POPULATION FLUCTUATIONS AND THE SPATIAL HABITAT USE BY AMPHIBIANS IN A HUMAN MODIFIED LANDSCAPE

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SUMMARY. In this study we present the start of the breeding season and the long term population fluctuations of amphibians in a permanent pond and a number of temporary ponds in a human modified landscape in the middle section of the Târnava Mare basin. The start of breeding was strongly influenced by temperature in spring. The populations of *Hyla arborea* and *R. temporaria* were in decline whereas *Pelobates fuscus* and *Bufo bufo* were stable during this time. *Pelobates fuscus* is represented by a small population and is probably maintained by immigration from other areas. *Bufo bufo* and *P. fuscus* use only the permanent pond for reproduction. More egg masses of *R. temporaria* were found in the temporary ponds than in the permanent pond. The reproductive success was yearly observed in the temporary ponds but not in the permanent pond. We assume that the permanent pond is a source habitat for *B. bufo* and represents sink for *R. temporaria* whereas the temporary ponds from the forest represent source habitats for the last species. This and the previous studies on this community suggest that in this landscape, both the permanent and temporary ponds and the landscape connectivity are crucial for the maintenance of rich amphibian communities.

Keywords: amphibian populations, climate, long term fluctuations, landscape

Introduction

The human domination on the earth ecosystems substantially altered the Earth systems through several interacting processes (Vitousek *et al*., 1997). It was recognized that designing protected areas does not guarantee the protection of biodiversity because (i) the protected area covered is still too small (Hoekstra *et al.*, 2005), (ii) human interests are continuously growing (Liu *et al.*, 2001). It is increasingly recognized that there is a significant biodiversity outside of the protected areas that should be also properly managed and protected (Daily et al. 2001, „2010 Biodiversity Indicators Partnership" http://www.twentyten.net/target.htm).

Habitat and population based studies in Romania are more important now than ever. Firstly, with the recent EU adhesion new land use practices will be (quickly) adopted, and this will lead to drastic changes in the landscape structure. Secondly, we have (maybe the last) opportunity to study populations in clutural landscapes, thus, to get information about the ways at which human perturbations in the landscape represented by traditional land use practices affect the distribution and persistence of the natural populations. New developements in population /

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metapopulation theory, island biogeography and landscape ecology and their integration in ecology, represent useful modern insights about how hypoheses should be formulated, data gathered and interpreted to achieve this goal (Sandersen *et al*., 2002; Fischer *et al*., 2004; Manning *et al*., 2004; Fischer *et al*., 2006; Fischer and Lindenmayer, 2007; Lindenmayer *et al*., 2007; Schneider and Willig, 2007).

Pond breeding amphibians are important focal group for conservation for a number of reasons: (i) They have complex life cycles and need high spatial heterogeneity of the landscape to complete it. These life history traits makes them sensitive to habitat loss and fragmentation. (ii) They often are organized in metapopulations (Alford and Richards, 1991; but see Smith and Green, 2005). Loss of connectivity between local populations may expose them to genetic depletion, this making them susceptible for a number of other natural and anthropogenic stressors (Edenhamn *et al*., 2000). (iii) Amphibians are in global decline (Stuart *et al*., 2004) for various and often complexly interacting causes (Beebee and Griffiths, 2005).

Monitoring amphibian populations and the spatial use of the habitats provide information about the factors (natural and anthropogenic) causing long term fluctuations and their potential role as source populations at landscape level. In this paper we present the population fluctuation in 11 years of four amphibian species in the middle section of the Târnava Mare basin. The area is a mosaic patch that has natural – seminatural (ponds, river, grassland, forest) and anthropogenic (railway, arable lands, built areas) origins. The composition and configuration of the above mentioned landscape elements allow the study of the role of spatial arrangement of these landscape elements in determining the spatial extent of habitat use by amphibians. Previous studies have shown that the distribution of the migrating and dispersing Common Toads can be related to these land use types, the grassland between the pond and the forest being of critical importance (Hartel and Demeter, 2005; Hartel *upublished results*). In a larger scale, both the landscape composition and configuration was found to be important predictors of the Common Toad population sizes (Hartel and Moga, 2007 *in press*). Moreover, the long term study regarding the temporary habitat use by the Yellow Bellied Toad (a Habitat Directive species) in this area (Hartel *et al*., 2007a) shows that this species use the most durable ponds for reproduction in dry years. Temporary ponds that act as sink habitats in dry years represent high quality breeding habitats in rainy years. Because of the large and stable populations of some species the area was proposed as Natura 2000 Site of Community Importance (Ministerial Order 776/2007). Yet the whole area where the temporary ponds occur is seriously impacted by the drainage ditches that were made in order to facilitate gas extraction and the movement of heavy vehicles towards and from the gas source stations (2007).

