BIOLOGÍA

FOOD AVAILABILITY'S ROLE IN THE REDUCTION IN BODY SIZE ASSOCIATED WITH SANDY SUBSTRATE IN *Pelobates cultripes*

El rol de la disponibilidad de alimento en la reducción del tamaño corporal asociada al sustrato arenoso en Pelobates cultripes

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ABSTRACT: Body size is the most relevant trait with the greatest number of implications in an organism's life. Studying its variation is essential for understanding the evolution of many others morphological and physiological characteristics. *Pelobates cultripes* exhibit variation in body size, related to a geological transition from hercinic substrates to sandy ones in southwestern Spain. Here, we analyze if this variation can be explained by differences in the diversity and abundance of potential preys between the two environments. We found 443 potential prey in the hercinic environment, identified in 11 orders and 3 classes, and 792 in the sandy environment identified in 7 orders and 2 classes. The diversity was significantly higher in the hercinic environment, while the dominance index was significantly higher in the sandy one. In both environments, Hymenoptera and Diptera were dominant, but Coleoptera exhibited greater dominance in the hercinic environment compared to the sandy one. In addition, the preys were on average significantly heavier in the hercinic environment than in the sandy one. This might suggest that the differences in observed body size in *P. cultripes* could be explained by differences in diversity of food available and energy consumption. However, we think it is necessary to increase the sample size in future studies of food availability between the two environments studied, as well as diversify the methods used in describing the diet of *P. cultripes*, to arrive at more robust conclusions.

KEYWORDS: Food availability, body size, *Pelobates cultripes*

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RESUMEN: El tamaño corporal es el rasgo con mayor número de implicaciones en la vida de un organismo. Estudiar su variación ayuda a entender la evolución de otras características morfológicas y fisiológicas. *Pelobates cultripes* muestra una variación en el tamaño corporal, relacionada con una transición geológica de substratos hercínicos a arenosos, en el suroeste de España. Aquí, analizamos si esta variación puede ser explicada por la existencia de diferencias en la diversidad y abundancia de potenciales presas entre los dos ambientes. Encontramos 443 potenciales presas en el ambiente hercínico, identificadas en 11 órdenes y 3 clases, y 792 en el ambiente arenoso identificadas en 7 órdenes y dos clases. El índice de diversidad fue significativamente más alto en el ambiente hercínico; mientras el índice de dominancia fue significativamente más alto en el arenoso. En ambos ambientes dominaron Hymenoptera y Diptera, pero Coleoptera exhibió una mayor dominancia en el ambiente hercínico, en comparación con el arenoso. Además, encontramos presas significativamente más pesadas en promedio en el ambiente hercínico que en el arenoso. Esto podría sugerir que las diferencias en el tamaño corporal observado en *P. cultripes*, se expliquen por diferencias en la diversidad de alimento disponible y energía consumida. Sin embargo, consideramos necesario incrementar el tamaño de muestra de disponibilidad de alimento entre los dos ambientes en futuros estudios, como así también los métodos utilizados en la descripción de la dieta of *P. cultripes*, que permitan llegar a conclusiones más robustas.

PALABRAS CLAVE: Disponibilidad de alimentos, tamaño corporal, *Pelobates cultripes.*

INTRODUCTION

Body size is a fundamental feature of all living organisms due to its relevance to its survival, longevity, fecundity, metabolic rate, competitive ability, or tolerance to starvation and desiccation, among many others (Schmidt-Nielsen, 1984; Blanckenhorn, 2000; Marangoni and Baldo, 2023). In a wide variety of organisms, the body size presents large within-species geographical variation, most of it associated with changes in the environments (Bergmann, 1847; Rensch, 1938; Marangoni *et al*., 2008; Goldberg *et al.*, 2018; Zheng *et al.*, 2023).

