# Is the Pyrenean newt (*Calotriton asper*) a thermoconformer? Cloacal and water temperature in two different thermal periods in a Pre-Pyrenean stream population

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In ectothermic animals, heat seems to be a determining factor because it influences many vital activities such as locomotion, the ability to escape, feeding, and reproduction, among others. In aquatic environments, physical characteristics of water prevent small ectotherms from thermoregulating and therefore it is expected that their body temperature remains similar to water temperature. Throughout its distribution and annual cycle, the Pyrenean newt (Calotriton asper) is exposed to a wide range of water temperatures that affect its biological and ecological traits like the standard metabolic rate, oxygen consumption, activity period and growth pattern. This study analyses the relationship between the cloacal and water temperatures in a Pre-Pyrenean population of C. asper in two periods with well differentiated water temperatures (July and September). The aims are to establish if there are differences between sexes in cloacal temperature, whether reproductive activity modifies cloacal temperature, and to analyse the degree of thermoconformity of the species. The results indicate that cloacal temperature depends on the water temperature, corroborating the idea that C. asper is mainly a thermoconformer, as it corresponds to an aquatic ectotherm. However, the cloacal temperature of newts was slightly higher than water temperature. In individuals in amplexus, cloacal temperature was significantly higher than in specimens that are not in this mating position, and in July, females showed much greater dispersion in cloacal temperature than males, which is probably related to spawning behaviour.

*Key words:* aquatic environment; *Calotriton*; cloacal temperature; ectotherms; newts; Salamandridae.

The maintenance of constant body temperatures by endotherms is partly due to the intrinsically high metabolic rates of their tissues (HULBERT & ELSE, 2000), and also due to the existence of homeostatic mechanisms (ANGILLETTA *et al.*, 2010). In contrast, ectotherms have lower metabolic rates with high rates of heat loss. Metabolic heat production therefore exerts little influence on body temperature (HUTCHISON *et al.*, 1966). The body temperature of ectothermic organisms is primarily determined by the heat obtained from the surrounding habitat and so they depend on environmental heat to maintain their physiological functions such as locomotion, the ability to escape, feeding, and reproduction. Thermoregulation in terres-

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trial amphibians (BRATTSTROM, 1963) is behavioural (emergence, retreat, selection of temperatures or basking, among others) and physiological (acclimation or evaporative cooling, among others). In aquatic newts, body temperatures usually are in accordance with the temperature of the water bodies where they are found, but they can absorb a small amount of heat, especially in shallow waters, from the pond substrate (BRATTSTROM, 1963). However, in strict stream dwelling newts like *Calotriton* species the thermoconformity with water temperature seems the most effective strategy.

The Pyrenean newt Calotriton asper mainly inhabits streams with cold and well oxygenated waters at medium and high altitude in the Pyrenean range. It can occasionally be found in lakes, wells and underground courses. The species is active in a range of water temperatures between 4° C and 17°C. This cold and relatively wide range affects its biological and ecological traits, such as standard metabolic rate (SMR), oxygen consumption, activity period, and morphometry, among others (Clergue-Gazeau, 1987; Serra-Cobo et al., 2000; TROCHET et al., 2018, 2019; DELUEN, 2022; DELUEN et al., 2022). At high altitude, the yearly window of favourable temperatures for reproduction is reduced to practically two months (CLERGUE-GAZEAU, 1983), while in the populations at medium or low altitude the range of optimal temperatures extends much longer, or may be temporarily reversed (Clergue-Gazeau, 1983, 1987).

Because the basal metabolism (SMR) varies with temperature (Deluen, 2022), it is generally accepted that ectotherms maximize their metabolism and their biological

functions by thermoregulating in the most favourable areas (ANGILLETA et al., 2002; Angilletta, 2009; Balogová & Gvoždík, 2015). Calotriton asper, despite being active also during the day, tends to avoid the sunniest areas of the streams and selects mainly the shaded sections (DESPAX, 1923; Montori & Herrero, 2004). This behaviour can be easily observed both in the field experimental and under conditions (TROCHET et al., 2018; DELUEN, 2022). The physical characteristics of water (specific heat and thermal conductivity) prevent the body temperature of small ectotherms from being very different from the water temperature. Consequently, most aquatic ectotherms are thermoconformers (BALOGOVÁ & GVOŽDÍK, 2015; PIASEČNÁ et al., 2015) despite being able to thermoregulate behaviourally (Hutchison & Dupré, 1992; DALIBARD et al., 2022; BALOGOVÁ & Gvoždík, 2015), or even to increase their body temperature from metabolic heat, as it occurs other in ectotherms (BROWN & AU, 2009). Then, certain plasticity of the basal metabolism in C. asper described by GUILLAUME et al. (2020) and DELUEN et al. (2022) could slightly increase body temperatures.

