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Long-term within pond variation of egg deposition sites in the agile frog, *Rana dalmatina*

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Abstract: This study presents long-term fluctuation, within pond distribution and mortality of egg masses in a population of *Rana dalmatina* reproducing in a permanent semi-natural pond in Romania. The average number of egg masses per year was 410 (SD = 114.56, min.–max. = 265–581). The mean spawning duration (days) was 26.72 (SD = 5.53). The egg mass distribution was not constant during the years. The long-term change in the distribution of egg masses can be related to changes in the reed cover (pond variable) and landscape structure, i.e. the presence of the forest and a pasture between the pond and the forest. There was a reduction in the number of dead egg masses during the study period. We suggest that the maintenance of the connectivity with the forest and the high amount of reed cover assure the main aquatic and terrestrial habitats for the agile frogs. This will allow agile frogs to shift breeding habitats in this area according to their ecological needs and find safe terrestrial habitats for summering and wintering.

Key words: *Rana dalmatina*; habitat use; Romania

Introduction

Pond breeding amphibians from temperate areas have complex life cycles and habitat requirements; they require three types of habitats for completing their yearly life cycles. These are the breeding habitat (most commonly represented by a pond), the feeding habitat, and the overwintering habitat. The survival of individuals depends on the quality of these habitats and is influenced by many factors, all linked to habitat quality: the availability of shelter and food, predation pressure, chemical properties of the water, and the microclimate. Amphibians are able to assess these habitat properties and to decide if these will be used (and for how long time) to complete the different physiological needs and the life cycle (Méhely 1903; Hopey & Petranka 1994; Viertel 1999; Petranka et al. 2004; Ficetola et al. 2006). Moreover, shifts in habitat use are often recorded in amphibians (Petranka et al. 2004). A common case of such shifts that is relatively easy to measure is when amphibians change their oviposition site within a pond or when new sites become available and get to be used (Gollmann et al. 2002; Ficetola et al. 2006). Finding the causes of such variations may help managers to develop adequate conservation strategies for the target species and to better know the reaction of the focal species to changes in the habitat quality.

In this paper the long-term fluctuation in population size and changes in the within pond distribution of egg masses of the agile frog, *Rana dalmatina* (Bonaparte, 1839) are presented. This species was selected because of the easiness of egg mass counts (Hartel 2003) and the relatively small populations reported in Western Europe. Moreover, the agile frog is listed as endangered species in the Appendix II of the Bern Convention.

Material and methods

The study area lies in the central part of the Târnava Mare basin, Romania (46°13′47.8″ N; 24°46′47.6″ E, 345 m altitude). The detailed description of the area was presented in Hartel (2003). The studied pond has an area of approximately 2.2 ha and a maximum depth of about four meters. About 35% of the pond is currently covered by species of *Typha* and *Phragmites.* The emergent macrophyte vegetation cover changed over the years: about 25% until 2002, to about 30% and 35% in 2005 and 2007, respectively. During the study period, the following fish species were present in the pond: *Pseudorasbora parva* (Temminck et Schlegel, 1842), *Carassius auratus* (L., 1758), *Cyprinus carpio* L., 1758 and *Leucaspius delineatus* (Heckel, 1843) (Hartel 2003). Negative effect on *R. dalmatina* larvae was experimentally found in *P. parva* (Teplitsky et al. 2003). There is a mixed deciduous forest on the eastern side of the pond, at a distance of about 400–600 m; and there is a grassland patch (green corridor), between the pond and the forest. This is the main migration corridor for amphibians in spring (Hartel & Demeter 2005; Hartel personal obs.). The pond is surrounded by arable lands in the northern and southern part. In the northern part the lands were intensively treated with chemicals until 2003 and again, after one year of abandonment in 2005, 2006 and 2007. In the southern part the agricultural activities were resumed in 2007 in a very small (25 × 25 m) patch after two years of abandonment (note that no fertilizers were used here). There is a railway line in the southern

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Table 1. The beginning and duration (in days) of the spawning period and the annual number of egg masses in *Rana dalmatina*.

