Weather conditions, breeding date and population fluctuation in *Rana dalmatina* from central Romania

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As ectotherm vertebrates, amphibians are sensitive to weather conditions, which may influence the start of the breeding season, body condition and survival during the winter. If climatic conditions change at large spatial and temporal scales, this can have adverse effects on amphibians, including susceptibility to disease. In this study, I recorded the start of the breeding season (males and females separately) and monitored population size in a *Rana dalmatina* population over an 11-year period. I found no indication of an early extension of the breeding season or of significant population trends. Initiation of breeding and the size of the population were significantly negatively correlated with temperature and precipitation in February. The rate of change in the egg mass number seems to be influenced by density. This result suggests that if the climate change results in drier springs, this population of *R. dalmatina* may be adversely affected.

Key words: breeding, climatic conditions, Târnava Mare basin

INTRODUCTION

Amphibians are ectotherm vertebrates with complex life cycles and have a permeable skin that necessitates certain moisture and microclimate conditions to assure physiological function. Due to these developmental and physiological constraints, amphibians are sensitive to climatic conditions and, at the same time, good candidates for monitoring the (multiple) effects of climatic changes on natural populations (see Corn, 2005; Araujo et al., 2006; Pounds et al., 2006). Climate change (including global warming, often associated with long periods of drought or other changes in rainfall patterns) may have a wide range of effects on amphibians. Temperate amphibians may shift the start of their breeding season following an increase in air temperature through time; such shifts have been noted in Europe (Beebee, 1995; Tryjanovski et al., 2003) and North America (Gibbs & Breisch, 2001; but see Blaustein et al., 2001). Early breeding may expose amphibians to unpredictable and drastic temperature changes in spring that may cause severe mortality (Corn & Muths, 2002). Moreover, amphibians are forced to use refugia against extreme temperatures in extremely dry and warm summers and to reduce their activity. This may limit energy intake, affecting both fecundity and fat deposition needed to overwinter, and thus result in decreased reproduction and survivorship potential (Reading, 2007). Climate warming may also result in potential changes in the geographic ranges of some species, including range expansions if dispersal is not limited (see Araujo et al., 2006). Climate change has also been implicated as a key factor in facilitating pathogen outbreaks, especially of amphibian chytrid, *Batrachochytrium dendrobatidis* (Pounds et al., 2006).

Amphibian populations show wide fluctuations, the factors causing this being multiple (ranging from natural ones to those having anthropogenic origins) and often difficult to separate and interpret (Pechmann et al., 1991).

Field studies need to be conducted in order to assess the importance of environmental factors in determining habitat use and population fluctuations of amphibians. Baseline data can be gathered on a regional scale through amphibian habitat surveys and on a local scale through long-term monitoring projects on specific amphibian populations (Cogălniceanu et al., 2006; Dodd & Barichivich, 2007). The importance of these studies is enhanced in central and eastern Europe where many ecosystems have been less impacted by humans than in other parts of Europe (Palang et al., 2006). However, although the stress caused by the anthropogenic impact on amphibians may be lower in this part of Europe, available studies (both habitat association and long-term studies) from this region are scarce.

The present study was conducted on a population of *Rana dalmatina* in a semi-natural hilly forested landscape of central Romania between 1997 and 2007. In this paper I examine 1) if the start of breeding activity by males and females is related to weather variables, 2) the yearly egg mass number in relation to weather conditions and 3) the relationship between climatic variables, population density (i.e. the number of egg masses) and the rate of change in the egg mass number. *Rana dalmatina* was selected because it is common in Târnava Mare basin (Hartel et al., 2006), its breeding population size is relatively easy to estimate using egg mass counts, and it is considered to be locally represented by small populations in western Europe. Moreover, long-term studies on this species are available from different parts of its range (Sofianidou & Kyriakopoulou-Sklavounou, 1983; Strömberg, 1988; Waringer-Löschenkohl, 1991; Gollmann et al., 1998, 2002).

MATERIALS AND METHODS

The study area is situated in the middle region of the Târnava Mare Valley, Romania (46°13'47.8"N; 24°46'47.6"E, 345 m altitude). The study population of *R.*

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Table 1. The descriptive statistics of the variables presented in this study for an 11-year period. SD = Standard Deviation, LCI and UCI = lower and upper confidence intervals.