In this study we present:
a) aspects of the breeding phenology (start of breeding season) in two species,
b) the long term fluctuations in the amphibian populations,

    c) the correlation of the start of breeding period and population fluctuations with a number of climatic variables.

Materials and Methods

Study area. The study area is situated in the middle section of the Târnava Mare Valley, Romania (46º13'47.8''N; 24º46'47.6''E, 345 m altitude) and has approximately 3 km² area. A permanent pond and up to 80 temporary ponds were studied in this area (Fig. 1).

The permanent pond has 2.2 ha area with a maximum depth of about 4 m. The reed cover in the pond shows gradual increase during the years in the south eastern part of the pond, probably linked to the changed light condition due to the cutting of the trees along one shore of the pond. In 2007, approximately 35% of the pond was covered by *Typha* sp. and *Phragmites* sp. Four fish species, *Pseudorasbora parva*, *Carassus auratus*, *Cyprinus carpio* and *Leucaspius delineatus* were constantly present during the 11 years. The temporary ponds are situated in the forest to a distance from 800 to 1500 m. Their number varies greatly with the precipitation (Hartel *et al*., 2007b), the maximum number being generally recorded in spring. The average pond area is 22 m^2 (range 1-250), the depth varies between 3 to 100 cm (Hartel and Nemes, 2006).

The terrestrial area surrounding the permanent pond is represented by arable lands, a railway, the Târnava River, and a grassland patch between the pond and the forest (Hartel, 2004) (Fig. 1). We created a database regarding the land use types in this area using maps, aerial and other photographies. The landscape composition (in this case the land use types and their amount in the landscape) and configuration (i.e. the spatial arrangement of these) was not changed significantly during the 11 years.

The studies on the permanent pond were started in 1997 whereas in the temporary ponds in 2002. The methodology used for estimating "size of the populations" was presented in detail in Hartel (2004; 2005). The fieldwork started in the middle of February and lasted until the first part of June. Two aspects of the beginning of the breeding season were considered in *R. temporaria* and *B. bufo*: (i) the start of male activity (first day), meaning the first chorus in *R. temporaria* and the first adult males found in the water in *B. bufo* and (ii) the first day when deposited eggs were found. Since it is assumed that females lay maximum one egg mass every year, and the egg masses are easy to count, we used the number of egg masses as indicators of population size in the case of *R. temporaria*. In the case of the Common Toad (*Bufo bufo*) we counted the active individuals in the water until about 1 m from the water shore on land. This method provided ecological meaningful informations about the pond and landscape characteristics influencing the common toads in the Târnava Mare basin (see Hartel et al. 2007b and the references cited therein for the use of "head count" methods"). The population