Year	Beginning (month. day)	Duration (days)	Number of egg masses
1997	III. 14	24	408
1998	III. 5	31	233
1999	III. 5	28	536
2000	III. 7	33	581
2001	III. 4	18	376
2002	II. 28	32	368
2003	III. 16	23	415
2004	III. 11	24	442
2005	III. 20	28	570
2006	III. 20	19	285
2007	III. 1	34	270

part, at approximately 70 m, and the Târnava Mare River is at approximately 200 m from the western edge of the pond.

Data collection regarding egg masses was made from 1997 to 2007. Data on egg mass distribution in the pond were gathered between 1999 and 2007, excepting the year 2000. The female population sizes were estimated using egg mass counts. Field studies were made from the end of February to the second half of April. The number of field days per season in the egg deposition period varied between 6 and 25. Since the egg masses are deposited in the shallow, warm areas of the pond, their counts could be easily realized. Intensive searches carried out with dip net in the deep parts of the pond, using large jackboots, and a boat in one of the years (up to 2 m depth) showed that the deep parts of the pond are not used for reproduction by this species.

The different parts of the pond were named according to the surrounding land use type: "forest side" (hereafter FS) is the part of the pond that is connected with a forest through a grassland patch (not used for agriculture since the 1970s), "arable A side" (AA) is the northern part of the pond surrounded by arable area, "railway and river side" (RRS) is the part of the pond close to the railway and the river, and finally the "arable B side" (AB) is the southern part surrounded by arable land. The amount of vegetation was constant in the RRS (40%), AA (5%) and FS (40%) sites and increased from 5% (2002) to 15% (2005) and 20% (2007) in the AB site. This was most probably caused by the cutting of trees on the waterfront. As a consequence, large parts of the site became sun-exposed. Considering the amount of vegetation cover (%) and its change during the years in the different parts of the pond, four parts were differentiated into three categories: "high vegetation" (FS and RRS), "low vegetation" (AA) and "increasing vegetation". The mortality of egg masses was recorded for seven years. All non-hatching egg masses were considered dead.

Variables were related to each other with Spearman correlation (r). Kruskal-Wallis ANOVA was used to compare the percentage of egg masses deposited in each side of the pond. Pairwise comparisons were made with Mann-Whitney U-tests (with the pond side as grouping variable and the percentage of the egg masses in each year as dependent variable).

Results

The average number of egg masses per year was 410 (SD = 114.56, min.–max. = 265–581) (Table 1). The population showed no overall significant trend of increasing or decreasing egg mass numbers in the period studied ($r = -0.11$, $P = 0.77$). The first egg mass appeared in average on day 70.18 (Julian calendar was considered with January 1 being day 1) (SD = 7.37) and the mean spawning duration (days) was 26.72 (SD = 5.53). The annual variation of the spawning duration (i.e. the number of days between the first and last deposited egg mass) is presented in Table 1. The FS and the AB sites were those where most *R. dalmatina* egg masses were deposited during the eight years. The percentage of egg masses from the AB site showed the largest variation (Kruskal-Wallis ANOVA, $H_{(3, N=32)} = 19.97$, $P = 0.0002$), although the AB part showed a significant increase in egg mass percentage over the years ($r = 0.90$, $P = 0.02$) (Fig. 1). Egg mass percentage showed no trend in the AA and RRS parts of the pond (Spearman$_r$, $P > 0.05$ in both cases) whereas there was a decreasing tendency at the FS side ($r = -0.89$, $P = 0.002$) (Fig. 1). The FS part of the pond contained the highest average percentage of egg masses in eight

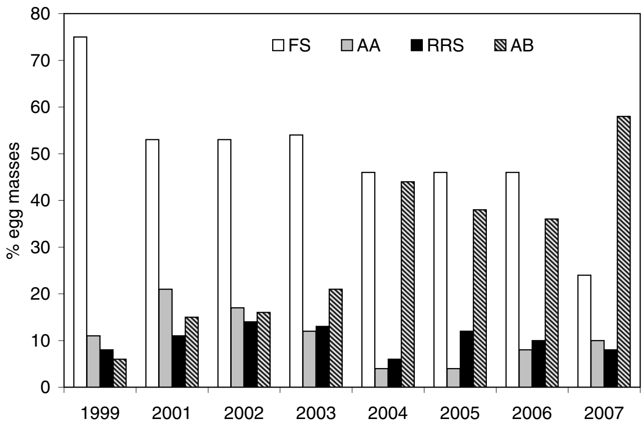

Fig. 1. Annual variation in the percentage of egg masses deposited by *R. dalmatina* at different sites of the pond. Abbreviations: AA –"arable A"; AB – "arable B"; RRS – "railway and river side"; FS – "forest side".

