

Aspects of breeding activity of *Rana dalmatina* and *Rana temporaria* reproducing in a seminatural pond

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Abstract. Although *Rana dalmatina* and *Rana temporaria* are widely distributed species throughout Europe, studies regarding the breeding biology of *R. dalmatina* and the long term fluctuations of the populations of these species, when they are syntopic are scarce. In this study I present data gathered during eight years, regarding the breeding activity and long term trend of two populations that reproduces in a seminatural pond. The prespawning period lasts in average 5.6 days in *R. dalmatina* and 4 days in *R. temporaria*. The male (i.e. calling) and female (i.e. spawning) activities are correlated. A significant negative correlation between the average temperature in February and the beginning of the breeding activity in both sexes of both *R. dalmatina* and *R. temporaria* populations was observed. The intensity of the spawning is varying between the years, and is influenced by temperature. Egg masses of both *R. dalmatina* and *R. temporaria* were deposited in shallow water, close to the shore. A negative correlation was found between the number of deposited egg masses in the two species.

Key-words: Breeding activity, *Rana dalmatina*, *R. temporaria*, population trends

Introduction

Rana dalmatina and *Rana temporaria* are widely distributed species throughout Europe (Fuhn 1960, Cogălniceanu et al. 2000). However, studies regarding the breeding biology of *R. dalmatina* and long term fluctuations of populations, when it is syntopic with *R. temporaria* are scarce (Sofianidou and Kyriakopoulou-Sklavounou 1983, Strömberg 1988, Kecskés & Puky 1992, Ponsero & Joly 1998, Lesbarreres 2001, Waringer-Löschenkohl 1991, Gollmann et al. 1999, 2002, Hartel 2003, 2004). A reason of the scarcity of studies on *R. dalmatina* could be the relatively small population sizes that locally represent the species. A recent study shows that small populations of *R. dalmatina* in France are affected by inbreeding (Lesbarreres et al. 2003). The reason of scarcity of long term population studies of *R. dalmatina* and *R. temporaria* when they are syntopic could be the small number of habitats in which they occur together. It is hypothesised that the two species are competitively excluding each other (Riis 1988). To my knowledge, long term studies on the two syntopic species have been carried out only in Austria (Gollmann et al. 1999, 2002) and Romania (Hartel 2004).

In this study I present long term data on aspects regarding breeding activity (length and intensity) and the annual fluctuation of two syntopic populations of *R. dalmatina* and *R. temporaria*.

Study area

The pond (46°13'47.8''N; 24°46'47.6''E; 349 m elevation) is situated near Sighișoara, Romania. It covers approximately 2.2 ha and has a maximum depth of about four meters. About 15% of the pond has less than 30 cm depth, and about 30% is covered by macrophytes (*Typha* sp., *Phragmites* sp.). Other aquatic plant species, such as *Myriophyllum* sp., *Potamogeton* sp., and *Ceratophyllum* sp. appear only in the middle of May, therefore in the peak activity of the most amphibian species (end of March until end of April) the water is clear, allowing easy observations. The pond is used for reproduction by 10 other amphibian species (Hartel 2004.). In the eastern side of the pond, at a distance about 400-600 m is a mixed deciduous forest. The majority of the individuals of the studied anuran species migrate from the forest's direction to breed.

The climate on this part of Târnava Valley is continental and moderate. The annual temperature averaged 8.2°C, with an average temperature in January of -4.3°C and in July 18.6°C. The mean number of freezing days per year is about 120. The first freezing day is between 1-28 October, the last between 11 April-1 May. The mean amount of precipitation is between 650-700 mm per year (Hurubian, 1995).

Methods

The study was carried out during the years 1997-2004. I visited the pond each year from mid February to the end of April. I estimated the population size based on the number of females in two species: *R. dalmatina* and *R. temporaria*. Both species deposited egg masses along the edge of the pond in shallow warm areas. Consequently, and due to the absence of the floating vegetation in this period, egg masses could be counted easily. I estimated the length of the spawning period and prespawning period every year. The prespawning period is defined as the period between the first chorus and the first deposited egg mass. A chorus means at least 4 males were calling. The spawning period was estimated in *R. dalmatina* and *R. temporaria* and defined as the period between the first and last deposited egg mass

I measured the depth at which egg masses were laid in years 2001, 2002 and 2003 below surface to the nearest 1 cm, with a meter stick, immediately after spawning in both species. The effect of temperature and precipitation (January and February) on the start of the breeding season, and the population trends were tested with Pearson product moment correlation. The average lengths of the prespawning periods were compared using t-test (after the normal distribution of the data was tested with Kolmogorov-Smirnov test).