sizes of Common Spadefoot Toad (*Pelobates fuscus*), and Tree Frog *Hyla arborea* were estimated using the number of calling males.
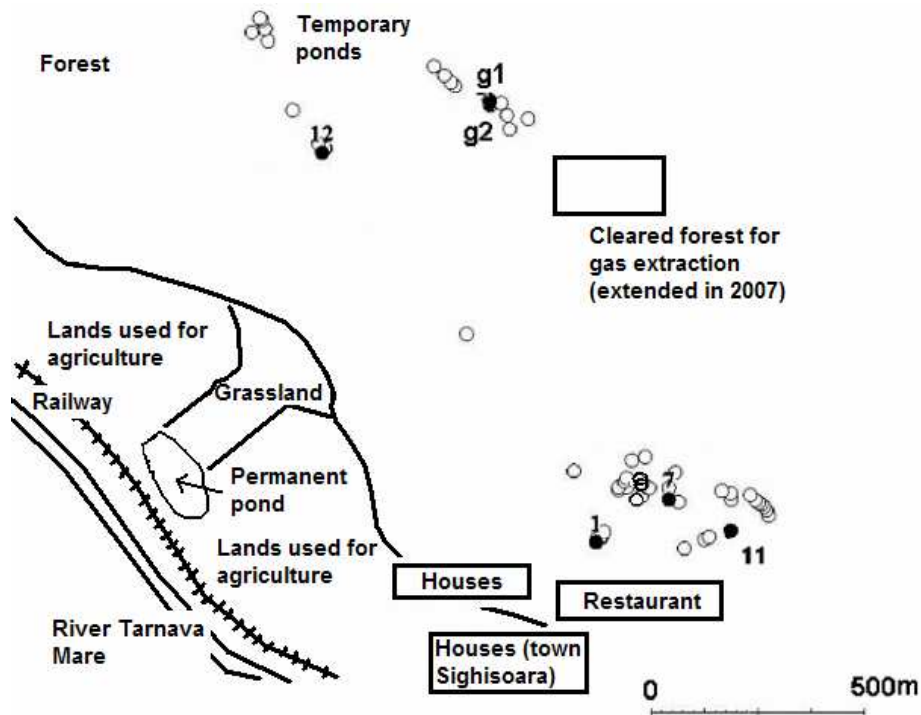
Fig. 1. The study area, the distribution of the ponds and the land use patterns. Black points represent the most stable temporary ponds that were used most frequently. Temporary ponds „g1" and „g2" were used by *Hyla arborea*

We used a number of climatic variables in this study: These variables were: (i) total amount of precipitation (l/mm) in January, and (ii) February in the years when egg masses were counted, (iii) total amount of precipitation during the active season of frogs (April to September) in the year previous to which eggs were counted, (iv) the mean air temperature in January and (v) the mean air temperature in February. These data were measured at the water station from Albeşti, at a distance of about 5 km from the population studied.

Besides the climatic variables (see above), the variables „year" and the number of egg masses recorded for *R. dalmatina* (in the case of *R. temporaria*) (Hartel *submitted*) were used as independent variables in a multipple regression analysis (forward stepwise). The dependent variables in the multipple regression were: the population size estimations (see above), the number of days in the year (using a Julian calendar whereby 1 January is day 1) when the first male called (*R.*

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temporaria) or was seen (*B. bufo*) and the Julian date when first egg mass was recorded (both species).

The number of egg masses deposited by *R. temporaria* in the permanent pond and the temporary ponds from the forest was compared using the nonparametric Mann - Whitney U test. The rate of change of population size was estimated using the ΔN method. According to this formula, changes in population sizes between years are related to each other by $\Delta N = \log(N+1)_t - \log(N+1)_{t-1}$, where N is the population size at time t (Houlahan *et al.* 2000).

Results and Discussions

The start of the breeding period. There was no significant difference between the start of the vocalization period in the males and the first egg mass deposited (mean: 71.50 days, SD = 11.84) and the first egg mass (74.22, 10.96) in *R. temporaria* (t = -0.56, df = 20, P = 0.58). In the case of *B. bufo* the differences were significant (males: 75.88, SD = 13.25; females: 88.44, SD = 8.62; t = -2.51, df = 18, P = 0.02). Although not significant in statistical terms (maybe because of low power of the test), the 2.72 day difference between the start of calling and the first egg mass deposited in *R. temporaria* may be significant from a biological point of view. As the number of individuals in the permanent pond is very small (see below) we assume that the vocalization in this case may be important in female attraction. Wells (1977) noted that the male mate locating strategies of explosive breeders, such as *B. bufo* may be dependent on the density. Calling at low density in this species may be advantageous but as the density increases (as more individuals migrate in short time) the calling activity is expected to cease. This was also observed in the population studied by us. In *R. temporaria* however, males in large / dense populations are expected to be more active in the breeding season than in small / less dense populations (Elmberg and Lundberg, 1991). *Bufo bufo* start breeding significantly later than *R. temporaria* (t test, P < 0.05). *R. temporaria* always finished breeding in 5-6 days, thus, the overlap between the reproduction periods of two species was minimal. *Bufo bufo* reproduced until the second part of April, active males being found even the first part of May (6th of May). High degree of interspecific spawning was found in the case of *R. temporaria* and *B. bufo* in a pond from England (Reading, 1984). Interspecific pairing between the two species was never observed in these two species due to the short overlap of the breeding seasons. However, the interspecific pairing between the *B. bufo* males and females of *R. ridibunda* were frequently observed. This may be because *R. ridibunda* appears in the pond toward the end of the breeding season of *B. bufo* when the operational sex ratio is strongly skewed toward the males. Size assortative pairing was not observed in *B. bufo* (Hartel and Demeter, 2005) nor in *R. temporaria* (Hartel *unpublished*).

