

# Life history of the European Common Frog (Rana temporaria) in the Pyrenees high mountain and the influence of temperature

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#### Abstract

We report an observation from a long-term study of low and high altitude populations of the European common frog (*Rana temporaria*, Linneaus, 1758) that shed light on the variation of adult frogs' numbers in wild populations during the post-breeding period as well as their biological cycle. Over a six-year period we identified the sex, considered two snout-vent-length sizes: sub-adults (1.6 - 3.9 cm) and adults ( $\geq$  4 cm), and characterized the biological cycle of 1704 frogs from our 600 km<sup>2</sup> study area consisting of lakes, ponds and wetlands. We considered two study areas low and high altitude (area A and B respectively). We placed two groups of temperature data loggers in both study areas, and together with bibliographical data we constructed the biological cycle of our populations studying the correlations between temperatures (maximum and minimum daily temperatures gathered fortnightly) and the postbreeding frog adult number during four annual time lapses (pre-wintering, wintering, breeding and post-breeding period). We found differences in the biological cycle of *Rana temporaria* between our two study areas. Indeed the number of post-breeding adult frogs depended on the mean minimum temperature during the breeding period in either low- or high-altitude populations, which might be a response to lack of food availability.

*Keywords*: *Rana temporaria*; biological cycle; Pyrenees; life history, mean minimum temperature.

#### Introduction

Previous studies have demonstrated ectothermic species especially from seasonally fluctuating environments show complex life histories (Wilbur 1980). So that some specific periods evolve into key factors in its dynamic and evolution constraining the viability of the species inhabiting these environments like our alpine populations of *Rana temporaria* (Fretwell, 1972; Enriquez-Urzelai, 2019). Due to the developmental stages sensitivity to a rapidly changing environment, the selection can facilitate specific stage adaptations promoting its viability, as specific thermal sensibilities and tolerances (McDermott et al., 2016). Other important factor in the life-history is the energy acquisition and expenditures necessary to accomplish among others the reproduction and survival.

In the reproduction case in energetic terms two strategies have been named capital and income breeders (Drent and Daan, 1980, Stearns, 1992, Jonsson, 1997), namely long-term storage and continuous energy intake without storage respectively. These strategies are not exclusive and they can show a high variation in a spectrum range from a pure capital to a pure continuous intake. Although the energy storage is costly for the ectothermic animals the effect is minimal. Indeed anurans breeding take place immediately after hibernation (Wells, 1977) like our study frog populations are adapted to use the long-term energy storage strategy, hence the survival during both the hibernation and breeding periods depends on the energy stored during the post-breeding period (Bonnet et al., 1998).

The European common frog is the most widespread anuran species of Europe inhabiting all kinds of permanent and temporary body waters from northern Spain to subarctic Fennoscandia, and all the way to the Ural mountains in the east (Gasc et al., 1997). Although other studies in *Rana temporaria* found migrating individuals (Montori, 1987), this is not the case in our study due to the pronounced natural geographical barriers.

In early studies, researchers tried to provide a foundation for further research of temperature effects on the development of biological cycles (van't Hoff 1884, Arrhenius 1915, Ludwig 1928, Powsner 1935, Harries and Douglass 1948, Wigglesworth 1972). In 1977 Sharpe and DeMichelle, designed a biophysical model to explain the relationship between developmental rates and temperature in poikilotherms. Since the degree-days approach presents some advantages such as minimal data for formulation, it has been widely used in various studies (Candolle 1855, Reibisch 1902, Sanderson and Peairs 1913, Arnold 1960, Baskerville and

Emin 1969, Abrami 1972, Allen 1976, Sevacherian et al. 1977; Beattie 1985; Diaz et al., 2008).

In this paper we have studied the temperature influence in the life cycle and post-breeding frog adult's numbers of *Rana temporaria* in both low- and high-altitude populations.

# MATERIAL AND METHODS

# 2.1. Area of study

Our study area in the Central Pyrenees consisted of high-mountain valleys that can only be reached on foot and are mostly free of contaminants (approx. 600 km<sup>2</sup>) where we studied the main lakes, ponds and wetlands, with the only noticeable human impact coming from hikers and the occasional camper. It is located from 0°42' W to 0°16' E and 42°53' N to 42°42' N (**Figure 1**).