Results and discussion

Climatic factors and the activity in the breeding period

The activity of males of both species begins earlier than that of females. In Sweden, the same pattern was observed in Umea region (*R. temporaria*), however, in Blekinge region (*R. dalmatina*) there was no significant difference between the beginning of the breeding activity of sexes (Table 1).

Table 1. Comparison of the spring activity of different European brown frog populations based on long term (at least 3 years) studies. The average duration (days) is presented (1st of January is day 1). The *p* value is the significance level of the t-test. NS=nonsignificant.

Species/population	First calling male	First deposited egg mass	<i>p</i>	Reference
<i>R. dalmatina</i> (Romania)	64.8 (54-76)	69.2 (59-81)	0.01	Present study
<i>R. temporaria</i> (Romania, Târnava Mare valley)	73.1 (49-90)	77.2 (61-95)	0.04	Present study
<i>R. dalmatina</i> (Sweden, Blekinge)	91.8 (57-106)	92 (53-106)	NS	Strömberg (1988)
<i>R. dalmatina</i> (Greece, Gallikos valley)	-	35 (25-30)	-	Sofianidou & Kyriakopoulou-Sklavounou (1983)
<i>R. temporaria</i> (Sweden, Umea)	120.7 (114-126)	126.1 (119-130)	0.00 001	Elmberg (1990)

Table 2. The Pearson correlation between the activity of the first males (calling) and that of females (spawning) NS=the correlation is not significant. n=8.

Species/population	Pearson r	<i>p</i>
<i>R. dalmatina</i> (present study)	0.89	0.003
<i>R. dalmatina</i> (Sweden, Blekinge) (Strömberg 1988)	0.73	NS
<i>R. temporaria</i> (present study)	0.95	0.001
<i>R. temporaria</i> (Sweden, Umea) (Elmberg 1990)	0.84	0.002

Table 3. The Pearson correlation between the average temperature in January and February and the beginning of the breeding activity in the two *Rana* species n=8.

Month	<i>R. dalmatina</i>		<i>R. temporaria</i>	
	Male	Female	Male	Female
January	r=0.04 p=0.9	r=0.04 p=0.9	r=0.34 p=0.4	r=0.2 p=0.66
February	r=-0.78 p=0.03	r=-0.84 p=0.009	r=-0.86 p=0.01	r=-0.89 p=0.007

The prespawning period (i.e. the period in days between the first calling males and the first egg mass deposited) in the two *R.* species studied lasts on average 5.6 days (SD=4.74, range 0-13) in *R. dalmatina* and 4 days (SD=3.69, range=2.12) in *R. temporaria*. There is a significant positive correlation between the beginning of the male (i.e. calling) and female (i.e. spawning) activity (Table 2). In two years, the first egg mass appeared in the same day with the first chorus in the *R. dalmatina* population. Strömberg (1988) found in a long term study (seven years) that the prespawning period lasted on average 0.7 days in this species. In that population, in two years the first egg mass was deposited before the first calling male appeared (with 4 and 11 days respectively). Elmberg (1990) in a long

term study (10 years) found a prespawning period that averages 5.4 days in *R. temporaria*. These findings suggest (i) a latitudinal trend toward a late breeding (spawning) in the northern populations compared with those in south and (ii) the prespawning period is shorter in northern populations (*R. dalmatina*). In a recent review, Miaud & Merilä (2000) explain the variation of the beginning of reproduction among populations along an altitudinal/latitudinal gradient as the result of adaptation and differentiation between local populations inhabiting environments differing in their selective regimes. The short prespawning season (and the annual variation of its length) could be caused by the feeding conditions in the activity period before hibernation and/or the long hibernation period (Elmberg & Lundberg, 1991). Both may reduce the energy reserves of males and females during the hibernation, and also could have consequences on the breeding activity. Male *R. temporaria* that reproduce at high altitudes call more sparsely, are generally more sluggish and allocate less into mating activities than males in the lowland (Elmberg & Lundberg 1991). This was related to the relative fat body mass, that was significantly higher in lowland males than in mid-altitude and alpine males (Elmberg & Lundberg 1991).