A relevant factor in the analysis of geographic variation in body size in amphibian, is the pattern of indeterminate growth they exhibit. This is a continuous growth that becomes asymptotic after reaching sexual maturity, due to a trade-off in resource allocation occurs between reproduction and growth (Heino and Kaitala, 1999). This growth pattern will determine that interdemic variations in both juvenile growth rates to

maturity and age of maturation, can promote divergences in adult size among populations (Marangoni *et al.*, 2008, 2021). Food availability is one of the most important factors determining juveniles and adult growth rate and consequently has strong effects on life-history traits such as survival and age at first reproduction (Arlettaz *et al.*, 2017). The variation in growth rate and body size associated with food availability is a common phenomenon, and it is generally consistent with predictions made by life history models (Berrigan and Charnov, 1994; Atkinson and Sibly, 1997).

The Iberian spadefoot toad (*Pelobates cultripes*) exhibit a extreme reduction in body size and reproductive traits within a short geographical range in southwestern Spain, associated with changes in the geological substrate (Fig. 1, Marangoni *et al.*, 2008). They exhibit a decrease of 71.6% and 36.8%, in body mass SVL respectively, over a mere 60 km transect. This geographic variation is associated to a geological transition from Hercinic to sandy substrates (Fig. 1, Marangoni *et al.*, 2008). We also found considerable differences in larval development among populations of *P. cultripes* through common garden experiments, consistent with the geographic variation observed for adult body size (Marangoni and Tejedo, 2008; Marangoni *et al.*, 2008). In addition, in our most recent study, comparing age structure and growth patterns across *P. cultripes* populations, we suggest both environmental variations in resources availability and divergent growth trajectories among populations (Marangoni *et al.*, 2021). This steep variation also affects other amphibian species that inhabit the same studied area such as *Epidalea calamita* and newts, suggesting a strong environmental influence (Diaz-Paniagua *et al.*, 1996; Díaz-Paniagua and Mateo, 1999; Marangoni *et al.*, 2008; Hyeun-Ji *et al.*, 2020 Refs; Marangoni *et al.* 2021). In addition, reduced life-span was suggested as the main factor of body size reduction of *Pelobates cultripes* inhabiting sandy substrates of Sierra de Ariça (Portugal) (Leclair *et al.*, 2005).
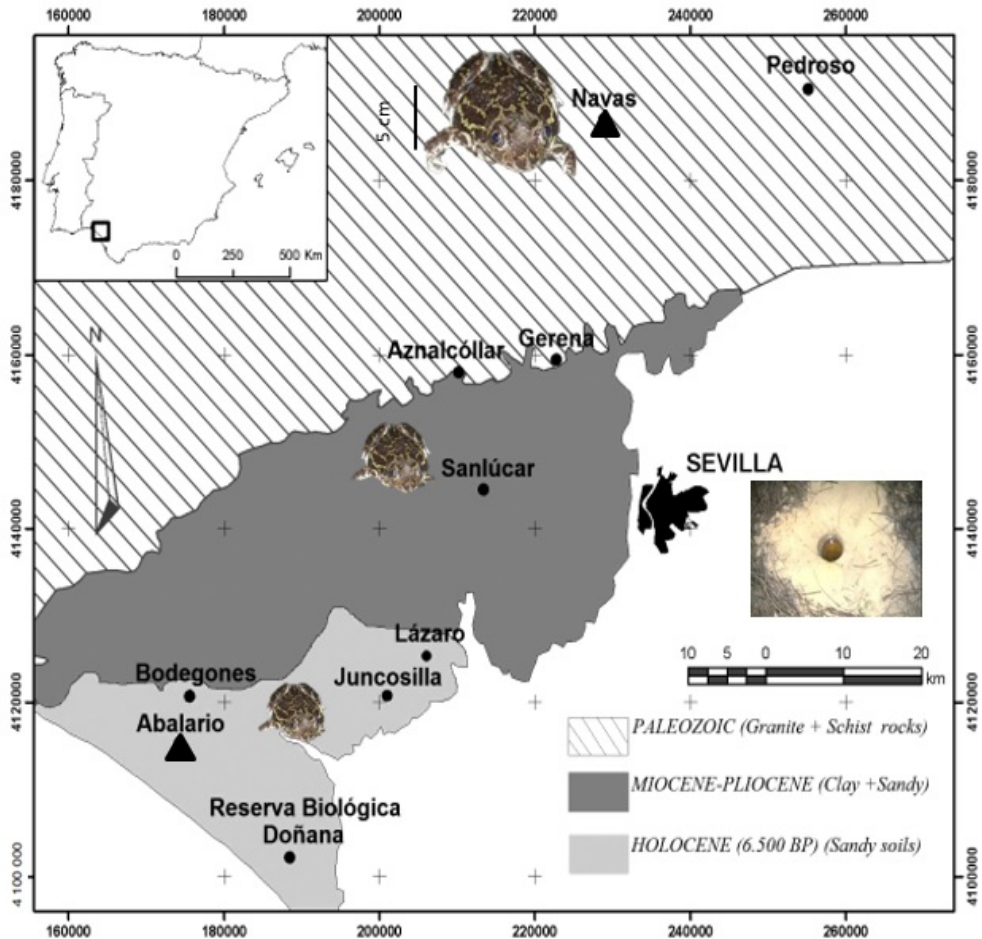
Figure 1. Location and geological substrate of the ten *Pelobates cultripes* population, studied by Marangoni *et al.* (2008, 2021). Abbreviated names of sampling localities, geographic coordinates (Coordinates UTM x/y in meters, Datum European 1950, Spain and Portugal, Zone: 30) and elevation are as follows: PED (255170/4190574, 395 m); NAV (229255/4187617, 420 m); GER (222768/4159484, 100 m); AZN (210199/4158023, 130 m); SAN (213349/4144548, 34 m); LAZ (206339/4125928, 20 m); JUN (203208/4124509, 23 m); BOD (175577/4120711, 32 m); ABA (174267/4115417, 63 m); RBD (188450/4102197, 24 m). The populations where food availability was analyzed are denoted with triangles. Toad pictures are scaled to the mean snout-to-vent length of each geological substrate. The picture of the pitfall traps distributed in the Hercinic and Sandy substrate is embedded in the panel. Detailed description of samples size, body size, age, growth patterns and reproductive output of *P. cultripes* are given in Marangoni *et al.* (2008, 2021).

