) Acaria (17%)	Isopoda (53%) Collembola (17%)	Isopoda (61%) Collembola (13%)	Isopoda (40%) Collembola (12%) Formicidae (10%)	Isopoda (47%)	Isopoda (41%) Collembola (15%)
Mean trophic diversity (nr. of prey items/nr. of taxa) in SC	45.31	17.43	3	32.72	15.5	12.81	85.25
Mean trophic diversity (nr. of identified items/n. of taxa) in TO	123.29	58.58	105.08	255.20	209.66	208.66	960.5

Although Ivlev index points the fact that *P. syriacus* avoids consuming Araneae and Tenebrionidae, this two taxa are dominant in the stomach content from April and July. This fact indicates seasonality and opportunity in feeding of *P. syriacus*, when preferred prey items are scarce.

Table. V.2. Ivlev selectivity index (*-avoided taxa, **-preferred taxa)

Taxonomic group	Ivlev index (E_i)
Acarina	-0.96*
Isopoda	-0.90*
Araneae	-0.14*
Coleoptera Carabidae imago	0.60**
Coleoptera Coccinelidae imago	0.95**
Coleoptera Curculionidae imago	0.93**
Coleoptera Larvae	0.01
Coleoptera Staphylinidae imago	-0.20*
Coleoptera Tenebrionidae imago	-0.51*
Coleoptera varia imago	0.67**
Collembola	-0.99*
Gastropoda	0.68**
Hemiptera Cicadidae	-0.37*
Hemiptera Aphidae	0.58**
Heteroptera	0.41**
Hymenoptera Formicidae	0.69**
Hymenoptera varia	-0.44*
Lepidoptera larvae	0.96**
Orthoptera varia	0.64**
Insecta varia	0.87**
Anelida	0.89**

APMHIBIAN POPULATIONS MANAGEMENT RECOMMENDATIONS FOR THE STUDIED AREA

The human impact effect on the amphibian populations, it is present in the studied areas from RBDD, due to different activities such as: reed harvesting and everything that this activity impplies, standing out the vulnerability of the studied species to traffic, showed in the last chapter of the present paper; the danger represented by abandoned concrete foundations, because of which every spring thousands of amphibians and dozens of European pond turtle die by falling into these traps, being unable to escape due to high concrete walls. In the aim of reducing the negative effects of human impact on the health of the studied amphibian populations I propose a series of recommendations regarding the conservation management measures:

1. Access limitation of vehicles and machineries used in the activity of reed harvesting in the studied protected areas, during night time, from March until October. This measure assists in avoiding mass roadkills of amphibians and other nocturnal species.
2. Prohibiting reed harvesting during the amphibian maximum activity periods (March-June), avoiding the destruction of clutches and high mortality of tadpoles and metamoprhs, ensuring the minimum water level necessary for tadpoles' development and metamorphosis.
3. The rearrangement of the abandoned concrete foundations, in order to help the individuals that fall into them to escape, avoiding the high mortalities recorded every spring.

For this purpose, I submitted a collaboration proposal to the Danube Delta Biosphere Reserve Administration, for making a redevelopment project of these foundations. Currently the steps to formalize the collaboration and starting the project are ongoing.

Conclusions

I emphasized the evolutive life cycle particularities of the three amphibian species studied, by characterizing and evaluating the influence of biotic and abiotic factors on the general development of the studied individuals, following each ontogenetic stage. The main conclusions are listed below:

- ✓ The results of the present study reveal no sexual dimorphism in *P. syriacus,* even though in previous studies the males are slightly larger than the females. In *P. fuscus* and *T. dobrogicus,* sexual dimorphism is highlighted, the females being larger than the males. These size differences between sexes reflect the breeding behaviour of each species (i.e. selective breeding behavior) in *P. fuscus* through the breeding call and in *T. dobrogicus* through the mating parade, in both cases the females choosing the males. Reproductive characteristics analysis highlighted that *P. syriacus* did not show assortative mating. Explosive breeding in just few days, the sex ratio strongly male biased, and the almost absent breeding vocalization in males determines unselective formation of the pairs.
- ✓ *P. syriacus* shows a high fidellity for the breeding pond.
- ✓ The results of the comparative study on the breeding behavior between *P. syriacus* and *T. dobrogicus* highlight the differences between their reproductive strategies. Due to differences in egg deposition period (i.e. one day in *P. syriacus* and over 30 days in *T. dobrogicus*) differences in the reproductive effort appeared. *P. syriacus* showed a more pronounced reproductive effort compared to *T. dobrogicus*, reflected in the differences between females body mass before and after reproduction. The prolonged egg deposition in *T. dobrogicus* females, allows them to feed during this period, benefiting of an energetic intake in compensation of the body mass loss.
- ✓ In *T. dobrogicus* female body length (SVL) influences the egg volume, larger females depositing eggs with larger volume. Correlating this data with the results of a previous study (Cogălniceanu et al., 2013a), in which female SVL growth is positively correlated with age, I suggest that egg volume is influenced by female age. In the end, egg dimension of the two studied species are similar. Thus, the parental care given to their eggs by *T. dobrogicus* females it represents a behavior strategy contributing to increasing reproductive success in compensation for the small number of eggs deposited and for the high mortality rate at embryo level due to a chromosoal mutation.