The air temperature in February was the most important variable affecting the start of the reproduction in both species: there was a negative relationship

between the beginning of the reproduction and the air temperature in February (Table 1 and 2), in *B. bufo* the precipitation in January being important determinant of the start of males activity (Table 2). These results confirm the previous findings on the role of the climatic conditions in the initiation of reproduction of temperate explosive-breeding amphibians (Duellmann and Trueb, 1986; Sofianidou and Kyriakopoulou-Sklavounou, 1986; Reading, 1998), including the population of *R. damatina* (Hartel 2005, Hartel *unpublished*). We assume that any long term increase in the air temperature in spring may be reflected by earlier dates at which breeding begins for the populations of these two species. Moreover, the identified climatic variables explains overall a small variation in the begining of thereproduction period of these two species (R^2 is small, the largest value being for the beginning of the males activity in *B. bufo*). This means that other, unrecorded factors may be also important in the start of reproduction of these two species.

Population sizes fluctuations. *Bufo bufo*, and *P. fuscus* used only the permanent pond for reproduction, whereas *H. arborea* occurred in two sunny temporary ponds (Fig. 1), with longer duration. The temporary ponds from the forest are small, shadowed (thus cold and unproductive relatively to the temporary ponds from the open areas) and have short hydroperiod (Hartel *unpublished*), these conditions not being preferred by these two species (Laurila, 2000; Pellet and Hoehn, 2004; Nyström *et al.*, 2007).

The average size of the populations of the four species in the permanent pond was: 27.27 (SD = 15.10) egg masses in *R. temporaria*, 1372.80 (SD = 475.69) individuals in *B. bufo*, 46.63 (SD = 23.17) calling males in *H. arborea* and 7.18 (SD = 5.75) calling males in *P. fuscus*. The fluctuation of the population sizes are presented in Fig. 2, 3 and 4. The ΔN is negative in *R. temporaria* (-0.07) and *H. arborea* (-0.07) whereas it is positive in *P. fuscus* (0.09) and *B. bufo* (0.01). The number of the calling males in *H. arborea* and the number of egg masses in *R. temporaria* shows negative trends during the 11 year period (Table 1 and 2). The number of males of *H. arborea* show sharp decline from 2004 (Fig. 3). The number of egg masses deposited by *R. temporaria* was significantly positively associated with the amount of precipitations in January, and negatively associated with the number of the *R. dalmatina* egg masses (Table 1).