Table 4. Beginning and duration of the spawning season of *R. dalmatina* in Târnava-Mare Valley. The total number of egg masses and the temperature and precipitation are also shown.

Year	Starting date	Duration (days)	No. egg masses	Mean air temp °C			Precipitation mm	
				January	February	March	January	February
1997	III. 14	24	408	-1.1	-0.9	2	6	33.3
1998	III. 5	31	233	-0.5	0.5	1.4	33.8	10.3
1999	III. 5	28	536	-1.9	-1.4	4	16.4	37.5
2000	III. 7	33	581	-6.3	-1	3.7	14.1	20.6
2001	III. 4	18	376	-1.1	0.2	6.5	19.4	15
2002	II. 28	32	368	-3.5	2.9	5.6	9	15.6
2003	III.16	23	415	-1	-4.9	0.62	39.4	28.8
2004	III.11	24	442	-4.53	-1.6	4.57	39.7	47.6

It is known that males of many explosive breeders (a breeding type to which both species belong) form choruses during the breeding period. Wells (1977) stated that in explosive breeders, the chorus could have a role in the attraction of females to the spawning site, but after the females arrive, they may not differentiate between calling individuals due to the large overlap of individual calls. However, the above presented data show that

females may arrive at the breeding site and spawning may start before chorus activity begins. I suggest that the calling activity in this circumstance (i.e. when the breeding activity of the males is limited by the low energy reserves after hibernation) could be a sign of the male fitness and increase the mating probability of the more fit males. This could lead to sexual selection (however, if the female approaching to a calling male is not „caught” by a „silent” satellite male). To my knowledge this hypothesis has been never tested in these two species.

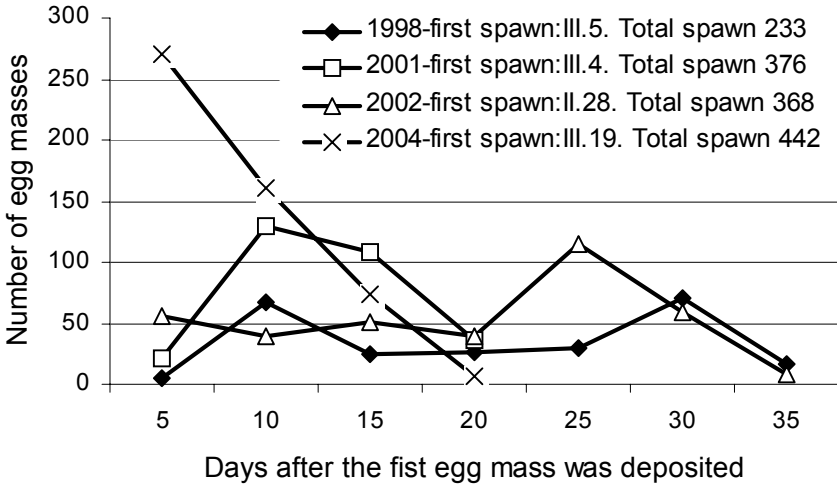


Figure 1. The spawning duration and intensity in four years in *R. dalmatina*. The figure shows the egg masses deposited in five day intervals. The day of first spawn and the total deposited egg masses/year are also showed

There is a significant negative correlation between the average temperature of February and the beginning of the breeding activity in both males and females of *R. dalmatina* and *R. temporaria* populations (Pearson r , $p < 0.05$, $n = 8$) (Table 3). The precipitation in February is positively correlated with the beginning of calling and spawning activity in both species, but only in *R. dalmatina* the correlation closely approaches a statistical significance ($p = 0.09$ and 0.07 for both sexes, $n = 8$). The duration of the egg deposition period never exceeds six days in *R. temporaria*. In the case of *R. dalmatina* the egg deposition period ranges between 18 and 33 days (Table 4), lasting for 26.62 days on average.

There is no significant correlation between the last complete ice cover that covers the pond (measured in days between 2000 and 2004) and the beginning of the calling and spawning activity of the two species ($p > 0.7$ in both cases). This could probably be caused by the low number of individuals that hibernate in the pond. The pond is deep and contains fish in high densities, and large quantities of aquatic plants (*Myriophyllum* sp. and *Ceratophyllum* sp.) therefore there is a high chance for the drop in oxygen concentration during winter. These, together with the pollution caused mass winter mortalities in other taxa such as the fish *Lepomis gibbosus* and crayfish *Astacus astacus* in previous years

(Hartel 2004). Consequently, the pond does not represent a suitable hibernation habitat for the two amphibian species. During the years, I never observed semnificative migrations from the terrestrial habitats into the pond in autumn.