We hypothesized that differences in food availability between hercinic and sandy substrates could be one of the most important environmental

factors driving the local divergence in body size and age structure observed in *Pelobates cultripes* (Lack, 1954; Marangoni *et al.*, 2008, 2021). We aimed to ascertain whether differences in food availability between these environments (hercinic and sandy subtrates) could account for the observed variation in body size in *P. cultripes.*

MATERIALS AND METHODS

Geographical area

The *Pelobates cultripes* populations studied in Southwestern Spain (Marangoni *et al.*, 2008, 2021) distributed along a 60 km stretch, coincidental with the transition between two geological substrates: old hercinic soils composed of granites and schists from Sierra Morena (hercinic, hereafter), and sandy soils from quaternary aeolian deposits (sandy, hereafter) (Fig. 1). These sandy soils originated in recent times (<6500 years BP, Zazo *et al.*, 1994; Lario *et al.*, 1995; Rodriguez-Ramirez *et al.*, 1995) by the filling of the former Guadalquivir River estuary by aeolic deposits. Mainly as sand sheets, that became a large system of fixed and mobile dunes that ranges from the coastline to more than 40 km inland, and covers over 2500 km2. Both in hercinic and sandy area, all populations of *P. cultripes* breed in small and shallow temporary ponds that become flooded in the fall and dry up in the summer (Gomez-Mestre, 2014; Recuero, 2014). The environmental condition (climate, soil and vegetation) differs considerably across the transect (see Hyeun-Ji *et al.*, 2020).

Food availability Sampling

We used pitfall traps to assess differences in the diversity and abundance of potential prey of *P. cultripes*, between the two environments studied (hercinic and sandy). Within each environment, six traps were arranged in two sets of three (triplet). The triplets were spaced 20 meters apart from each other, and pitfalls within each triplet were 5 m apart from each other (distributed as if they were the three vertices of an equilateral angle). Pitfall traps were 60 mm in diameter and 70 mm deep plastic vials partially

filled with water, ethanol and soap. Sampling of potential prey spanned from November 2002 to February 2004 (Appendix A), coinciding with six mark-recapture sampling events in our reciprocal transplant study on juveniles of *Pelobates cultripes* (Marangoni, 2006; Marangoni and Gomez-Mestre, *in prep.*). In each event (done on the same day in each environment), traps were left open for 24 h. Thus, each pitfall was operating six days during the study (Appendix A). All potential prey (see criteria below) fallen in each trap (except small mice for example) were brought to the laboratory and stored separately in plastic tubes (100 mL) filled with 70% ethanol. We identified the prey to the order or class level, using the keys in Roth (1973). The number of prey items per traps was recorded, to analyze their diversity and abundance per environment.

