

Ecological features of a *Rana iberica* population inhabiting a human-altered ecosystem

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In this work, data are presented about spatial use and behaviour of Iberian brown frog (*Rana iberica*) during and after the breeding season in Pontevedra (north-western Spain). Field data were collected between November 2008 and February 2010 at a human-altered stream. The results suggest that there was a change in habitat use between the breeding and the non-breeding seasons. *Rana iberica* individuals concentrated in the upper part of the stream during the breeding season (November-March), moving to lower parts of the stream during late spring and summer. The estimated monthly frog abundance was inversely correlated to the recorded mean daily minimum temperatures.

Key words: Galicia; habitat use; Iberian brown frog.

Dispersal through a landscape can be performed in two different ways: (i) as a result of routine movements associated with resource exploitation (like foraging or mate-searching) with frequent return to the original site, or (ii) as special, fast and directed movements meant to displacement or migration (VAN DYCK & BAGUETTE, 2005). SEMLITSCH (2008) divided, in the case of amphibians, the latter process in two types of dispersal; first, he defined amphibian migrations as movements performed primarily by resident adults towards and away from aquatic breeding sites. The second type was defined as dispersal and consists of unidirectional movements from natal sites to breeding sites that are different from the pond of birth and are not part of the habitat of the origin population.

The influence of landscape and local

factors on amphibian distribution is well known for lentic habitats (VAN BUSKIRK, 2005; WERNER *et al.* 2007), but has been little studied in running waters (LOWE & BOLGER, 2002; MANENTI *et al.*, 2009). It is thus important to evaluate the relationship between the presence of the different species in given habitats and the features of both terrestrial and aquatic ecosystems in those habitats (DENOËL & FICETOLA, 2008).

The Iberian brown frog (*Rana iberica*) is an endemic species from the northern and western parts of the Iberian Peninsula, with some isolated locations along the Central Range in central Spain (SALVADOR & GARCÍA-PARÍS, 2001). Despite being one of the most common amphibian species in some parts of the Galicia region, relatively little is known about its ecology in that region (ESTEBAN & SANCHIZ, 2000; RODRÍGUEZ-PRIETO & FERNÁNDEZ-JURICIC,

2005; BOSCH *et al.*, 2006; ROSA & PENADO, 2013).

The aim of this study was to investigate the distribution and movements of adult individuals from a *R. iberica* population during the breeding and post-breeding seasons in a human-altered area, and the relationship of those parameters with the environmental features of the stream and upland habitats.

Between November 2008 and February 2010, a human-altered stream located in Lourizan (Pontevedra, NW Spain, 8°39'N, 42°24'E) was monitored. The stream was visited at least once per month, with increased number of visits during the breeding season (3-7 visits per month between November and March). The study area is an Atlantic coast locality, with a mixture of autochthonous and allochthonous vegetation. The stream vegetation cover is dominated by *Magnolia* sp. and the stream banks are partially covered by shadow grass and *Tradescantia fluminensis*. The stream runs downhill between 80 and 50 meters above sea level, with some waterfalls. The streambed has been partially modified, with some ponds created in the lower part of the stream that reduce water flow and speed. Diurnal visual encounter surveys were used to evaluate the presence and location of the frogs. During each survey, the banks of the stream were monitored in order to detect *R. iberica* individuals, and their positions were recorded with a Magellan GPS device. Sex of the detected frogs was recorded when possible, and so were the presence of amplexa and clutches, which were used to determine the onset of the breeding period.

The study of the biology of adult Iberi-

an brown frogs was approached by dividing it into two aspects: (i) the spatial one, which deals with how frogs distribute in the space during and out of the mating season, and (ii) the environmental one, which aims to unveil the climatic factors shaping changes in the abundance of frogs in the study area. Each topic merits some separate methodological words.

For the spatial analysis, kernel probability distributions were obtained (probably the most common way to study spatial use by animals, CLOBERT *et al.*, 2012) separately considering the recorded positions for animals in and out the breeding season. Kernels were calculated using a Gaussian function in GRASS GIS (GRASS DEVELOPMENT TEAM, 2012). After that, the breeding and non-breeding kernel distributions were compared by means of a non-parametric paired sign test. This statistical procedure allowed for detecting differences between seasons in the space use by frogs.

Climatic factors are known to affect the phenology of amphibians (LLORENTE *et al.*, 2006) because they contribute to determine the physiological limits of these animals (KÖHLER *et al.*, 2011). Here, I explored whether peaks in frog abundance observed throughout the year were shaped by climatic influence. With this purpose, I estimated monthly frog abundance using the open population model described by DAIL & MADSEN (2011), considering each visit to the stream as a primary survey period and then estimating the abundance for each month. The used dynamical model (DAIL & MADSEN, 2011) accommodates animals that are both undetected (i.e. not detected but present during the surveys)

and not available for detection (i.e. not present at the time of a given survey but present at other times). This method allows for correcting field estimates, and in further analyses I used the corrected abundance estimates.