It was showed that the sensitivity of females toward the climatic conditions has increased toward the end of the winter dormancy period due to the depletion of energy reserves (Reading, 2007). In dry springs a certain proportion of females may fail to reproduce or mortality could increase.
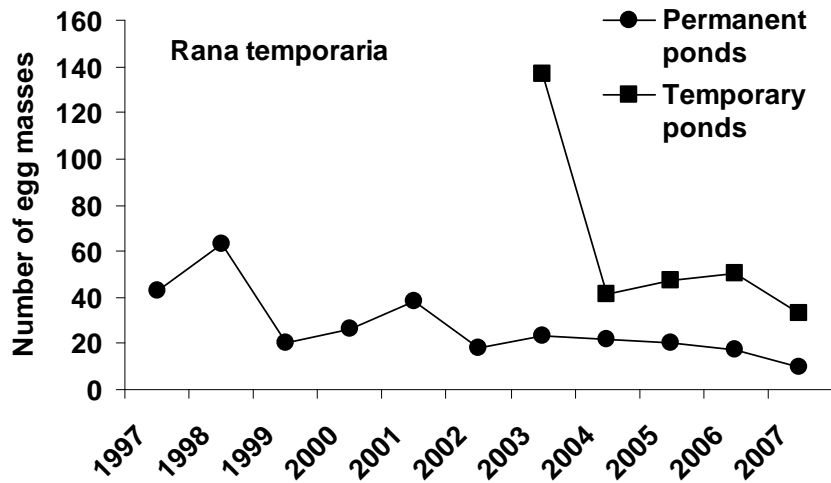
Fig. 2. The fluctuation of the egg mass number in the permanent pond and the temporary ponds in *R. temporaria.* Data from all the temporary ponds are pooled
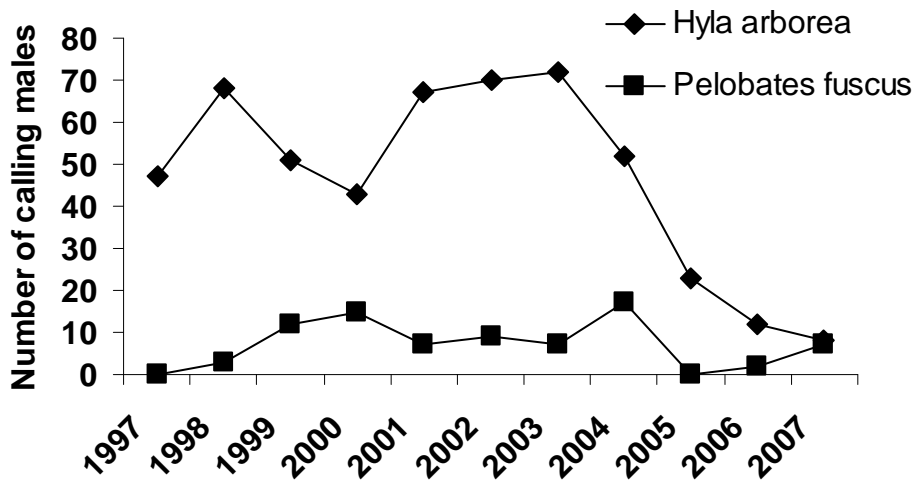

Fig. 3. The fluctuation of the number of calling males in *P. fuscus* and *H. arborea*
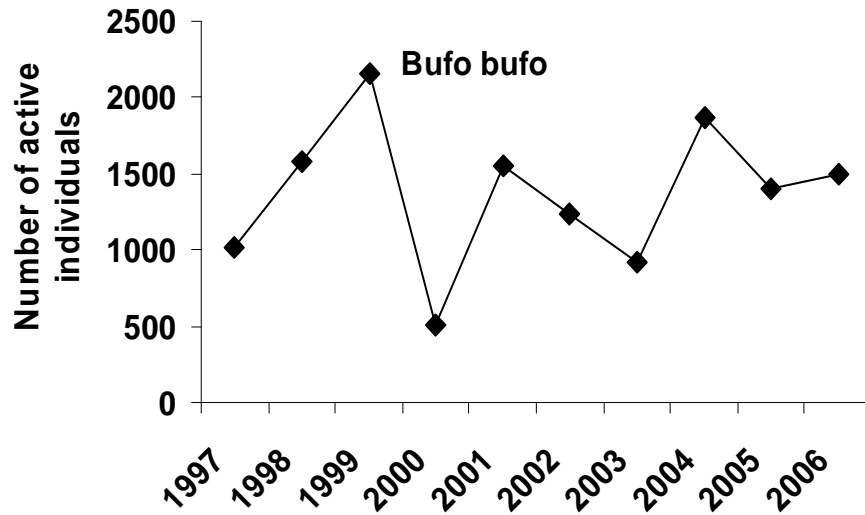
Fig. 4. The fluctuation of the number of active individuals in *B. bufo*

Table 1.

The summary of the multiple regression analysis on the relationship between the climatic variables and the start of reproduction and population size in *Rana temporaria*. The population size of *R. dalmatina* was used as predictor variable for the number of egg masses.

Variable	β (±SE)	t	p	R^2
Beginning of calling activity				
Air temperature in February	-0.69 (0.23)	-2.93	0.01	0.48
Multiple regression $F_{(1,9)} = 8.60$, $p < 0.01$				
First egg mass				
Air temperature in February	-0.64 (0.25)	-2.51	0.03	0.41
Multiple regression $F_{(1,9)} = 6.33$, $p < 0.03$				
Number of egg masses				
Year	-0.94 (0.15)	-6.14	<0.001	0.85
Population size of *R. dalmatina*	-0.51 (0.15)	-3.34	0.001	
Precipitations in Ianuary	0.43 (0.25)	2.77	0.02	
Multiple regression $F_{(1,10)} = 13.77$, $p < 0.002$				

Table 2.