In this study I analysed the relationship between the cloacal and water temperature in two periods (July and September) with well differentiated water temperatures. The aims of the study were to (i) establish whether cloacal temperatures differ between sexes, (ii) evaluate whether reproductive activity modifies cloacal temperature, (iii) analyse the degree of thermoconformity of the species, and (iv) test if variation in SMR could slightly modify body temperature as a consequence of the metabolic plasticity described in previous studies.

#### MATERIALS AND METHODS

Sampling was carried out in the Pi stream, located on the north slope of Serra del Cadí (1225 m asl; geographic coordinates ETRS89: 1.753987°E - 42.333520°N). On July 20, 1994, 31 adults (17 males and 14 females) and on September 20, 1995, 40 adults (23 males and 17 females) were captured by hand. All captures were made in the same stretch in both periods with active searching. The length of the sampled section of the stream was 50 m. The following variables were recorded for each captured individual: sex, snout-vent length (SVL in mm with a calliper of 0.01 mm of accuracy), cloacal and water temperature at the capture site (with Tempecon 98 thermometer with type K thermocouple of 0.5 mm diameter and accuracy of 0.1°C), newt activity (in amplexus, active, hidden), stream depth, distance from the margin, shaded or sunny area and the characteristics of the capture site (waterfall, gorge, shallow pond, margin, backwater and uncovered or under stone). All captured specimens were released immediately at the same capture site.

Cloacal temperature was measured immediately after capture to avoid changes in the newt's body temperature due to handling. Throughout the study it was assumed that cloacal temperature could be assimilated to body temperature. The sex ratio was estimated as the ratio of the number of males relative to the total number of individuals [ $\sigma\sigma/(\sigma\sigma+QQ)$ ] following the criteria of WILSON & HARDY (2002). In order to test for thermoconformity I per-

formed ordinary least squares regression considering body temperature as a response variable and calculated Pearson's correlation coefficient. In addition, the association of water and cloacal temperatures for each capture at different months and for different sexes was tested using Wilcoxon Matched Paired tests. The difference between sampling periods in the collected environmental variables (distance of the newts from the water body margins, depth they were found, occurrence in sunny or shaded areas, and water body type) variables were tested with one-way analyses of the variance (ANOVAs). To test how newt SVL, as well as water and cloacal temperatures, differed between sampling months and within sexes, one-way ANOVAs were also performed. All analyses were run with Statistica 10.0 software.

#### Results

The average stream width in the sampling section was 148.2 cm (range 98-240 cm). Most of the individuals were located near the margin and mostly in shaded areas at a maximum depth of about 30 cm (Table 1). No significant differences between sampling months were found in the distance of the newts from the margin, the depth they were located, the relative frequency of occurrence in sunny or shaded areas, or the water body type (Table 1).

Ten amplexa were observed (three in July and seven in September), which altogether represent 28.1% of the captures. The sex ratio ( $\sigma\sigma/(\sigma\sigma+QQ)$ ) was in both periods favourable to males (0.55 in July and 0.58 in September). No significant differences were found in body length between males and females, either considering all sam-

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		July				September			
		n	Mean	SD	Range	n	Mean	SD	Range
SVL (mm)	All	31	60.71	4.56	52.80-68.20	40	60.72	4.22	53.10-68.90
	Males	17	60.51	4.78	52.80-68.20	23	61.29	4.08	53.10-68.90
	Females	14	60.96	4.45	54.70-67.10	17	59.95	4.40	53.20-67.10
Water temperature (°C)		31	13.37	0.22	13.00-13.90	40	9.44	0.14	9.20-9.65
Cloacal temperature (°C)	All	31	14.65	1.08	13.40-17.70	40	10.12	0.59	9.40-12.20
	Males	17	14.31	0.58	13.70-15.50	23	9.93	0.49	9.40-11.60
	Females	14	15.05	1.40	13.40-17.70	17	10.38	0.62	9.70-12.20
Depth (cm)		31	11.92	7.16	0.00-31.00	40	10.93	7.29	2.00-30.00
Distance to margin (cm)		31	32.94	26.92	0.00-110.00	40	41.08	28.89	5.00-120.00
Insolation (shadow = 1; sunny = 2)		31	1.29	0.46	1.00-2.00	40	1.28	0.45	1.00-2.00

**Table 1:** Descriptive statistic (sample size, mean, range and standard deviation) of biometrical (SVL = snout-vent length), temperature and environmental variables analysed per month.

pled newts together or considering the two periods separately.