Due to the importance of the isolation effect of the high mountain topography (**Figure 2**) we detailed the wetlands location, trekking kilometres and hours per year in our study area. We sampled the same 30 wetlands in July and August over a six year period (1997, 1998, 2001, 2013, 2014 and 2016). We followed the GR-11 trekking route to get to the different sampling sites: Formigal-Ibón de las Ranas (11 km, 6 hours trek), Guarrinza-Candanchu (20.8 km, 7.3 hours trek), Candanchú-Sallent de Gállego through the Canal de Izas (21.8 km, 8.4 hours trek), Candanchú-Canal Roya to Sallent de Gállego (22.2 km, 8 hours trek), Sallent de Gállego-Balneario de Panticosa (22.5 km, 11.2 hours trek) all of which totalled 88 km and 35 hours of trekking per year. In addition to this we trekked another 99 km to get to the different parts of the GR-11, which ultimately meant that we had completed a total of 181 km treks and 145 hours during each sampling year.

We grouped together the wetlands below 1.500m a.s.l (area A) and over 2.000 m a.s.l. (area B), low- and high altitude respectively to compare both study areas where the water surface was similar.

# 2.2. Animal Sampling

In total, we gently captured 1704 frogs with a fine net, measured and sexed them. Once the procedures were done we released them at the place of capture. We considered two SVL sizes: sub-adults (SVL 1.6- 3.9 cm) and adults (SVL  $\geq$  4 cm). We identified sex unambiguously using external morphological sexual characters (nuptial pads, size, and white

throat in males). We have only studied the adults that had already mated in the breeding period.

# 2.3. Temperature data

The temperature information was prepared using our data loggers (iButton) information from two data loggers groups (5-10 devices per location, **figure 1**) located around "1" at 1500m a.s.l. (42°49'16.3115"N to 0°19'31.5885" W) in area A, and "2" at 2157m a.s.l. (42°48'53.0686" N to 0°16' 52.5277" W) in area B. Every summer we downloaded the temperature recorded every four hours during the previous year. In area B we placed the data loggers in two environments above-and under-water.

# 2.4. Data analysis

Using our observations and data reported in previous studies from our study areas (Balcells 1955, 1956, 1957a, 1957b, 1975; Savage 1962; Montori and Pascual 1987), we constructed a diagram of the biological cycle of *R. temporaria* for our study areas (**Figure 1**). The percentage of the mean snowfall in both study areas was calculated taking as reference the maximum snowfall in the area B that reached 200 cm in February.

Because many studies reported the influence of environmental (air) temperature on the biological cycle of *R. temporaria* (Savage 1962, Beattie 1985) we examined correlations, positives and negatives, between different annual time-lapses' (pre-wintering, wintering, breeding and post-breeding), temperatures (maximum and minimum daily temperatures gathered fortnightly) and post-breeding frog adult number to test for its influence in our populations.

In order to test the influence of temperature along the biological cycle of *R. temporaria* we conducted a non-linear regression analysis to carry out five different correlation tests (linear, quadratic, logarithmic, cubic, and exponential) taking as the independent variable the total mean minimum and maximum temperature and post-breeding adult's numbers as the dependent variable for every annual time-lapse (pre-wintering, wintering, breeding and post-breeding) gathering both study areas (A and B) along six years, using the IBM SPSS Statistics 19.0 (SPSS, Inc., Chicago, IL) statistical package for Windows.



**Figure 1.** The figure displays the altitude profile of the trekking GR-11 route showing the position of the lake Ibón de las Ranas and the other sampled lakes and ponds along the route. Numbers 1, 2 and 3 represent the iButton data loggers' locations where temperatures were recorded along the study. In the bottom the biological cycles of Rana temporaria in both study areas. Panel A represents the study area located at 1500 m a.s.l. (e.g. Formigal) and panel B represents locations at 2200 m a.s.l. (e.g. Responuso). Purple (column 1) represents the juveniles stages, the red (column 2) represents sub-adults, light blue (columns 3 and 4) represents adults, Green (column 4) represents tadpoles, orange (column 4) represents clutches and dark blue (column 4) represents the hibernation period. Column 5 represents the months and column 6 represents the mean snow height in centimetres. This diagram was elaborated based on data from our samplings and Balcells (1975). Light red squares represent the breeding periods where the frog adults recently emerged from hibernation are susceptible to die if they do not find food. Images representing the five annual time-lapse in the study area B (high-altitude) are present in the last column on the right hand side



Figure 2. Panoramic view showing landscape complexity and isolation of high-elevation study area (B).