Variable	Mean	SD	LCI95%	UCI95%	Range
First call (Julian calendar date)	66.09	7.98	60.72	71.45	54–76
First egg mass	70.18	7.37	65.22	75.13	59–81
Number of egg masses	409.72	114.56	332.75	486.69	265–581
Amount of rain in the active season in previous year	447.47	119.19	367	527	287–662
Amount of rain in the previous year	649	139.22	555.54	742.61	414.6–868.3
Precipitation January	21.24	13.45	12.20	30.28	7.00–40.70
Precipitation February	28.06	13.57	18.94	37.18	10.00–47.60
Temperature January (°C)	-2.24	2.06	-3.63	-0.85	-6.30–0.2
Temperature February (°C)	-0.78	1.95	-2.09	0.53	-4.37–2.90

dalmatina breeds in a 2.2 ha pond with a maximum depth of about 4 m. Approximately 35% of the pond is covered by *Typha* sp. and *Phragmites* sp. The terrestrial area surrounding the pond is represented by arable lands, a railway line, the Târnava Mare River and a grassland patch between the pond and the forest (Hartel, 2003).

Field studies were carried out during 1997–2007. The pond was visited each year from the middle/end of February until the end of the breeding season (the second part of April). Female population size was estimated by counting the number of egg masses which were deposited in shallow, warm areas of the pond. Using large jackboots (and a boat in one year) the deeper parts (more than 1.5 m) of the pond were also searched with a dip net. The number of days when egg masses were counted varied between six and 25 per spring. Additional details of egg mass counts and study area are in Hartel (2003, 2005). The beginning of male vocalization and the day of the first egg deposition were recorded each year. The weather data were gathered from a hydrological station from Albeşti that is around 5 km in a direct line from the study site. The mean monthly temperatures were calculated from the daily minimum and maximum temperatures, and these values were used in statistical analysis (see below).

The rate of yearly change in egg mass number was measured using ΔN method (Houlahan et al., 2000): $\Delta N = \log(N+1)_t - \log(N+1)_{t-1}$, where N represents the population size (number of the egg masses) at time t.

The relationship between the independent and dependent variables was tested using a multiple regression approach (see below). The dependent variables were: 1) the start of the breeding season (the Julian date when the first calling male and egg mass were recorded), 2) the yearly egg mass counts, 3) the yearly egg mass counts and 4) rate of the change of the egg mass number. The following seven independent variables were used: 1) year, 2) total amount of precipitation (l/m²) in January and 3) February in the years when egg masses were counted, 4) total amount of precipitation during the frogs' active season (April to September) in the year previous to that when eggs were counted, 5) the total amount of precipitation in the year previous to that when the eggs were counted, 6) the mean monthly air temperature (°C) in January (i.e. around two months before the beginning of reproduction) and 7) the mean monthly air temperature in February (i.e. around one month before the beginning of

reproduction). In the case of the population growth rate the variable "year" was not used as a predictor.

RESULTS

No significant correlation was found between the year of the study and the precipitation or mean air temperature in February ($r=0.23$ for precipitation and –0.12 for air temperature, $P>0.05$ in both cases). Descriptive statistics for each variable are provided in Table 1. Males started calling at a mean of 66.09 days (SD=7.98). The first egg mass appeared on average at 70.18 days (SD=7.37). Multiple regression analysis revealed that the mean air temperature in February was a good predictor of both the start of male calling activity and the start of egg mass deposition. This variable explained 66% of the observed variation in the start of calling activity and 74% of the observed variation in the start of egg deposition (Table 2).

The amount of precipitation in February was the only predictor for the number of egg masses deposited (the fluctuation of these two variables is presented in Figure 1), explaining 41% of the variation in egg mass number (Table 2). The overall mean value of the population growth rate was slightly negative ($\Delta N = -0.01$, SD=0.17), suggesting a slight decrease. Declines were found in 50% of years with mean $\Delta N = -0.14$ (SD=0.12). The mean value of ΔN for population increases was 0.10 (SD=0.11). The number of egg masses deposited (i.e. density) was the only variable that (negatively) affected the rate of change in the egg mass number, accounting for 42% of the variation in this dependent variable (Table 2).
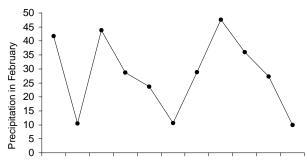
DISCUSSION

There was no measurable trend among any of the climatic variables over the 11-year period. Although the start of reproductive activity was correlated with climatic factors (the temperature in February) in both sexes, there was no trend towards earlier breeding during the course of the study (but see Beebee, 1995; Tryjanowski et al., 2003). The initiation of reproduction by explosive-breeding amphibians in temperate regions is triggered by climatic conditions in spring (see, for example, Sofianidou & Kyriakopoulou-Sklavounou, 1983, for *R. dalmatina* and Reading, 1998, for *Bufo bufo*). This study showed that the mean air temperature in February has an important effect on the start of local breeding activity. Based on the strong

Table 2. Multiple regression results of the association between weather variables and dependent variables (first call, first egg mass and egg mass number).