- ✓ Observing the influence of females characteristics on clutch features we than analyzed how parental body size influences larvae body size. The result of this analysis showed that mean body length and growth rate of *P. syriacus* larvae is influenced by parental body dimensions. The larvae of large dimension adults showed higher mean body length and growth rate than larvae of the small body size pair. Final body length of metamorphs was similar. The larvae of large body size adults experienced a shorter larval period than larvae of small body size *P. syriacus* adults. This results, showed a possible genetic influence of adult age on larvae development.
- ✓ Larvae growth rate, larvae age and survival rate are influenced by density level. Larvae bred in high density levels had a decreased growth rate, advanced age and high mortality rate. Also, in high density levels, larvae experienced mutations (e.g. backbone deviations).
- ✓ Food availability influenced *P. syriacus* and *P. fuscus* juvenile growth rate and growth efficiency. *P. fuscus* showed a lower adaptability to variation in food availability than *P. syriacus* juveniles.
- ✓ Stomach content analysis highlighted that *P. syriacus* did not specialise in the consumption of specific prey items but is a selective species regarding the trophic resource. *P. syriacus* feeding behavior is opportunist in low food availability and cannibalistic behavior is present.
- ✓ In the studiee area human impact is affecting amphibians, reptiles and mammals populations. Therefore, I developed a series of recommendations in order to reduce the negative effects of the human impact.

The final conclusion of the present paper is that, from the three studied species (*P. fuscus, P. syriacus* and*T. dobrogicus*) *Pelobates syriacus* is better adapted to rough environmental conditions In terms of evolutionary aspects *P. syriacus* showed the best physiological characteristics for adaptation in oscillatory conditions.

Selective literature

1. **Alford, R. A. and Richards, S. J**. (1999). Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics, 133-165.
2. **Alvarez, D. and Nicieza, A. G.** (2002). Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia, 131:186-195
3. **Asquith, C. M. and Vonesh, J. R.** (2012). Effects of size structure on predation risk and inter-cohort competition in red-eyed treefrog tadpoles. Oecologia, 170:629-639.
4. **Beck, C. W.** (1997). Effect of changes in resource level on age and sizeat metamorphosis in *Hyla squirella*. Oecologia, 112:187-192.
5. **Beebee, T. J. C**. (1995). Amphibian breeding and climate. Nature, 374: 219-220.
6. **Becker, C. G., Fonseca, C. R., Haddad, C. F. B., Batista, R. F. and Prado, P. I.** (2007). Habitat split and the global decline of amphibians. Science, 318(5857): 1775-1777.
7. **Bernard, M. F. and Middlemis, M. J.** (2011). Consequences of intraspecific niche variation: phenotypic similarity increases competition among recently metamorphosed frogs. Oecologia, 166: 585-59.
8. **Bernardo, J.** (1996). Maternal effects in animal ecology. American Zoologist, 36:83-105.
9. **Berven, K.A. and Chadra, B.G**. (1988). The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). Oecologia, 75:67–72.
10. **Blanckenhorn, W.U.** (1999). Different growth responses to temperature and resource limitation in three fly species with similar life histories. Evolutionary Ecology,13: 395- 409
11. **Brokelman, W. Y.** (1969). An analysis of density effects and predation in *Bufo americanus* tadpoles. Ecology, 50:632–644.
12. **Cogălniceanu, D., Buhaciuc, E., Tudor, M. and Roșioru, D.** (2013a). Is Reproductive Effort Environmentally or Energetically Controlled? The Case of the Danube Crested Newt (*Triturus dobrogicus*). Zoological Science, 30(11): 924-928.
13. **Cogălniceanu, D., Székely, P., Székely, D., Roșioru, D., Băncilă, R. I. and Miaud, C.** (2013b). When Males Are Larger than Females in Ecthotherms: Reproductive Investment in the Eastern Spadefoot Toad *Pelobates syriacus*. Copeia, 4: 699-706.
14. **Cogălniceanu, D., Rozylowicz, L., Székely, P., Samoilă, C., Stănescu, F., Tudor, M., Székely, D and Iosif, R**. (2013c). Diversity and distribution of reptiles in Romania. *ZooKeys*, 341: 49.
15. **Cogălniceanu, D., Székely, P., Samoilă, C., Ruben, I., Tudor, M., Plăiaşu, R**., **Stănescu, F. and Rozylowicz, L.** (2013d). Diversity and distribution of amphibians in Romania. *ZooKeys*, 296: 35.
16. **Cogălniceanu, D., Roșioru, D., Székely, P., Székely, D., Buhaciuc, E., Stănescu, F. and Miaud, C.** (2014). Age and Body Size in Populations of Two Syntopic Spadefoot Toads (Genus *Pelobates*) at the Limit of Their Ranges. Journal of Herpetology, 48:537-545.
17. **Cone, R.S.** (1989). The need to reconsider the use of condition indexes in fishery science. Transactions of the American Fisheries Society, 118(5): 510-514.
18. **Cvijanović, M., Ivanović, A., Tomašević, N., Džukić, G. and Kalezić, M.L.** (2009). Early ontogeny shows the same interspecific variation as natural history parameters in the crested newt (*Triturus cristatus* superspecies) (Caudata, Salamandridae). Contributions to Zoology, 78 (2): 43-50
19. **Duellman, W.E.** (1992). Reproductive strategies of frogs. Scientific American, 267(1): 80-87.
20. **Duellman, W.E. and Trueb, L.** (1994). Biology of Amphibians. The Johns Hopkins University Press, Baltimore and London.
21. **Dmitriew, C.M.** (2011). The evolution of growth trajectories: what limits growth rate? Biological Reviews, 86: 97-116.