Finally, we used mean prey weight from each trap ("prey-weight", hereafter) as another estimate differences in food availability between hercinic and sandy environments. We oven-dried all the invertebrates sampled in each trap together for 24 h and then weighed them. Two categories of samples were created to analyze differences in "prey-weight" between environments, considering whether or not the prey could be eaten by juveniles or adults of *Pelobates cultripes*. To do this, we first measured the total body length of each coleopteran fallen in each trap with calipers to the nearest 0.01 mm. Second, taking into account the gape size of juveniles and adult of *P. cultripes* (Tejedo, unpublished data), mean adult size (Marangoni *et al.*, 2008) and the prey size described in the literature (Valverde, 1967; Recuero, 2014), the category were: 1 = included coleopterans smaller than 20 mm in length or 10 mm in thickness, and category 2 = includes beetles that exceed those sizes (were considered to be too large for juveniles).

Statistical analysis

We estimated prey diversity using the Shannon's diversity index H (Shannon and Weaver, 1949) and Simpson dominance D, using the Past 4.03 software (Hammer *et al.*, 2001). We also tested the diversity and dominance differences in food availability between environments using a Diversity t-test.

In addition, we use the rarefaction/extrapolation curves based on the

Hill's numbers (q0, q1 and q2) with 95% confidence intervals based on a resampling method with 1000 replicates (Felsenstein, 1985), to a) compare diversity of food availability between hercinic and sandy environments, and b) to check differences between the food availability recorded in our study in the sandy environment with pitfall traps, and the existing data in the literature on the diet of *Pelobates cultripes* inhabiting that environment. Hill (1973) integrated species richness (in our case = Items prey, hereafter) and their abundances into a set of diversity measures later called the "Hill numbers" or effective number of species, defined for q ≠ 1 as:

$$^qD = \left(\sum_{i=1}^{S} p_i^q \right)^{1/(1-q)}$$

Where the parameter q determines the measure's sensitivity to relative frequencies. In other words, it is a measure of the number of species when each species is weighted by its relative abundance (Hill, 1973; Magurran, 2004). Among the entire series, the most important are: q0 = total number of species (S). When q = 0, the abundances of species do not contribute to the summation in equation 2. Only presences are counted, so q0 is simply species richness. q1 = number of abundant species = $\in$ H". As the limit of Equation approaches 1, it becomes the exponential of the familiar Shannon index, also referred to as Shannon diversity. q2 = number of very abundant species = 1/λ. When q = 2, it produces the inverse of the Simpson index, which gives more weight to the frequencies of abundant species and does not consider rare species. Statistical analyses were performed with the iNEXT package (iNterpolation/EXTrapolation) (Hsieh *et al.*, 2016) in R (R Development Core Team, 2011).

In addition, we did a non-parametric Mann-Whitney test for two independent samples in order test to check for differences in the food availability recorded in our study in the sandy environment with pitfall traps, and the existing data in the literature on the diet of *Pelobates cultripes* inhabiting that environment, using the Past 4.03 software (Hammer *et al.*, 2001).

The similarity in prey items between environments was estimated using qualitative (presence/absence, Jaccard (1908), usually, Sorensen

similarity index), and quantitative indexes (species and their abundances in common, Sorensen index; Magurran, 1988) (see Duré *et al.*, 2008).

We used type III general linear models using the STATISTICA 6.0 statistical package (StatSoft Inc. 2001), to compare the "preys-weight" between environments and the category of prey. Mean values were compared using post-hoc Scheffé multiple comparison test, at α = 0.05.