Variable	(β±SE)	*t*	*P*	*R²*	Multiple regression
First calling male					
Temperature February	-0.81 (0.19)	-4.20	0.002	0.66	$F_{1,9}=17.65, P<0.002$
First egg mass					
Temperature February	-0.86 (0.16)	-5.06	0.006	0.74	$F_{1,9}=25.66, P<0.001$
Number of egg masses					
Precipitation February	0.64	2.51	0.03	0.41	$F_{1,9}=6.34, P<0.03$
Population growth rate					
The number of egg masses	-0.65 (0.26)	-2.44	0.04	0.42	$F_{1,8}=5.97, P<0.04$

relationship between the mean temperature in February and the start of breeding in both sexes (*R²* being more than 66%), I hypothesize that any long-term increase in this variable may ultimately be reflected by earlier Julian dates for the onset of breeding in this population. The lack of evidence for an earlier shift in the start of breeding activity at my study site does not necessarily mean that trends toward earlier breeding do not occur elsewhere or at a larger temporal scale in this population and the climatic conditions.

I found no evidence for population decline in *R. dalmatina*. Fluctuations in population sizes are a com-
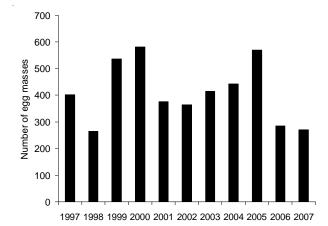

Fig. 1. Egg mass fluctuations in *R. dalmatina* and the total amount of precipitation in February.

mon phenomenon in pond-breeding amphibians and can be caused by competition, predation (Hairston, 1996), density (Meyer et al., 1998) and climatic conditions such as precipitation and air temperature (Pechmann et al., 1991; this study). In the Târnava Mare basin, the number of *R. dalmatina* egg masses deposited yearly is positively related to the amount of precipitation in February, the month preceding spawning. This variable explains only 41% of the variation of egg masses, suggesting that other, unrecorded variables may also be important predictors for the breeding female population size. Jensen et al. (2003) also found that the rainfall prior to egg deposition was an important predictor for the egg mass number of *R. capito*. Females are sensitive to climatic conditions towards the end of the winter dormancy period because of the depletion of energy reserves (Ryser, 1989; Reading & Clarke, 1995). In dry springs during the breeding season, a certain proportion of females may fail to reproduce or mortality could increase due to loss of body fat reserves during prolonged dormancy (see Jørgensen, 1984, for *B. viridis*). However, Meyer et al. (1998) did not find a significant relationship between the breeding population size of *R. temporaria* and rainfall immediately before spawning. In the Târnava Mare basin population of *R. dalmatina*, there was no evidence that rainfall in previous years affected the size of the breeding population over the 11-year period. Similar results were found by Meyer et al. (1998) in two of three populations of *R. temporaria* in Switzerland (but see Reading & Clark, 1995, for *B. bufo* and the third population in Meyer et al., 1998). One possible explanation for the lack of such a relationship in our study area is the large percentage of forest cover near the pond that may buffer extreme hot summer temperatures. The deciduous forest (*Fagus* sp., *Quercus* sp. and *Carpinus* sp.) should provide good shelter, moisture and a suitable microclimate, as well as good feeding conditions for frogs in warm and dry summers. I never found frogs in the pasture between the pond and the forest during the summer months, suggesting that this landscape element is not used by frogs for feeding (pers. obs.). Another possibility is that the annual precipitation and temperature fluctuations were not so extreme as to reach the tolerance limits of amphibians in this area.

There is no trend in the population I studied. This study indicates that the growth rate of this population is sensitive to density. However, only 42% of the variation

in the population growth rate was explained by density. The effects of density on the adult population growth rate in amphibians may be caused by the limitation of resources such as food and overwintering habitats (Harper & Semlitsch, 2007). Density may affect body condition, reproductive potential through delaying the age of first reproduction and small body size at maturity (and thus smaller egg masses), and this may lead to the decrease in the population growth rate (Reading & Clarke, 1995; Harper & Semlitsch, 2007). I cannot estimate if the "density effect" found in this study is on adult survival or reflects reduced spawning activity (this being the more likely possibility; see above). If female spawning activity was affected (i.e. some females did not breed every year), then the rate of yearly change of the female population may not be as great as the variation in egg masses.

In conclusion, the *R. dalmatina* population I studied is not declining. No temporal shift in the start of breeding activity was recorded in 11 years. The number of egg masses was significantly affected by the amount of precipitation in February, whereas the population growth rate was influenced by density. With this 11-year study, a reference database was created that will be useful in tracking further changes in the dynamics of this population. Further studies of the movements, terrestrial habitat use and feeding ecology of amphibians and of ovarian cyclicity in varying climatic conditions are needed. Such studies, if coupled with aspects of breeding ecology, may provide valuable information about amphibian habitat use and behavioural ecology in changing climatic conditions, and may improve the efficiency of management strategies for amphibians and their habitats.

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