The summary of the multiple regression analysis on the relationship between the climatic variables and the start of reproduction and population size in *Bufo bufo*

Variable	β (±SE)	t	p	R^2
Beginning of males activity				
Air temperature in February	-1.05 (0.24)	-4.31	0.003	0.72
Precipitation January	-0.53 (0.24)	-2.16	0.06	
Multiple regression $F_{(2,8)} = 9.44$, $p < 0.01$				
First egg mass				
Air temperature in February	-0.71 (0.25)	-2.82	0.02	0.59
Precipitation in previous year	0,57 (0.25)	2.28	0.06	
Multiple regression $F_{(1,9)} = 4.27$, $p < 0.05$				
Number of active individuals	No significant effect of climatic variables nor year was found			

Table 3.

The summary of the multiple regression analysis on the relationship between the climatic variables, and the population size in *Pelobates fuscus* and *Hyla arborea*

Variable	β (±SE)	t	p	R^2
Pelobates fuscus				
Temperature January	-0.58 (0.26)	-2.18	0.05	0.34
Multiple regression $F_{(1,9)} = 4.77$, $p < 0.05$				
Hyla arborea				
Year	-0.75 (0.22)	-3.34	0.01	0.67
Precipitation in January	0,70 (0.28)	2.44	0.04	
Multiple regression $F_{(2,8)} = 4.74$, $p < 0.04$				

The number of calling males of *P. fuscus* was negatively associated with the temperature in January whereas in the case of *H. arborea* there was a positive relationship between the number of calling males and the amount of precipitation in January (Table 3). It was suggested that calling in anurans may be energetically demanding behavior the calling intensity of the males being strictly dependent on the energy reserves remained after hibernation (Elmberg and Lundberg, 1991). Thus, what we registered may be the variation of the calling males and not those that survived from year to year. Even in this condition calling may be an indicator of the male's fitness, influencing the probability for finding a mate (Friedl and Klump, 2005). We mention that the recorded climatic variables explained only a small variance of the number of males in *P. fuscus* (34%).

The reasons for the decline of *R. temporaria* and *H. arborea* (in the last species the decline being sharp in the last years) in the permanent pond are not yet known. Both species are susceptible to predation by fish (*Carassus auratus*,

Pseudorasbora parva) (Meyer *et al.*, 1998, Teplicky, 2003; Hartel *et al.*, 2007c for local study). We have not recorded the changes of the density of these fish species during the years, but it is possible that changes in this variable affected the two species. In the case of *R. temporaria*, the population size was negatively associated with the population size of *R. dalmatina*. *Rana dalmatina* is represented by a large and stable population in this area, and is not negatively associated with predatory fish. Interspecific competition in larval stages may be a cause of the negative correlation between the population sizes of the two species (Riis, 1988). The observations made in the Târnava Mare basin (Hartel *unpublished*) suggest that *R. dalmatina* is more represented in the permanent ponds than *R. temporaria* whereas *R. temporaria* is more efficient in temporary ponds than *R. dalmatina* (Hartel, *unpublished*).