#### RESULTS

#### Biological cycle in areas A and B

When comparing bibliographic data (Balcells E. 1955, 1956, 1957a, 1957b, 1975), and personal previous observations from our two study areas, we found a significant data difference between the biological cycles of *R. temporaria*. In low-elevation populations (Area A, 1500m a.s.l), sub-adults were seen as early as the second week of March with the presence of juveniles and adults being observed from the second week of May until November. Conversely, clutches and tadpole sightings increased during the month of May but declined during the second week of June. From this, it was deduced that the hibernating period for this population ranged from the second fortnight of November to the first fortnight of March, four months. The breeding period ranged from the second fortnight of March to end of April, the post-breeding from May to the end of August and the pre-wintering period from September to the first fortnight of November (**Figure 1A**).

On the other hand, high-elevation population (Area B, 2.000m a.s.l.) sightings varied greatly with the presence of adults being greatest from May to October and juveniles being observed later in July. Similarly, sightings of tadpoles were high in July but declined in August, whereas, clutches were present from May to June and sub-adults from June to October. From these sightings, it was deduced that the hibernation period for high elevation populations occurred over an eight month period from October to May, the breeding period ranged from the second fortnight of May to the end of June, the post-breeding period from July to the end of August, and the pre-wintering period from September to October (**Figure 1B**).

The snowfall also differed in both study areas, in area A the snowfall started in the second fortnight of November until the first fortnight of Mars, whereas in area B the snowfall started in the second fortnight of November until the second fortnight of June. Both areas showed their maximum percentage of snowfall in February. In area B the snow was persistent during most of the breeding period.

#### Temperature and its relationship with frog adults' numbers in the post-breeding period

The total mean maximum temperature did not show a significant result. Whereas the total minimum temperature from the breeding period showed only a significant correlation (exponential) with the post-breeding frog adults' number (Beta= 0.993, P=0.000) (**Table 1**).

The mean monthly temperatures above- and under-water along the six-year period study showed differences. In high-altitude the mean monthly environmental temperatures were lower than the under-water along the biological cycle reaching the maximum differences during the post-breeding period up to 17 °C (**Figure 3**).

**Table 1.** Standardized coefficients of non-linear regression analysis (exponential correlation). We used the total mean minimum temperature as the independent variable and post-breeding adult's numbers as the dependent variable.

Year	Sampling area	Post-breeding adults	Total prewintering period mean minimum temperature (°C) (from the previous year)	Total wintering period mean minimum temperature (°C)	Total breeding period mean minimum temperature (°C)	Total post-breeding period mean minimum temperature (°C)
1997	A Low elevation	13	1.89	1.76	1.62	6.81
1998	B High elevation	97	6.27	3.48	4.17	8.30
2001	B High elevation	349	3.99	-3.02	5.64	8.29
2013	A Low elevation	7	6.18	-1.24	1.15	9.94
2014	A Low elevation	158	6.73	0.20	5.03	10.26
	B High elevation	42	4.63	-0.04	3.15	7.37
2016	A Low elevation	11	6.83	0.00	1.91	7.76
	B High elevation	23	2.41	-3.11	2.26	7.63
Total		700				
Beta *			0.093	-0.276	0.993	0.211
Sig			0.827	0.509	0.000	0.616



**Figure 3.** Graphic of mean monthly temperatures along the six-year period studied in Area B, both above- and under-water along the frogs' life-cycle. Ibon de las Ranas picture detailing data loggers' locations.

# DISCUSSION

The breeding season is a physically demanding period when the food consumption is greater in larger animals, females and breeding individuals (Duellman and Trueb, 1994), thus the adult frogs need a high input of energy which makes them in this sense more sensible to food availability variations when compared to sub-adults. Our studied *R. temporaria* populations mainly feed on beetles (44.9%), two-winged flies (12.9%), bees, wasps and ants ... (11.4%) (Rodriguez Vieites et al., 1997). But as the mating males are mainly focused on mating, they spend most of their time searching for the opportunity to mate, even using different strategies, among them the clutch piracy (Vieites et al., 2004). Indeed they did not be able to feed due to the snowfall prevalence in high-land populations (B) until the second fortnight of June, which did not allow the presence of their prey. At the end of June, overlapping with the end of the breeding period, usually the snow melted and it was concentrated in snow patches where the frogs could find insects around the borders.

In low-land populations (A) the snowfall prevalence lasted until the first fortnight of Mars, and as the mean minimum temperatures were low and the frogs' preys were not yet present.

Consequently the mean minimum temperatures during the breeding period were decisive for the prey presence essential to the adult frogs after mating. Subsequently the frog adults may die at the end of the breeding period due to starvation.