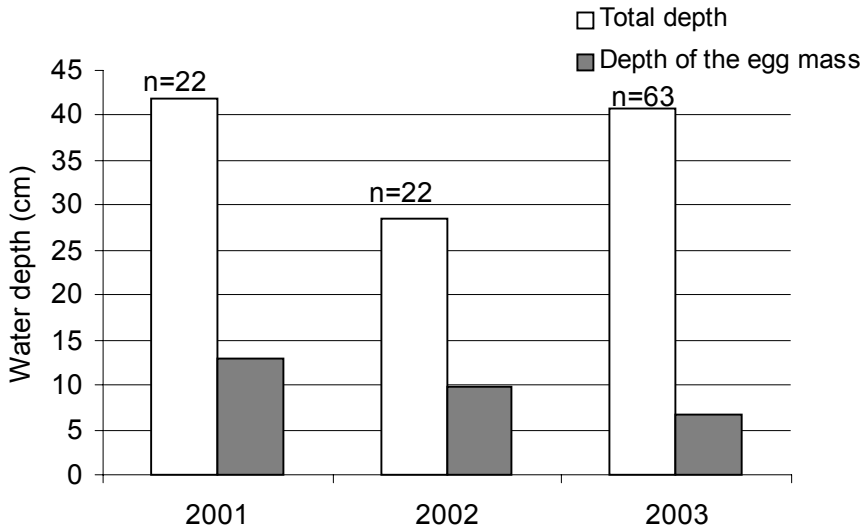


Figure 2. Water depth at the spawning site: the average of the total water depth where deposited egg masses were found and the depth in which the egg masses were laid in *R. dalmatina*. n=sample size.

Data on egg deposition were available only for *R. dalmatina* for four years: 1998, 2001, 2002 and 2004. Figure 1 shows that the intensity of spawning varied between the years: 1998 and 2002 were the longest spawning periods, when the intensity of spawning was low. In 2001 and 2004 the spawning intensity was high, and the duration was relatively short. However, there is no significant correlation between the spawning period and the number of deposited egg masses (Pearson r , $p > 0.5$, $n = 8$) (Table 4). The few data available on intensity of egg deposition (Figure 1) allow only speculations regarding the factors involved in causing such differences between years. The effect of air temperature fluctuations is highly probable. Sofianidou & Kyriakopoulou-Sklavounou (1983) in Greece found that the air and water temperature fluctuations affect the spawning intensity of *R. dalmatina*.

The high intensity of egg deposition in 2001 and 2004 (Figure 1) is probably related to the high average temperature of March (5.6 and 4.6°C). Based on these data, it can be stated that 2001 and 2004 were „good years” because once started, spawning occurred very intensely and within a short time period (less than 3 weeks). If spawning begins late, the possibilities for unpredictable unfavorable changes in climatic conditions are expected to be low. If spawning begins early (i.e. in the middle of February) a high probability exists for periods, with low temperatures that hamper the breeding activity.

Both *Rana* species deposited egg masses in a depth of less than 15 cm, close to the water shore. Figure 2 shows that the average depth of the egg masses in *Rana dalmatina* is constant in three years. The reason could be the constant water depth in spring during the years. Gollmann et al. (2002) showed in a long term study that the depth in which the egg

masses of *R. dalmatina* and *R. temporaria* eggs are deposited may change due to the overall shift of the water depth.

Long term changes of the two Rana populations

Figure 3(a,b) shows the fluctuation of some *R. dalmatina* (Figure 3a) and *R. temporaria* (Figure 3b) populations in Europe, including the data of this study.