RESULTS

The prey categories captured (n/relative abundance %) in each environment in the six sampling events is shown in Appendix A. We sampled 443 potential prey in hercinic environment identified in 11 orders and 3 classes (17 preys were not identified). Whereas we sampled 792 potential preys in sandy environment identified in 7 orders (14 preys were not identified) and 2 classes. In both environments the food available was dominated numerically by Hymenoptera and Diptera, but Coleoptera exhibits higher dominance in the hercinic environment (22.1%) compared to the sandy environment (8.2%).

Shannon's index showed a significantly higher mean diversity in food available in hercinic (H'=1.779, variance = 0.002) than sandy (H'=1.204, variance = 0.001) (t-test = 9.430, df = 861.34, P < 0.0001). However, the dominance index was significantly higher in sandy (D = 0.410, variance = 0.0002) than hercinic (D = 0.224, variance < 0.0001) (t-test = -10.042, df = 1162.9, P < 0.0001).

The hercinic environment had a greater diversity of available food items compared to the sandy environment across all orders of q, and the differences in diversity were significant for all orders of q, as there was no overlap in confidence intervals in any case (Table 1 and Fig. 2). On the contrary, there were no differences in diversity for all orders of q, as there was a high overlap in confidence intervals between the food availability recorded in our study in the sandy environment with pitfall traps, and the existing data in the literature on the diet of *Pelobates cultripes* inhabiting that environment (Table 1 and Fig. 2). In addition, the statistical analysis showed that the difference between the samples (food available vs. literature of diet) is not significant (Mann-Whitney *U* test, Z = 0.105, *P* = 0.916).

Table 1. Diversity profiles estimated in each environment studied, the food availability recorded in our study in the sandy environment with pitfall traps, and the existing data in the literature on the diet of *Pelobates cultripes* inhabiting that environment (Valverde, 1967; Díaz-Paniagua *et al.*, 2005). Mean ± SE (LCL-UCL). LCL = lower control limit, UCL = upper control limit.

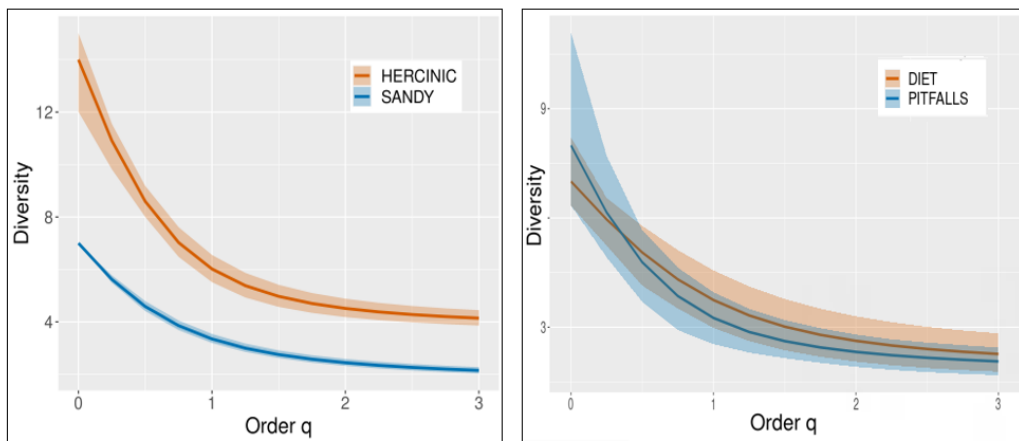| Environments / Order q.	Hercinic vs. Sandy		Pitfall- vs. Diet traps	
q0	14 ± 0.82	7 ± 0	7.99 ± 1.04	7 ± 0.94
	(12.01 – 15.01)	(7 – 7)	(6.49 – 10.25)	(5.51 – 9.49)
q1	6.02 ± 0.26	3.35 ± 0.10	3.25 ± 0.32	3.74 ± 0.33
	(5.5 – 6.55)	(3.19 – 3.54)	(2.63 – 3.84)	(3.16 – 437)
q2	4.51 ± 0.19	2.44 ± 0.08	2.32 ± 0.24	2.62 ± 0.31
	(4.18 – 4.88)	(2.32 – 2.59)	(1.94 – 2.8)	(2.05 – 3.33)

Figure 2. Diversity profiles of available food Items for the hercinic and sandy environment (left) and the comparison between the food availability recorded in our study in the sandy environment with pitfall traps, and the existing data in the literature on the diet of *Pelobates cultripes* inhabiting that environment (right) (Valverde, 1967; Díaz-Paniagua *et al.*, 2005).