22. **Ebenman, B.** (1992). Evolution in organisms that change their niches during the life cycle. American Naturalist, 990-1021.
23. **Enriquez-Urzelai, U., San Sebastian, O., Garriga, N. and Llorente, G.A.** (2013). Food availability determines the response to pond desiccation in anuran tadpoles. Oecologia, 173:117-127.
24. **Fuhn, I.** (1960) Amphibia – Fauna R.P.R., Ed. Acad. R.P.R., 14 (1).
25. **Furtula, M., Todorovic, B., Simic, V. and Ivanović, A.** (2009). Interspecific differences in early life-history traits in crested newts (*Triturus cristatus* superspecies, Caudata, Salamandridae) from the Balkan Peninsula. Journal of Natural History, 43(7–8): 469–477.
26. **Gamble, L., Ravela, S. and McGarigal, K.** (2008). Multi ‐ scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the marbled salamander *Ambystoma opacum*. Journal of Applied Ecology, 45: 170-180.
27. **Grayson, K. L., De Lisle, S. P., Jackson, J. E., Black, S. J. and Crespi, E. J.** (2012). Behavioral and physiological female responses to male sex ratio bias in a pond-breeding amphibian. Frontiers in Zoology, 9(24): 9994-9.
28. **Halliday, T. R. and Verrell, P. A.** (1988). Body size and age in Amphibian and Reptiles. Journal of Herpetology, 22(3):253-65.
29. **Heino, M. and Kaitala, V.** (1999). Evolution of resources allocation between growth and reproduction in animals with indeterminate growth. Journal of Evolutionary Biology, 12: 423-429.
30. **Horner, H. A. and Macgregor, H. C.** (1985). Normal development in newts (*Triturus*) and its arrest as a consequence of an unusual chromosomal situation. Journal of Herpetology, 261-270.
31. **Jørgensen, C.B.** (1982). Factors controlling the ovarian cycle in a temperate zone anuran, the toad *Bufo bufo*: food uptake, nutrional state, and gonadotropin. Journal of Experimental Zoology, 224: 437–443.
32. **Jørgensen, C.B.** (1989). Pattern of growth and fattening in young toads *Bufo bufo* fed mealworms. Effects of growth hormone and feeding regimen. Copeia, 124-128.
33. **Jørgensen, C.B.** (1995). Growth and reproduction. In Feder, M. E. *Environmental physiology of the amphibians*. University of Chicago Press. 439-466.
34. **Kaplan, R.H.** (1980). The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (*Ambystoma*). Evolution, 34:51–64.
35. **Kaplan, R.H. and King, E.G.** (1997). Egg size is a developmentally plastic trait: evidence from long term studies in the frog *Bombina orientalis*. Herpetologica, 53(2): 149-165.
36. **Kirițescu, C.** (1930). Cercetări asupra faunei herpetologice a României. Cartea românească
37. **Kozlowski, J.** (1992). Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. Trends in Ecology and Evolution, 7: 15-19.
38. **Lardner, B. and Loman, J.** (2003). Growth or reproduction? Resource allocation by female frogs *Rana temporaria*. Oecologia, 137: 541 -546.
39. **Lehtinen, R. M., Green, S. E. and Pringle, J. L.** (2014). Impacts of Paternal Care and Seasonal Change on Offspring Survival: A Multiseason Experimental Study of a Caribbean Frog. Ethology, 120(4): 400-409.
40. **Martin, R. and Pfenning D.W.** (2010). Maternal investment influences expression of resource polymorphism in Amphibians: implications for the evolution of novel resource-use phenotypes. PloS One, e9117.
41. **Michimae, H., Nishimura, K., Tamori, Y. and Wakahar, M.** (2009). Maternal efects on phenotypic plasticity in larvae of the salamander *Hynobius retardatus*. Oecologia, 160:601–608.
42. **Mousseau, T. A. and Fox, C. W.** (1998). The adaptive significance of maternal effects. Trends in Ecology & Evolution, 13:403-407.
43. **Pakkasmaa, S., Merila, J. and O'Hara, R.B.** (2003). Genetic and maternal effect influences on viability of common frog tadpoles under different environmental conditions. Heredity, 91:117–124.