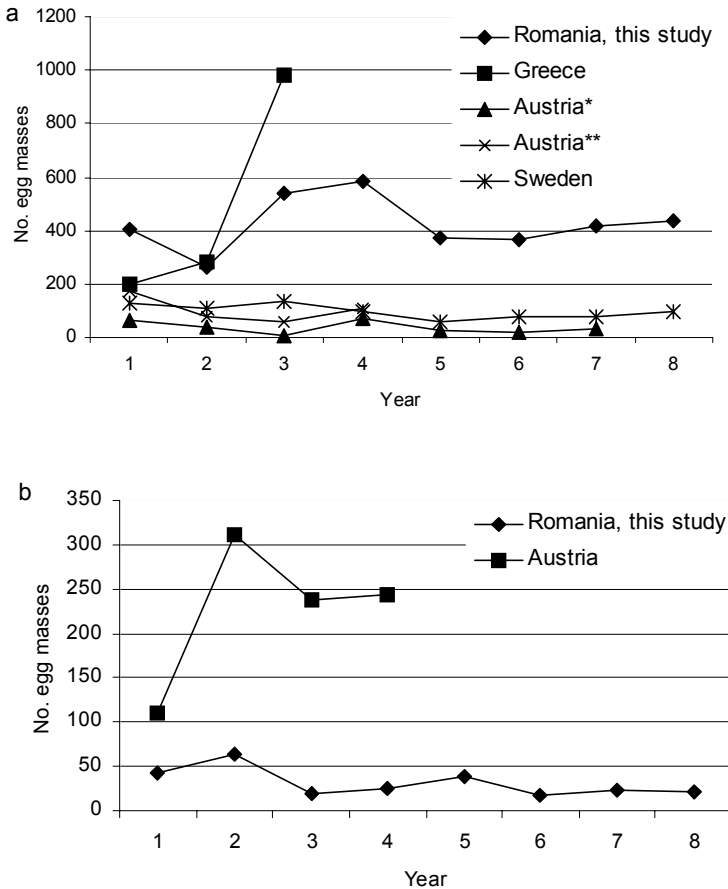


Figure 3. The long term fluctuation of some populations of the two *Rana* species in Europe, based on long term data. a) The fluctuation of five populations of *R. dalmatina* in Greece (Sofianidou & Kyriakopoulou-Sklawounou 1983), Austria (Gollmann et al. 1999**, Waringer-Löschenkohl 1991*) Sweden (Strömberg 1988) and Romania (this study). b) The fluctuation of two *R. temporaria* populations. The data on Austrian population are from Gollmann et al. (1999)

The long term study of another three anuran populations (*Hyla arborea*, *Bufo bufo* and *Pelobates fuscus*) shows that none of them are in decline (Hartel 2004). Based on these data I conclude that the amphibian community in this pond seems to be stable in time, and in the recent past no drastic changes occurred in the area.

To my knowledge there are only a few long term studies which surveyed the yearly spawn production in *R. dalmatina* (reviewed in Figure 3a). The longest study on *R. temporaria* in Europe was carried out by Meyer et al. (1998) (covering 23-28 year periods).

Fluctuations in population sizes are characteristic for amphibian populations (Alford & Richards 1999), and a decrease over a short time period does not necessarily mean that the population is in decline in the long term (Pechmann *et al.* 1991, Semlitsch *et al.* 1996). In fact Alford & Richards (1999) noted that „It is clear that a population decreasing in more years than it increases is not necessarily in decline”. In a simulation model –fitted with data sets gathered with long term studies– they found that amphibian populations may be –naturally– „in decline” more than 50% of time.

As noted by Gollmann *et al.* (1999), habitats where the two brown frog species (*R. dalmatina* and *R. temporaria*) are syntopic are rare in Europe. The reason suggested by some authors is interspecific competition, that may contribute to niche separation of these two species (Riis 1988), and may lead to reproductive failure as shown for another coexisting ranid species (Hettyey & Pearman 2003). I analysed the correlation between the number of the yearly deposited spawn clumps by the two species, and found that the correlation is negative, and closely approaches statistical significance ($r=-0.65$, $p=0.07$, $n=8$). A recent study along the Târnava Valleys shows that the domination of *R. dalmatina* upon *R. temporaria* is a general phenomenon in the lower-middle part of the Valley, whereas in the upper section (>600 m elevation) *R. temporaria* begins to dominate (Hartel *unpublished data*). Other long term studies in Western Europe (Gollmann *et al.* 2002) show an inverse relationship (i.e. the domination of *R. temporaria* upon *R. dalmatina*).

Further studies (i. e. competitive coexistence of larvae, age distribution in the two syntopic species, the spatial and temporal dynamic of the two populations) are needed to understand this. There are evidences that the interspecific competition between amphibian larvae (*Ambystoma*) could result in fluctuations of population densities (Brodman 1996; Jaskula & Brodman 2000).

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