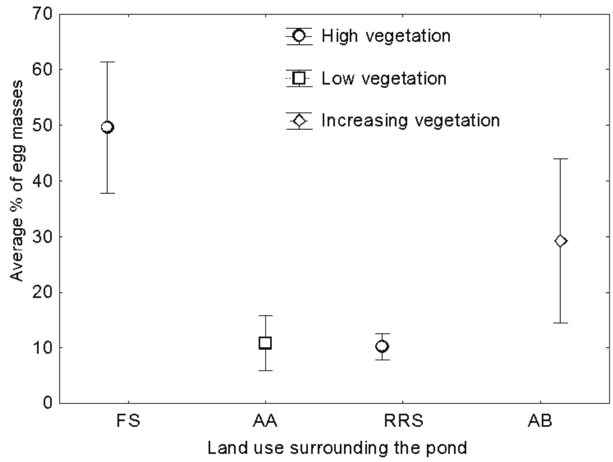

Fig. 2. The average percentage of the egg masses deposited by *R. dalmatina* relative to the emergent vegetation cover and the land use types surrounding the pond. Error bars represent confidence intervals.

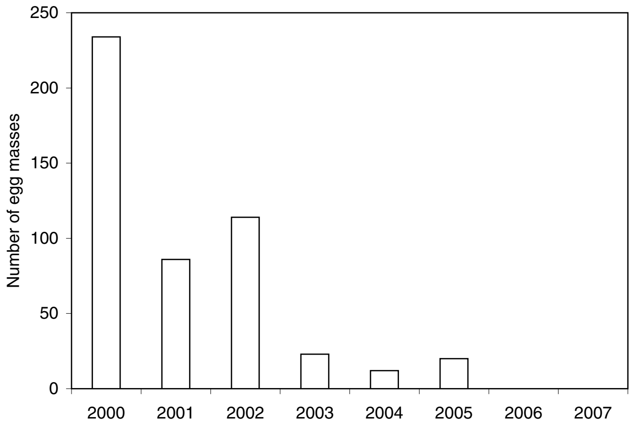

Fig. 3. Egg mass mortality in *R. dalmatina* per year in the study pond.

years (Mann-Whitney *U*-test, $P < 0.02$ for all comparisons). Moreover, the AB part of the pond contained significantly more egg masses than the AA and RRS parts ($P < 0.01$ for both cases). No significant difference was found between the AA and RRS sides ($P > 0.01$) (Fig. 3). As shown in Fig. 2, the largest average percentage of egg masses within the pond is associated with the part that contains both high cover of emergent vegetation and green connecting corridor between the pond and forest (FS). The percentage of the egg masses deposited at the AB site was negatively correlated with the percentage of egg masses at the FS and RRS sites ($P = 0.02$ for both cases).

The egg mass mortality is presented in Fig. 3. There was a reduction in the number of dead egg masses during the study period. The number of dead egg masses was positively correlated with the percentage of eggs deposited in the FS part ($r = 0.83$, $P = 0.02$) and the RRS part of the pond ($r = 0.81$, $P = 0.04$).

Discussion

The spawning duration in the studied population of the agile frog is up to a month, in early spring, similar as in explosive breeder anurans (Wells 1977). Despite high variation between years, the population size (as measured by number of egg masses) showed no trend during the study period of 11 years. To our knowledge only a few long-term studies are available for this species. Sofianidou & Kyriakopoulou-Sklavounou (1983) found a mean number of egg masses of 985 (445–1761) in Greece (three year study), Strömberg (1988) found in average 97 egg masses (60–133) in Sweden in seven years, Waringer-Löschenkohl (1991), studying two ponds in Austria, found on average 36.85 (7–68) and 25.71 (6–82) egg masses in seven years. Finally, Gollmann et al. (1999) found in average 103.25 egg masses (60–171) in Austria in four years. Fluctuation in population size is a common phenomenon in amphibians (Pechmann et al. 1991; Semlitsch et al. 1996; Meyer et al. 1998; Loman & Anderson 2007) and can be caused by competition, predation (Hairston 1996), density (Scott 1990, 1994; Meyer et al. 1998) and climatic conditions such as precipitation and air temperature (Semlitsch et al. 1996; Reading 2007). The climate, especially the amount of precipitation in February, seems to cause egg mass fluctuation in *R. dalmatina* in the study area (Hartel, unpublished data).