The open population model described by DAIL & MADSEN (2011) allows to directly model frog abundance as a function of covariates, such as climatic variables, by using a Poisson-log Generalized Linear Model (GLM) to relate covariates to abundance. Climatic data were available from a meteorological station located close to the sampled stream (METEOGALICIA, 2019). Monthly changes in frog abundance were studied as a function of five climatic variables collected on a month by month basis: mean daily maximum temperature in °C (Tmin), mean minimum daily temperature in °C (Tmax), mean relative humidity in % (Humidity), mean monthly rainfall in mm (Rainfall), and mean monthly temperature 10 cm above ground in °C (T10cm). More specifically, and in line with good statistical practice (BURNHAM & ANDERSON, 2002), we proposed an *a priori* set of statistical models that represented our hypotheses about how the climatic covariates influence frog abundance. In other words, this set of models were our alternative hypotheses about the functioning of the system to be tested through model selection procedures. This is akin to classical hypothesis testing approaches, but considering multiple models and the models as the quantitative hypotheses. The objective is to evaluate these alternative hypotheses and not to conduct a data dredging exercise to find possible relationships between the different covariates and the frog abundance. All

models were ranked based on their Akaike's Information Criteria (AIC) score (BURNHAM & ANDERSON, 2002), using both the standard and corrected (AICc) values. AIC scores balance the explanatory capacities of the models with the risk of overfitting, and the model with the lower score is considered the best-fitting model and used for subsequent statistical inference.

All the statistical analyses were done in R (R DEVELOPMENT CORE TEAM, 2010) using the package unmarked (FISKE & CHANDLER, 2011) for the dynamic population models (DAIL & MADSEN, 2011).

A total of 582 frog locations were recorded during the study, 502 of them corresponding to the breeding and 80 to the non-breeding period (Fig. 1). Kernel bivariate functions reflect the existence of changes in the spatial distribution of the frogs. In particular, the use of the space along the stream by Iberian brown frogs show statistically significant differences between the breeding and the post-breeding periods (Fig. 2; Sign test performed over the bivariate kernels: $z = 2.5$, 11 d.f., $P < 0.01$).

During the breeding season, frogs tend to move to the upper part of the stream, right downstream two waterfalls located in that edge of the sampling area. There, a previously non-described reproductive behaviour of *R. iberica* has been observed, consisting of the aggregation of up to 30 males in a small stretch of the stream (Fig. 1). After breeding, bivariate kernels show that frogs disperse towards downstream zones, that are more shadowed and vegetated than upstream ones (Fig. 1).

The best fitting model as identified by AIC and AICc values (Table 1) related the monthly frog abundance to mean daily

Figure 1: *Rana iberica* locations along the studied stream during the breeding (left) and non-breeding (right) seasons. The waterfalls located in the upper part of the study area are indicated with arrows and the ponds located in the lower part of the study area are indicated with stars.

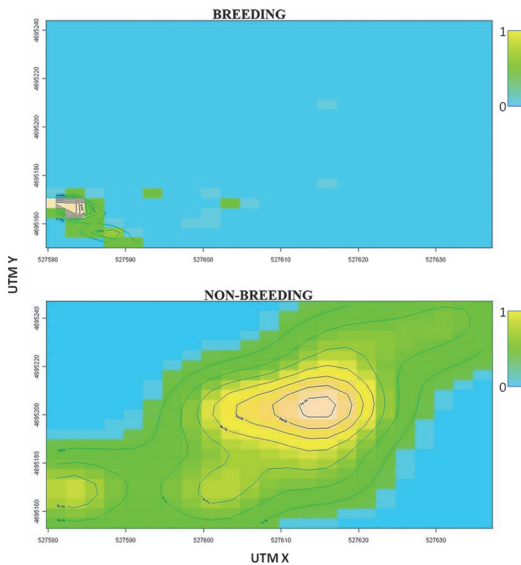
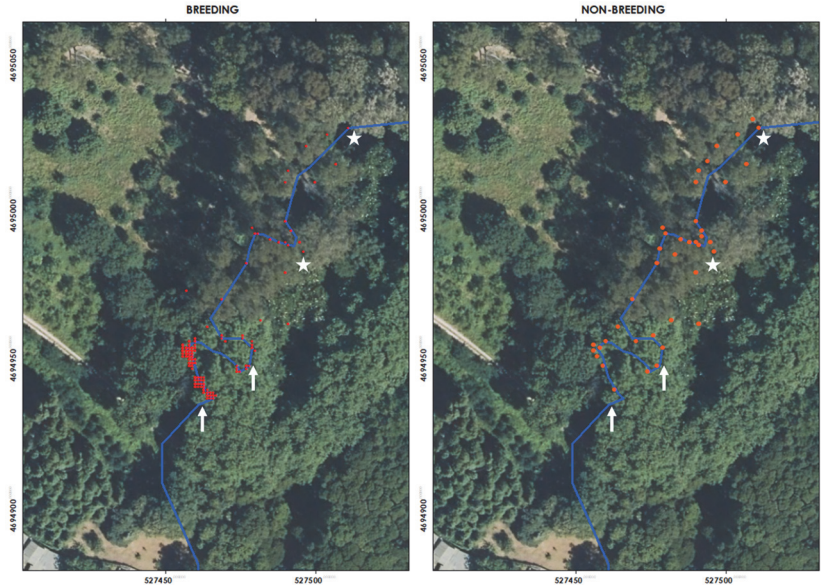