Cloacal temperature of newts and water temperatures at the capture site were significantly different for both sexes and periods (Wilcoxon Matched Pairs test:  $T_{males-july}$ : z = 3.62; p < 0.001.  $T_{females-july}$ : z = 3.29; p < 0.001.  $T_{males-setember}$ ; z = 4.20; p < 0.001,  $T_{females-september}$ : z = 3.62; p < 0.001). The water and cloacal temperature showed significant differences between the two periods (F<sub>water(1,69)</sub> = 8743.0; p < 0.001; F<sub>cloacal</sub> (1,69) = 506.90; p < 0.001). No significant differences were obtained between the cloacal temperature of males and females in July (F<sub>1,29</sub> = 3.9000, p = 0.0579), while there were significant differences in September (F<sub>1,38</sub> = 6.5777, p = 0.014) (Fig. 1). In both periods the cloacal temperatures of females were higher than those of males (Table 1).

During both sampling periods, the clo-

**Figure 1:** Cloacal temperatures of male and female *Calotriton asper* and water temperature in the two sampling periods. Diamonds indicate mean values and bars indicate 95% confidence interval of 95%.



**Figure 2:** Cloacal temperatures of *Calotriton asper* individuals depending on whether they were in amplexus or not during the September sampling and water temperature. Diamonds indicate mean values and bars indicate 95% confidence interval.



acal temperature of individuals in amplexus was higher than the temperature of those newts that were not displaying this behaviour at the moment of the capture. However, while in July the differences were not significant, in September the cloacal temperatures of the individuals was significantly different as a function of their status in amplexus or not ( $F_{1,38}$  = 12.826, p = 0.00096). The newts in amplexus had an average cloacal temperature about 0.6°C higher than that of those who were not in amplexus (Fig. 2). No significant differences in cloacal temperatures were observed between males and females in amplexus, although the average cloacal temperature of females in amplexus was slightly higher than that of males.

The analysis of the relationship between the cloacal and water temperatures per sex and study period revealed that only for the males in September there was a significant correlation between the cloacal temperature and water temperature ( $R^2$ = 0.277, p = 0.0289), although with a very small coefficient. The dispersion in the cloacal temperatures in July was much higher for females than for males, whereas this difference between sexes did not occur in September (Fig. 3). The estimated coefficients of variation for cloacal temperatures in July were 9.33 for females and 4.08 for males, and in September they were 5.99 for females and 4.92 for males.

#### Discussion

The differences in water temperature found between the two periods in the brook are consistent with data published in other studies for stream environments on both sides of the Pyrenees (MONTORI & LLORENTE, 2014; LORAS, 2019; DALIBART et al., 2021, 2022; POTTIER et al., 2021). The preferent location of the individuals in the shaded areas of the stream and the significantly high percentage of activity close to the water surface are consistent with the diurnal activity of the species its preference for not sunny places, as previously reported by Montori & Herrero (2004), MONTORI & LLORENTE (2014) and POTTIER et al. (2021).



**Figure 3:** Cloacal temperatures for males (red squares) and females (green diamonds) in the two sampling periods. Blue line represents the theoretical line of thermoconformity with water temperature. For a proper visualization of the figure, the reader is referred to the online version of the article.

Differences between sexes in cloacal temperatures

Although significant differences in the cloacal temperature between males and females were only observed in September (the studied period with the lowest water temperatures), females clearly had higher cloacal temperatures than males, with average differences around 0.74°C in July and 0.45°C in September (Table 1).

The results obtained by DELUEN *et al.* (2022) indicate that *C. asper* females have a higher SMR than males, which could be a strategy to ensure reproduction under situations of short favourable thermal periods in cold environments. This could be the reason why females have a slightly higher cloacal temperature. It is known that reproduction in females demands a large proportion of energy, and oogenesis will be more efficient with higher body temperatures (ORRELL & KUNZ, 2016). The most likely hypothesis is that a greater amount of energy is required for females, compared to males, to prepare for egg pro-

duction and laying in the short time available in cold water streams, and this would be reflected in higher body temperatures. The results obtained by MONTORI (1988) support this hypothesis. In the studied *C. asper* population, spawning begins in May and ends in July. In September, coinciding with the drop in water temperatures, oogenesis already takes place to prepare for next year's spawn. It remains unknow what happens in the other months of the biological cycle, just after or before wintering.

