Effects of temperature on embryonic and larval development and growth in the natterjack toad (*Bufo calamita*) in a semi–arid zone

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Abstract

*Effects of temperature on embryonic and larval development and growth in the natterjack toad (*Bufo calamita*) in a semi–arid zone.—* Temperature affects the duration of embryonic and larval periods in amphibians. Plasticity in time to metamorphosis is especially important in amphibian populations of Mediterranean semi–arid zones where temperatures are high and precipitation is low, increasing the rate of pond desiccation. In order to test the influence of water temperature on the larval development and growth of the natterjack toad (*Bufo calamita*), we collected two spawns in a semi–arid zone at Balaguer (Lleida, NE Iberian peninsula). Approximately 50 (± 10) eggs (stage 14–16) were raised in the lab at different temperature conditions: 10, 15, 20, 22.5 and 25ºC with 12:12 photoperiod. The results show a lengthening of development time with decreasing temperatures and a better survival performance of *B. calamita* to high temperatures. However, mean size at metamorphosis was not different across treatments, thus, suggesting that this population of *B. calamita* requires a minimum size to complete the metamorphosis. This study is the first approach to examine the effects that climatic factors have on the growth and development of *B. calamita* in semi–arid zones.

Key words: Amphibians, Development, Growth, Semi–arid zone, Temperature.

Resumen

*Efectos de la temperatura sobre el desarrollo y crecimiento de embriones y larvas en el sapo corredor (*Bufo calamita*) en zonas semiáridas*.— La temperatura afecta la duración de los períodos embrionarios y larvarios de los anfibios. La plasticidad en el momento de la metamorfosis es especialmente importante en las poblaciones de anfibios del Mediterráneo y de zonas semiáridas donde las temperaturas son elevadas y las precipitaciones son escasas, lo que incrementa la desecación de las charcas. Con el fin de comprobar la influencia de la temperatura del agua en el desarrollo de las larvas y crecimiento de la sapo corredor (*Bufo calamita*), se recolectaron huevos de dos puestas procedentes de una zona semiárida en Balaguer (Lleida, NE península ibérica). Aproximadamente unos 50 (± 10), huevos (etapa 14–16) se sometieron en el laboratorio a diferentes condiciones de temperatura: 10, 15, 20, 22,5 y 25ºC con un fotoperíodo 12:12. Los resultados muestran un alargamiento del tiempo de desarrollo con la disminución de las temperaturas y una mejor supervivencia de *B. calamita* a temperaturas mas altas. Sin embargo, el tamaño medio de los ejemplares metamórficos no fue diferente entre los tratamientos, lo que sugiere que esta población de *B. calamita* requiere un tamaño mínimo corporal para completar la metamorfosis. Este trabajo es un estudio preliminar para examinar los efectos que los factores climáticos tienen en el crecimiento y el desarrollo de *B. calamita* en las zonas semiáridas.

Palabras clave: Anfibios, Desarrollo, Crecimiento, Zonas semiáridas, Temperatura.

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Introduction

Populations of ectotherm animals have a strong dependence on ambient temperature because they do not have an efficient mechanism for physiological thermoregulation (Brattstrom, 1963). Climatic variation is an important selective factor for life history trait differentiation, so populations of ectotherms are expected to diverge in their thermal optima for development and growth if they are exposed to different temperature environments. Amphibians have been widely used as model systems for the study of physiological ecology and temperature adaptation due to the easy manipulation of tadpoles in control experiments (Feber & Burggren, 1992).

In temporary ponds, time to metamorphosis is influenced by temperature and duration of the larval stage is highly plastic, especially in semi–arid environments where rainfall and pond duration are unpredictable (Newman 1989; Tejedo & Reques, 1994). In these environments, larvae need to accelerate metamorphosis when ponds dry out and delay the process when ponds dry later in the season. When pond duration is extremely short larvae should be the most adaptive strategy. It would be to express not a plastic but a quick canalized development (Tejedo & Reques, 1994). Predation and pond desiccation have been identified as major causes of larval mortality in permanent and transient aquatic environments, respectively (Brockelman, 1969; Calef, 1973; Smith, 1983; Newman, 1987).

In anuran populations, larger metamorphs may exhibit higher terrestrial survival since they can cope with different stressors such as predators and desiccation (Reques & Tejedo, 1997; Altwegg & Reyer, 2003). Smith–Gill & Berven (1979) considered that low temperatures retard differentiation more than growth and increase the stage–specific size. Therefore, larvae growing at cold temperatures have prolonged developmental periods but they may increase their size at metamorphosis. This phenomenon is considered a general rule for ectotherms (Atkinson, 1994, 1996). This trade–off in fitness, mediated by the influence of temperature, largely conditions the expression of an optimal phenotype at metamorphosis in amphibians and other aquatic organisms with complex life cycles (Etkin, 1964; Smith–Gill & Berven, 1979; Semlitsch et al., 1988; Hayes et al., 1993).