   The spatial distribution of the pond use and the reproductive success of Rana temporaria. The average number of the egg masses of *R. temporaria* in the temporary ponds for the five years was 61.60 (SD = 42.64). The median value of the *R. temporaria* egg masses deposited in the temporary ponds were significantly larger (47) than that of those deposited in the permanent pond (22) (Mann – Whitney U test, Z = -2.32, P = 0.02). The reproductive success was more constant in the temporary ponds than in the permanent one. In the permanent pond, reproductive success was recorded only in four years (1997, 1998, 2001 and 2005), these years having larger amount of precipitation in early summer than the other years). In the case of the temporary pond system, the number of the ponds where eggs were deposited varied yearly but metamorphosis occurred in all years (Fig. 5).
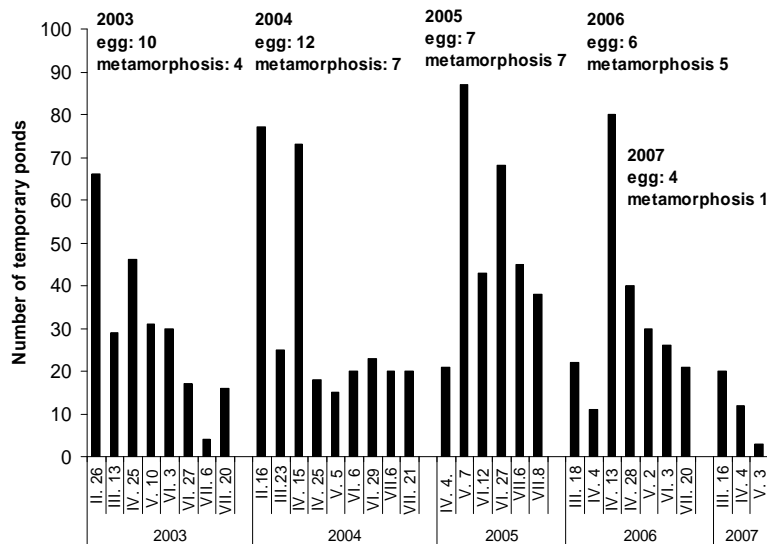
Fig. 5. The fluctuation of the number of temporary ponds after the snow melt until the first part of July (the end of metamorphosis in the majority of *R. temporaria* larvae)

The reproductive success observed in the permanent pond when the water level was increased suggest that the shallow parts of the pond are important for the larval growth and development, most probably due to the temperature regimes, increased productivity and safe against predatory fish. An other temporary pond breeder, *B. variegata* also used this pond for reproduction when the precipitation increased the water level (1998 and 2005) (Hartel, 2004; Hartel *unpublished results*). These results show that the temporary ponds from the forest are more important breeding habitats for *R. temporaria* (probably acting as sources, *sensu* Pulliam 1988) than the permanent pond (that is sink in some years, Pulliam 1988). Temporary ponds are fish free but pose the risk of reproductive failure due to drying. *Rana temporaria* is physiologically adapted to temporary ponds due to its ability to efficiently exploit the resources in temporary ponds and its high phenotypic plasticity (Hartel et al. 2005 and the references cited there).

The number of the temporary ponds with metamorphosis was positively related to the amount of precipitation in May – July (Spearman $r = 0.87$, $P = 0.05$). No relationship was found between the total amount of precipitation in spring (January-April) and the number of ponds used for reproduction. These results show the importance of the precipitations that fill the ponds toward the end of the larval period. At this stage, the larval crowding is high, the nutrient necessity is increased (but the resources are depleted) the waste elements are accumulated and the oxygen concentration is also dropped. According to the model of Wilbur and Collins (1973), there is a threshold larval body size/developmental stage that must be attained to be capable for initiating and completing metamorphosis, in response to environmental deterioration. Refilling the ponds toward the end of the larval stage may contribute to the attainance of the critical development stage at which the metamorphosis can occur. An experimental study (Hartel and Nemes, 2007 in press) indicated that under low energy intake, common frog tadpoles from this population fail to adapt to changing environmental conditions decreasing not only the chance of survival until metamorphosis but the post metamorphic fitness as well. The highest mortality was observed between the larvae having 34 - 38 Gossner stage (Gossner, 1960) (Hartel and Nemes, *in press*).

In conclusion, the climatic variables in spring are good predictors for the number of reproducing adults in *R. temporaria*, *P. fuscus* and *H. arborea*. *Rana temporaria* and *H. arborea* shows negative trends. It seems that the aquatic habitats from this landscape do not provide good habitats for *P. fuscus* and *H. arborea* the reasons not being known. This and the previous studies (Hartel, 2005; Hartel *et al.*, 2007a) show that maintaining a large variety of aquatic habitats at landscape scale is crucial for amphibian communities. Some species are more represented in the temporary ponds (i.e. *B. variegata* [Hartel *et al.,* 2007a], *R. temporaria* [this study]) whereas other species use the permanent pond (i.e. *B. bufo*). As it was suggested before (Hartel, 2004; Hartel and Demeter, 2005) the maintenance of the

green corridor between the permanent pond and the forest (the grassland in the Fig. 1) is crucial for this amphibian community.

We strongly encourage studies that aim to find how natural populations "behave" in different landscape types in Romania. Further studies will elucidate if the protected status of this landscape will really contribute to the conservation of this amphibian community.

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