## Cloacal temperature during amplexus

The cloacal temperature of newts in amplexus was significantly higher than in individuals that were not in this mating position (Fig. 2). This increase in temperature is probably due to the increase in SMR that the amplexus requires. It has been described that the amplexus in this species has a long duration and also that reproduction involves high energy demands (HARSHMAN & ZERA, 2007; COGĂLNICEANU *et al.*, 2013; REEDY *et al.*, 2014), but there are no previous data about what happens specifically during amplexus and no causality can be associated with the observed increase in cloacal temperature. As explained above, in cold-water amphibian populations like those of *C. asper*, the window of favourable temperatures for the reproduction is small (15°C, limited to July and August; CLERGUE-GAZEAU, 1983). Newts seem to choose the opportunity to maximize metabolism modifying their SMR during favourable periods, and *C. asper* is known to have a slight phenotypic plasticity in SMR (GUILLAUME *et al.*, 2020).

DELUEN et al. (2022) highlighted an increase in SMR in C. asper as temperature increased. This increase in SMR reflects an increase in metabolic activity that may be advantageous for ectotherms, leading to a faster growth rate in females, as observed by MONTORI (1990) in C. asper, or to a longer period of activity (ZAGAR et al., 2015), although this specific response has not been reported in C. asper. In general, studies on the growth of *C. asper* provide very different data depending on the population (Miaud and Guillaume, 2005). Looking at what has been described for other species of aquatic urodeles, these differences could be explained by the great variability of environmental conditions in which the populations inhabit (CAMARASA et al., 2020; DALIBARD et al., 2022), the genetic structure of the species (LUCATI et al., 2020), local adaptations (MIAUD & MERILÄ, 2001), or the phenotypic plasticity in SMR (GUILLAUME et al., 2020). However, the lack of studies in this regard for C. asper prevents from elucidating the specific mechanisms behind the observed responses, which are probably not because of a single

factor but of several causes that may be acting synergistically.

## Thermoconformity of the species

Temperate terrestrial ectotherms thermoregulate quite effectively despite variable environmental conditions, whereas species from tropical areas are usually thermoconformer (BALOGOVÁ & GVOŽDÍK, 2015). In the aquatic environment, although animals are more protected against extreme temperatures than in the land, body temperatures are generally lower than in their terrestrial counterparts, which only go to the water to reproduce (HUTCHISON & DUPRÉ, 1992).

In the present study, the cloacal and water temperatures showed significant differences between the two periods, corroborating the idea that the body temperature in C. asper depends mainly on the water temperature, as expected in an aquatic ectotherm (Angilletta et al., 2002; Balogová & Gvoždíк, 2015). In all sampled individuals, the cloacal temperature was slightly higher than the water temperature, both in July and in September (Fig. 3). This result is congruent with the hypothesis that in aquatic habitats the cost of thermoregulation is high compared to the benefits (PIASEČNÁ et al., (2015), and in consequence thermoconformity is an advatandgeous strategy. The results obtained by PIASEČNÁ et al. (2015) corroborate the prediction that if costs of thermoregulation are high compared to its benefits or if the thermal environment (cold water) constrains thermoregulation, ectotherms should abandon thermoregulatory behaviour (Angilletta et al., 2002), as it occurs with Salamandra salamandra larvae living in the wild (PIASEČNÁ et al., 2015).

In July, females showed a much greater dispersion in cloacal temperatures, and these temperatures were higher than in males. This higher temperature in females could be due to the search for more favourable (thermally) places to lay the eggs, or perhaps the females had a higher SMR associated with reproductive effort. So far, evidence from the scientific literature would only support the latter hypothesis. The SMR plasticity described in C. asper could slightly modify body temperature, as has been described in other ectotherms. For instance, BROWN & AU (2009) concluded that an increased metabolic rate due to specific dynamic action led to a small but significant elevation of body temperature in the green anole (Anolis carolinensis). This endogenous heat production could help elevate slightly body temperatures but would play a minor role in thermoregulation (BROWN & AU, 2009). In a species like C. asper, which inhabits cold water streams, this small increase of body temperature (0.6°C on average) would give females some advantage to face their comparatively large reproductive effort.

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