In this study, we examined the influence of temperature on the development and growth of the natterjack toad (*Bufo calamita)*. The study area is a semi–arid zone with a marked deficit of precipitation and high temperatures. Previous studies of the species in the study zone suggest that *B. calamita* adults (Miaud et al., 2000) presents different terrestrial behaviour with respect to other natterjack populations in other areas of Europe. In this study we focus on the response of natterjack larvae to increasing temperatures and analyze their metamorphic response.

Material and methods

The study area (Balaguer, 41° 46' N, 0° 46' E) is a semi–arid zone (Conesa et al., 1994) located in an agricultural setting of winter cereal fields. Mean annual temperature is about 14.5°C (January = 0.2°C and July = 32.2°C) and the mean annual rainfall is 400 mm (Balaguer meteorological station). The soil is poor and contains a high proportion of clay and lime. The evapo–transpiration is 805 mm (Thornthwaite & Mather, 1955). All these factors determine a harsh terrestrial environment with a particular flora and fauna that can tolerate such conditions (Conesa et al., 1994). As suitable breeding habitats for natterjack are temporary, tadpoles have to develop in a few days to complete the metamorphosis.

We examined the effects of temperature on size and time to metamorphosis in the natterjack toad, *Bufo calamita* (*Epidalea calamita* according to Frost et al., 2006), a common amphibian species in the study area. This species breeds in seasonal ponds. Breeding activity can occur throughout the year after rainfalls, except in the colder months, with an explosive peak in the periods of more intensive rains (Sanuy, unpublished data).

Two egg clutches were collected (approximately 100 eggs/spawn) in a temporal pond in the study area. At the lab where they were placed in a controlled temperature chamber (with an error of ± 0.5ºC) and immediately exposed to five different temperatures under a constant 12 hours light / 12 hours dark photoperiod. The temperature treatments were: 10, 15, 20, 22.5 and 25ºC. At the start of the experiment eggs were near Gosner stage 16 of development (14–16; Gosner, 1960). Approximately 50 eggs (± 10 eggs) from each spawn were raised in plastic recipients containing 1.3 L of dechlorinated water. Each spawn was replicated twice per experimental temperature camera. Larvae were fed fish food and the excess was removed daily. Water was changed every three days.

During the experiment the stage of development was checked and photographed every 12 hours. The total body length (BL) of each larva was estimated using the program corelDRAW 11. The stage of development and BL of larvae in each sample were measured on days 1, 5 and 19 of the experiment. Stage–size was measured for each spawn at each temperature condition, at stages: 16, 25 and 46 (Gosner, 1960). Due to development time differences between individuals of the same spawn, the developmental stage was considered to have changed when 70% of larvae in the same sample had reached a particular stage. After the experiment was completed (experimental time: 150 days), the surviving larvae were returned to the pond where they were collected.

The relationship between the stage of development (dependent variable) and time at each temperature and for each spawn was analysed using two–way ANOVA (PROC GLM version 9 SAS). The same type of analysis was also used to measure stage–size and the relationship between size and