44. **Petranka, J. W.** (1989). Denisty-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. Ecology, 70:1752-1767.
45. **Petranka, J.W.** (2007). Evolution of complex life cycles of amphibians: bridging the gap between metapopulation dynamics and life history evolution. Evolution Ecology, 21:751-764
46. **Rist, L., Semlitsch, R.D., Hotz, H. and Reyer, H.U.** (1997). Feeding behaviour, food consumption, and growth efficiency of hemiclonal and parental tadpoles of the *Rana esculenta* complex. Functional Ecology, 11:735-742.
47. **Scott, D. E.,** (1994). The effects of larval density on adult demographic traits in *Ambystoma opacum*. Ecology, 75:1383-1396.
48. **Sofianidou, T. S**. (1977). Studies on the biology and ecology of *Pelobates syriacus* Boettger (Anura: Pelobatidae). Ph. D. diss.
49. **Summers, K., McKeon, C. S. and Heying, H.** (2006). The evolution of parental care and egg size: a comparative analysis in frogs. Proceedings of the Royal Society B: Biological Sciences, 273(1587): 687-692.
50. **Székely, P.** (2010). Studii faunistice andecologiceasupra amfibienilor din Podișul Dobrogei. Ph.D. diss., Universitatea Babes-Bolyai, Cluj-Napoca.
51. **Székely, D., Denoël, M., Székely, P. and Cogălniceanu, D.** (2013). Differences in locomotor activity in two syntopic spadefoot toad species (Genus *Pelobates*). Societas Europaea Herpetologica (SEH) 17th European Congress of Herpetology, 22-27 August 2013 in Veszprém, Hungary.
52. **Székely, D., Székely, P. and Cogălniceanu, D.** (2015). *Pelobates syriacus* (Eastern spadefoot toad). Cannibalism. Herpetological Review, 46(1):76-77.
53. **Turner, F.B**. (1960). Postmetamorphic growth in anurans. The American. Midland. Naturalist, 64:327-338.
54. **Vitt, L. J. and Caldwell, J. P.** (2013). Herpetology: an introductory biology of amphibians and reptiles, Academic Press.
55. **Volker, H.W.R. and Rödel, M.O.** (2007). Phenotypic plasticity and optimal timing of metamorphosis under uncertain time constraints. Evolutionary Ecology, 21:121-142.
56. **Vonesh, J. R. and De La Cruz, O.** (2002). Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. Oecologia, 133:25–333.
57. **Vörös, J. andArntzen, J. W.** (2010). Weak population structuring in the Danube crested newt, Triturus dobrogicus, inferred from allozymes. Amphibia-Reptilia, 31(3):339-346.
58. **Wells, K.D.** (2007). The Ecology and Behavior of Amphibians. Chicago: Edit. University of Chicago Press.
59. **Werner, E.E.** (1986). Amphibian metamorphosis: growth rate, predation risk and the optimal size at transformation. The American Naturalist, 128:319-341.
60. **Wilbur, H. M. and Collins, J. P.** (1973). Ecological Aspects of Amphibian Metamorphosis. Nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. Science, 182 (4119): 1305-1314.
61. **Wildy, E. L., Chivers, D. P. Kiesecker, J. M. and Blaustein, A. R.** (2001). The effects of food level and conspecific density on biting and cannibalism in larval long-toed salamanders, *Ambystoma macrodactylum*. Oecologia, 128(2): 202-209
62. **Zuffi, M. A., Fornasiero, S., Picchiotti, R., Poli, P. and Mele, M.** (2010). Adaptive significance of food income in European snakes: body size is related to prey energetics. Biological Journal of the Linnean Society, 100(2): 307-31