The results indicate that the macrophyte vegetation (linked with tree cutting at the pond edge, see AB part of the pond) and the connectedness with a forest may be important habitat and landscape variables in determining the selection of the oviposition site by *R. dalmatina*. On regional level (middle Târnava Mare basin), the emergent macrophyte vegetation and the connection with a forest patch are also important determinants of the habitat use of amphibians (Hartel et al. 2007, 2008). Emergent macrophyte vegetation positively influenced the breeding habitat use of six amphibian species in this area, including *R. dalmatina* (Hartel et al. 2007). Reed represents support for the eggs and assures a complex microhabitat where the amphibian larvae can find shelter against fish predation. Experimental studies showed that fish like *P. parva* may increase the mortality rate, induce morphological (deeper tail muscles) and behavioural (refuge use and decreased activity) responses in *R. dalmatina* larvae (Teplitsky et al. 2003). These morphological and behavioural responses may increase the survivorship of *R. dalmatina* larvae in the presence of predatory fish, if habitat refuges are present. Our study that was carried out in the middle section of the Târnava Mare basin showed that aquatic habitat use of *R. dalmatina* was not negatively associated with predatory fish (Hartel et al. 2007).

The quality of terrestrial habitats is also important for pond breeding amphibians as these habitats represent dispersion corridors and should assure safe habitats for feeding and wintering. Recent studies have presented clear evidence of the importance of landscape elements (terrestrial habitats) in determining the aquatic habitat use of amphibians (e.g., Scribner et al. 2001; Mazerolle & Desrochers 2005; Van Buskirk 2005; Gagne & Fahrig 2007; Hartel et al. 2008). Similar pattern to the one found in this study was observed in some large fishponds close to this area (Hartel, unpublished data): up to 3000 *R. dalmatina* egg masses were deposited in those parts of a pond that had similar characteristics as the FS part of the study pond in the 2004–2007 period. However, no egg masses were deposited in areas with landscape configuration similar to the RRS part of the study pond (high amount of reed and unfavourable terrestrial areas).

The increase of the percentage of egg masses deposited at the AB site may have been caused by the change in habitat use by frogs that deposited at the FS and RRS sites (as found by correlation analysis). Both are close to the AB site and therefore this might indicate a shift in breeding habitat use. With the increase of the emergent aquatic vegetation cover at the AB site new suitable breeding habitats became available that were readily exploited by frogs. The abandonment of the RRS site and the deposition in the AB site may have been caused by water pollution from the pipeline that carries residual water of a town quarter to the water station (Hartel 2004). The reason of the abandonment of the FS part could be the large density of the egg masses deposited in that part every year. By reducing the egg mass density the larval density, thus the intraspecific larval competition can be reduced. Field experiments demonstrated that amphibian larval density negatively affects both the time and size of metamorphosis (Scott 1990) and the adult fitness (Scott 1994). As found by Waringer-Löschenkohl (1991), Gollmann et al. (2002) and Ficetola et al. (2006), *R. dalmatina* is able to shift the spawning site. These shifts are interpreted as having positive impact on the survival of larvae and thus the fitness of adults (Ficetola et al. 2006).

The maintenance of the high amount of reed cover and the connectivity with the forest most probably assure the main aquatic and terrestrial habitats for the agile frogs. This will allow agile frogs in this area to shift breeding habitats according to their ecological needs and find safe terrestrial habitats for summering and wintering. Timber harvesting may change the local microhabitat conditions and subsequently influence the distribution of egg masses but destroys potential good summer habitats of amphibians. Finally, many habitat specific variations in the spawning site selections would be possible because of variations in microhabitat conditions. Ficetola et al. (2006) found that these variations may occur even in different years within one habitat, which highlights the importance of local studies in the habitat selection of this species.

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