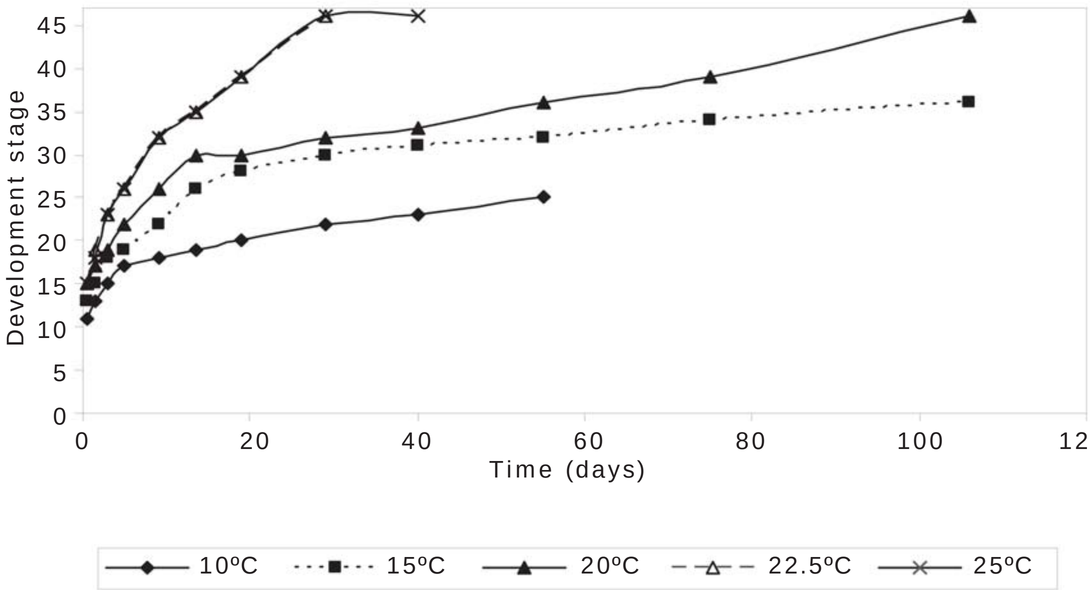

Fig. 1. Effect of temperature on *Bufo calamita* larval development (Gosner stages; Gosner, 1960).

Fig. 1. Efecto de la temperatura en el desarrollo larvario de Bufo calamita *(estadios de Gosner; Gosner, 1960).*

time. The significance level was set at µ = 0.05. All data are expressed as mean ± SD.

Results

Development time and survival.

Development time increased with decreasing temperature (fig. 1). Developmental stage differed for each temperature condition (5 day: F = 71.20; p < 0.001, 19 day: F = 339.5; p < 0.001) and spawn (only in the 5 first days; F = 0.20; p < 0.001). Similarities in development were only observed between 22.5ºC and 25ºC (post hoc Tukey test, 5 day = p = 0.99, 19 day = 0.99), with Individuals in both temperatures metamorphosing in 23 days (stage 42). The coldest temperature treatment (10ºC) had a negative effect on survival: tadpoles stopped development at stage 23 (55 days), dying in 10 days. In the 15ºC treatment tadpoles stopped developing at stage 35 (90 days) and they survived without further development until the last day of the experiment. The larval period of tadpoles reared at 20ºC was on average 88 days (stage 42).

Size at development stage

The size increased at each development stage. We found differences in the total size between temperatures at stage 25 (fig. 2, table 1). In stage 39 tadpoles attained the maximum body size and we found differences between 20, 22.5 and 20 and 25 temperature treatments but not between 22,5 and 25ºC (table 2). However, all individuals completed the metamorphosis with a similar size (7.6 ± 1.2 mm; F = 0.08; p = 0.92) across temperature treatments.

Discussion

Temperature affects development and growth and is an important factor for climate selection (Berven & Gill, 1983). As expected, in our study, development time increased with decreasing temperature. Development time is very important for *Bufo calamita* in the study area because the breeding sites are temporary ponds with a high risk of desiccation due to high water temperatures and shallowness. Pond temperature plays a major role in determining the duration of the larval period in amphibians (Loman, 2002). Our results support other laboratory studies along these lines that have shown the effects of lowered water level on anuran development, presumably an adaptation to survival in drying ponds (Tejedo & Reques, 1994; Loman, 1999; Ryan & Winne, 2001). If pond drying increases temperature, our findings could be considered the result of drying effects. However, the analysis of effects of pond drying is complicated

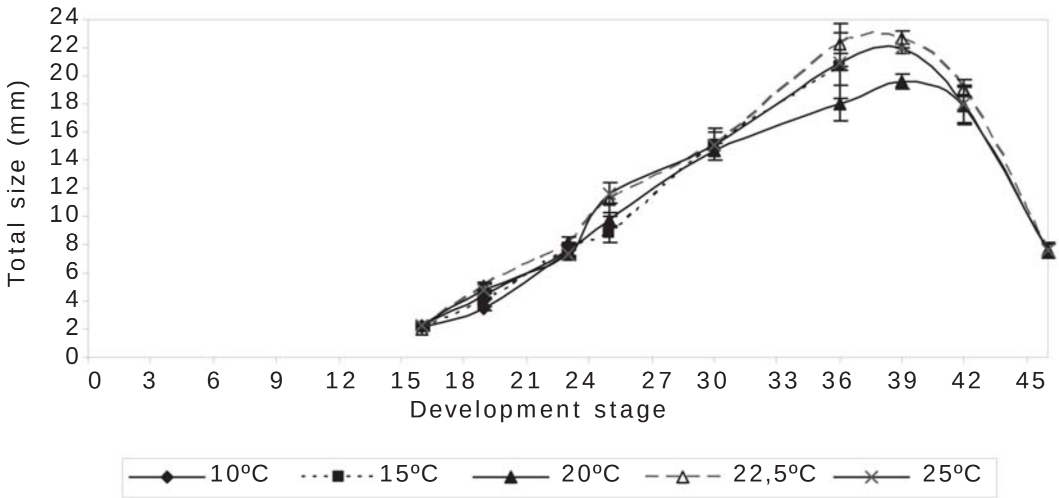

Fig. 2. Effect of temperature on larval size variation across developmental stages in *Bufo calamita.*

Fig. 2. Efecto de la temperatura sobre la variación del tamaño de las larvas, a través de todas las fases de desarrollo de Bufo calamita.