Scientific outputs of the author in ISI journals with impact factor

1. **Buhaciuc, E.,** Székely, P., Băncilă, R. and Cogălniceanu, D. (2015). Food availability influences postmetamorphic growth in two spadefoot toad species (genus *Pelobates*). Amphibia-Reptilia *(under review)*
2. Cogălniceanu, D., Roșioru, D., Székely, P., Székely, D., **Buhaciuc, E**., Stănescu, F. and Miaud, C. (2014). Age and body size in population of two syntopic spadefoot toads (genus *Pelobates*) at the limit of their ranges. Journal of Herpetology, 48:537-545
3. Cogălniceanu, D., **Buhaciuc, E**., Tudor, M., and Roșioru, D. (2013). Is Reproductive Effort Environmentally or Energetically Controlled? The Case of the Danube Crested Newt (*Triturus dobrogicus*). Zoological Science, 30(11): 924-928.
4. Stănescu, F., **Buhaciuc, E.,** Székely, P., Székely, D., and Cogălniceanu, D. (2014). Facultative paedomorphosis in a population of *Lissotriton vulgaris* (Amphibia: Salamandridae) from the Danube Delta Biosphere Reserve (Romania). Turkish Journal of Zoology, *38*(1): 114-117.

Scientific papers published in summary proceedings of international conferences

1. **Buhaciuc, E**. and Cogălniceanu, D. (2014). Effects of overpopulation in *Pelobates syriacus* larval development. Abstract, 6th Annual Zoological Congress of “Grigore Antipa” Museum (CZGA), Bucharest, Romania, pp.164.
2. **Buhaciuc, E.,** Stănescu, F., Székely, P., Székely, D., Samoilă, C., Iosif, R., Băncilă, R., Roșioru, D. and Cogălniceanu, D. (2014). What shapes species distribution? A case study on spadefoot toads (genus *Pelobates).* Abstract, Ecology and Evolutionary Symposium, Istanbul, Turkey, pp. 24.
3. Cogălniceanu, D., Székely P., Székely D., Stănescu, F., **Buhaciuc, E**., Samoilă, C., Iosif, R., Băncilă R. and Roşioru, D. (2013). Life history strategies of spadefoot toads (genus *Pelobates*). Abstracts, 5th Annual Zoological Congress of “Grigore Antipa” Museum (CZGA), Bucharest, Romania, pp. 35

4. **Buhaciuc, E.,** Cogălniceanu, D., Roşioru, D., Székely, P., Székely, D., Stănescu, F. and Miaud, C. (2013). Age and body size in populations of two syntopic spadefoot toads (genus *Pelobates*) at the limit of their ranges. Aquatic Biodiversity International Conference, Sibiu, Romania. pp. 41
5. **Buhaciuc, E.,** Székely, P., Székely, D., and Cogălniceanu, D. (2012). A comparative study of early post-metamorphic growth in two species of spadefoot toads (*Pelobates fuscus* and *Pelobates syriacus*). 4th Annual Zoological Congress of "Grigore Antipa" Museum (CZGA), Bucharest, Romania, pp. 85.

Scientific papers presented in national and international conferences

1. Iosif, R., Stănescu, F., Székely, P., Székely, D., **Buhaciuc, E.,** Roşioru, D., and Cogălniceanu, D. 2013. Life history strategies in spadefoot toads (genus *Pelobates*). BIOTA Symposium. Biodiversitate: Tradiţii şi Actualitate, Cluj-Napoca, Romania.
2. Roşioru, D., Székely, P.J., Székely, D., **Buhaciuc, E.,** Stănescu, F., Miaud, C. and Cogălniceanu, D. (2012). Population structure of two syntopic spadefoot toads (genus *Pelobates*) at the limit of their ranges. Abstracts, 12th ICZEGAR, Athens, Greece