The sub-adults were present after the adults breeding period, which could be explained due to the risk of being considered an energy source for adults (cannibalism). At the same time their preys were already present in this period (post-breeding) so they guaranteed their food sources. We have never found sub-adults died by starvation. Therefore they would become adults for the next breeding season assuring the next breeding adults generation.

This life cycle allowed maintaining the balance between sub-adults and adult frogs' numbers independently of the annual atmospheric variations. On the contrary, if a climate change produced a variation in the snowfall in quantity and permanence time may produce an imbalance in this life cycle and led to a fast amphibian's extinction rate.

We found that the biological cycle of *R. temporaria* differed between our two study populations (A, B). The high-land (B) population's biological cycle was shorter and frogs had to accomplish each stage of their life cycle quickly in order to survive their environmental conditions, mainly the snowfall that covers all the land acting as a barrier isolating and limiting the frogs' activity. Whereas in low-land populations (A), the biological cycle was not as constrained as in upland, and frogs had more time to go through developmental stages,

reproduction and post-breeding period, whereas the hibernation period was shorter and the breeding period started earlier. These substantial differences have similarly been reported in other *R. temporaria* populations (Beattie, 1987; Magnin, 1980; Ryser, 1996; Miaud, 1999; Laugen et al., 2003).

In both areas the adult frogs ended hibernation when the snow level in lakes and wetlands starts melting. Our study populations spent the hibernation inside the spaces underneath the shore and under the mood in the lakes bed. When the wintering period finished although the adult frogs were at a very low lipid content they straight went for breeding before searching for available food. Another specific characteristic of our populations is that they sang alone not chorus were found during breeding. Indeed our high mountain frog populations naturally sang under-water during the breeding and post-breeding seasons although their release call can be induced above- and under-water (Palanca-Castan et al., 2019; Miramontes-Sequeiros et al., 2019).

Once the mating was finished the frogs left the water and went feeding not far from their location during daylight and coming back to the water to spend the night since the water temperature was higher than the air temperature (up to 17°C). As soon as the clutch is laid and fertilized the adult frogs focus on feeding themselves and if they did not find sufficient available food they might die and we found their skeletons close and in the wetlands bed. Our results point out with no doubt and with a full probability that the mean minimum temperature during the breeding period might be responsible for adult frogs' numbers during the post-breeding period in both study areas which is supported by their biological cycles we

# described in the interpretation of our data.

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**Supplementary tables.** Summary tables for statistical tests. We used the IBM SPSS Statistics 19.0 (SPSS, Inc., Chicago, IL) statistical package for Windows.

**Supplementary Table 1.** Data summary of sampled adults and sub-adults of *Rana temporaria* in low- and high-elevation locations after the breeding season (both during the month of August), sexed adults and adults SVL size (SVL  $\geq$  4cm). SD is the Standard Deviation.

Year	Sampling area	Post- breeding adults	Post- breeding sub- adults	Total	Sex of post- breeding adults		Post-breeding adults SVL					
					female	male	female	mean	SD	male	Mean	SD
1997	Low elevation	13	20	33	4	9	4	8.00	0.75	9	7.53	0.55
1998	High elevation	97	99	165	20	77	20	4.64	0.69	46	4.80	0.53
2001	High elevation	349	523	872	119	230	119	6,87	52.00	230	6.58	0.46
2013	Low elevation	7	10	17	2	5	2	5.00	0.00	5	7.00	0.25
2014	Low elevation	158	237	395	47	111	47	5.97	1.21	111	6.21	0.65
	High elevation	42	63	105	12	30	12	4.44	0.13	30	6.06	0.60
2016	Low elevation	11	18	29	4	7	4	5.97	1.21	7	7.40	0,42
	High elevation	23	34	57	7	16	7	7.77	0.83	16	6.64	0.63
Total		700	1004	1704	215	485						

**Supplementary Tables 2, 3 and 4.** Model summary for the curve estimation regression statistics and related plots. We used the total mean minimum temperature as the independent variable and post-breeding adult's numbers as the dependent variable. DF are the degrees of freedom.

Model Summary							
R	R Square	Adjusted R Square	Standard Error of the Estimate				
0.993	0.987	0.985	0.173				

	Unstandardized Coefficients		Standardized Coefficients		
	В	Standard Error	Beta	t	Sig.
Total Mean minimum temperature in the critical period	0.832	0.039	0.993	21.215	0.000
(Constant)	2.903	0.397		7.316	0.000

ANOVA								
	Sum of Squares	df	Mean Square	F	Sig.			
Regression	13.454	1	13.454	450.094	0.000			
Residual	0.179	6	0.030					
Total	13.634	7						