Table 1. Effect of temperature on the total size at stage 25 (Gosner, 1960). Two–way factorial ANOVA.

Tabla 1. Efecto de la temperatura sobre el tamaño total del estadio 25 (Gosner, 1960). ANOVA factorial bidireccional.

Source	df	*F*	*P*
Temperature	3	10.11	0.0017
Spawn	1	0.87	0.3710

Table 2. Differences in maximum size reached due to temperature conditions at stage 39 (Gosner, 1960): PT. Post hoc Tukey test; T. Temperaure; P. Probability.

Tabla 2. Diferencias en el tamaño máximo alcanzado en la fase 39, debido a las condiciones de la temperatura (Gosner, 1960): PT. Test post hoc de Tukey; T. Temperatura; P. Probabilidad.

PT		T	*P*
T	LS mean	15 *vs* 20	0.3746
		15 *vs* 22.5	0.0074*
15	8.7792	15 *vs* 25	0.0024*
20	9.7645	20 *vs* 22.5	0.1201
22.5	11.2080	20 *vs* 25	0.0372*
25	11.6255	22.5 *vs* 25	0.8891

because this factor interacts with others, such as pond temperature and tadpole density (Loman, 2002). Other authors, studying *B. calamita*, did not find evidence in the response to desiccation *per se*, suggesting that development may differ under physiological constraints in relation to habitat types (Brady & Griffiths, 2000).

Laboratory studies have shown a pattern in which tadpoles develop and grow more slowly but metamorphose with a longer body size at low temperatures (Etkin, 1964; Smith–Gill & Berven, 1979; Hayes et al., 1993). This result was not found in our study. Individuals raised at different temperatures attained metamorphosis at different times but contrarily to expected, size at metamorphosis was similar for all the treatments. Therefore, higher temperature treatments determined both higher developmental rates and higher growth rates. All individuals that reached metamorphosis had the same body size. These results suggest that the population of *B. calamita* in the study zone requires a minimum size to complete metamorphosis.

Metabolic enzyme systems present high temperature sensitivity in an optimal range so growth and development can only proceed within a thermal window (e.g. Randall et al., 1997). The optimal temperature seems to be the higher temperatures (25 and 22.5ºC) as tadpoles reached metamorphosis in a shorter time and with larger size, thus with potential higher fitness. The results of this study do not allow us to determine higher limits of temperature tolerance for the species. The air temperatures in the study area can reach maximums of about 40ºC in summer, considerably higher than the maximum temperature used in our experiments. We cannot therefore determine a critical thermal maximum (CTM). On other hand, the minimum temperature that tadpoles needed to reach metamorphosis was 20ºC, which is a higher minimum temperature compared to the temperature tolerance in other anuran species in Europe (for example: *Rana arvali* Loman, 2002; *Rana temporaria* Laugen et al., 2003a, 2003b). Tadpoles attained a maximum size at stage 39 and decreased thereafter due to metamorphosis. The different sizes of larvae between temperatures at this stage, larger at higher temperatures, could be explained by the fact that the metabolism of growth factors is affected by temperature (Álvarez & Nicieza, 2002). Higher temperatures are well known to accelerating larval growth and development (Hayes et al., 1993). Growth rate depends on development rate, and both are functions of circulating hormone levels, which are in turn dependent upon the stage of differentiation reached. Nevertheless, growth is related to the duration of larval development (e.g. Wilbur & Collin, 1973) because conditions that are favorable for differentiation often are also favorable for growth (Smiths–Gill & Berven, 1979).

In conclusion, this study represents a first step towards determining the role of climatic factors in growth and development rates that are increased in response to the time constraint. One limitation of the study is that tadpoles can actively thermoregulate by moving between shallow and deep water, a fact that was not taken into consideration in this study. Nevertheless, the results suggest that temperatures below 20ºC are too low to allow normal tadpole development and growth, while higher temperatures could favour growth and developmental conditions. In further studies it would be interesting to examine the existence of adaptive plasticity in development and growth rates in response to pond drying, as well as investigate whether a process of local adaptation is emerging in the study area.

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