Both qualitatively and quantitatively, the similarity between environments was around 50% (IJ = 0.5 and IScuant = 0.58, respectively). We found on average heavier prey in hercinic than sandy in both category (Category 1: Hercinic = 0.0293 g ± 0.0055SE/0.0414SD, n = 31, Sandy = 0.0155 g ± 0.0052SE/0.0144SD, n = 34. Category 2: hercinic = 0.343 g ± 0.1534SE/0.7515SD, n = 24, sandy = 0.130 g ± 0.0312SE/0.1083SD, n = 12). Although, ANOVAs showed no significant "prey-weight" differences between environments in both category (hercinic vs sandy: Category 1 = $F_{1, 63}$ = 3.364, P = 0.071, Category 2 = $F_{1, 34}$ = 1.0218, P = 0.3192).

DISCUSSION

The present study is the first to describe the availability of potential prey for amphibians in southeastern Spain using pitfall traps. We assessed the diversity and abundance of potential prey for *Pelobates cultripes* within the two distinct environments, the hercinic and sandy substrates, of the short geographical range studied by Marangoni *et al.* (2008, 2021). Our results suggest that the differences in the diversity in food availability and energy consumption between these environments, could account for the extreme reduction in body size observed in *Pelobates cultripes* (Marangoni *et al.*, *2008*).

Amphibians are considered as carnivorous, generalist, and opportunistic predators, although certain specialist species do exist (Pough *et al.*, 2004). *Pelobates cultripes* is categorized as a generalist feeder with a broad trophic niche (Recuero, 2014). Their diet consists of a wide variety of invertebrates, including annelids, gastropods, orthopterans, and coleopterans (Recuero, 2014 Refs). In our studied sandy environment, the diet of *P. cultripes* is composed of coleopterans, (56.5%, including adults, terrestrial and aquatic larvae), followed by hemipterans, crickets, ants, dipterans, spiders, and gastropods (Valverde, 1967; Díaz-Paniagua *et al.*, 2005, see Appendix A). Few differences were found with coastal populations in the northeastern of Iberian Peninsula, but Coleoptera is also the most consumed prey category (35.2%) (Bea *et al.*, 1994). Similar results, were

found in other species of Pelobatids, such as *Pelobates fuscus* (Nicoara *et al.* 2005; Nicoara and Nicoara, 2008; Cogalniceanu *et al.*, 1998, 2000) and *P. syriacus* (Fathinia *et al.*, 2019), where the Coleoptera was the most consumed order, followed by Hymenoptera, Arachnida, and Diptera. With this background, our analysis of the rarefaction/extrapolation curves based on the Hill's numbers and non-parametric test, show that the diversity and abundance of food availability in sandy environment studied, typically reflected the dietary composition of *P. cultripes* that inhabit this environment (Valverde, 1967; Díaz-Paniagua *et al*., 2005).

We found a greater diversity of available food items in hercinic environment compared to the sandy environment across all orders of q, and the difference in diversity were significant for all orders of q (see Fig. 2). This difference was also conformed using a non-parametrical statistical analysis. In addition, we found higher abundance of Coleoptera in the hercinic environment, and it also had, on average, heavier prey than sandy environmet. Whereas, Hymenoptera was more abundant in the latter environment. The positive relationship between individual body size and the size of consumed prey, as well as between prey abundance in the environment and the diversity of items consumed, has been well-documented in amphibians (Cogalniceanu, 1998). Thus, we might expect that the observed differences in body size between the two species in the environments are related to differences in diversity of food available and energy consumption. However, we think that an increase in sample size could change the results of prey diversity and abundance found within each environment (see Kovacs and Torok, 1997), and we suggest the need for further studies on food availability in both environments and diversifying the methods for diet determination (García-Padrón, 2019) of *P. cultripes*, allowing for more robust conclusions.

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