Figure 2: Intensity of space use by *Rana iberica* at the studied stream, as estimated by kernel distributions, during breeding (top) and non-breeding (bottom) seasons. For a proper visualization of the figure, the reader is referred to the online version of the article.

minimum temperature. The z-test value for this best fitting model ($z = -10.24$, 11 d.f., $P < 0.001$) indicates that *R. iberica* abundance is higher during periods of lower minimum temperatures (Fig. 3). The influence of other climatic factors was not selected as part of our models, which suggests that their effects on frog abundance is relatively unimportant compared to that of the mean daily minimum temperature (Table 1).

Rana iberica adults are supposed to have a secretive way of life, living close to the water edge, showing little diurnal activity and with reproductive activity during the night. However, our results suggest that this is not true for the studied population. Breeding behaviour was easily detected at daylight, especially in central hours of the day, unlike to what was reported by GALÁN-REGALADO (1982) and NÖLLERT & NÖLLERT (1995).

Our results suggest a relationship be-

Table 1: Results of the Poisson-log GLM models relating monthly abundance of *Rana iberica* adults to some climatic factors. Fifteen different model designs were tested including individual explanatory variables and interactions. All the statistical comparisons using the z-test are based on 11 degrees of freedom. The best fitted model judged by its AIC and AICc values is emphasized in bold. See text for detailed description of the variables.

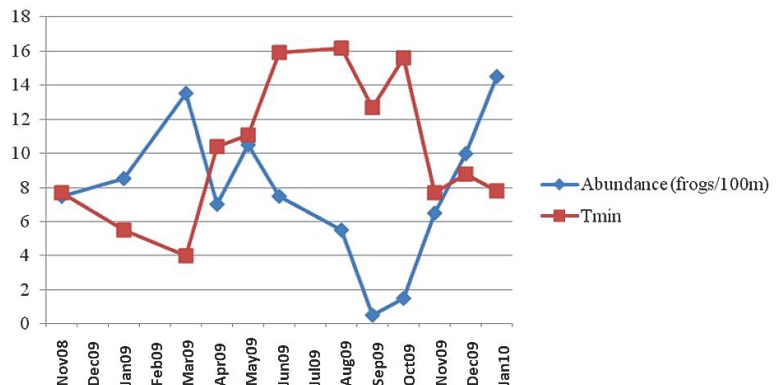
Model	z-test	AIC	AICc
Tmax	-5.46	444.90	453.2
Tmin	-10.24	364.27	372.58
Humidity	-1.45	473.55	481.83
Rainfall	6.64	434.80	443.11
T10cm	-8-80	395.81	404.12
Tmax x Tmin	-9.35	380.39	398.54
Tmax x Humidity	-7.16	423.43	441.97
Tmin x Humidity	-9.90	371.90	390.44
Tmax x Rainfall	5.86	443.45	461.99
Tmin x Rainfall	5.50	447.47	466.01
Humidity x Rainfall	6.68	434.38	452.92
Tmax x T10cm	-7.50	414.88	433.42
Tmin x T10cm	-9.70	372.90	391.44
Humidity x T10cm	-9.29	386.12	404.66
Rainfall x T10cm	5.48	447.29	465.83

tween activity peaks and low temperatures. It seems obvious breeding season happens during autumn and winter (November-March) in the study site. This could be related to increased water flow and water aeration during those months, and also to a strategy to avoid that eventual desiccation of the stream in summer happens before larvae complete their development.

The marked preference for the upper part of the stream could be explained by abiotic and biotic factors. That part of the stream presents high water flow, and in turn oxygenated water, as well as a low water depth. In addition, and probably associated with these characteristics, there is a low pressure from competitors or predators, as opposed to what happens in the low part of the stream, where the more lentic and deep waters stimulate the presence of marbled (*Triturus marmoratus*) and palmate (*Lissotriton helveticus*) newts, as well as of larvae of different dragonfly species, all of which could act as a predators or competitors of eggs and larvae of *R.iberica*.

It is noteworthy that, by using non-invasive techniques instead of the conven-

Figure 3: Monthly changes in *Rana iberica* abundance and in mean daily minimum temperature (Tmin) during the study period.



tional capture-mark-recapture protocols, which in amphibians use to be implemented through invasive procedures, it was possible to detect changes in habitat use, breeding areas and other ecological features (AYRES, 2008, 2009). The use of these data is essential for proper management of the stream, avoiding cleaning or drainage during critical periods of the life cycle of the species.

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