

## Article

# Morphological determinants of jumping performance in the Iberian green frog

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## Abstract

Predation is one of the main selective forces in nature, frequently selecting potential prey for developing escape strategies. Escape ability is typically influenced by several morphological parameters, such as morphology of the locomotor appendices, muscular capacity, body mass, or fluctuating asymmetry, and may differ between sexes and age classes. In this study, we tested the relationship among these variables and jumping performance in 712 Iberian green frogs *Pelophylax perezi* from an urban population. The results suggest that the main determinant of jumping capacity was body size (explaining 48% of variance). Larger frogs jumped farther, but jumping performance reached an asymptote for the largest frogs. Once controlled by structural body size, the heaviest frogs jumped shorter distances, suggesting a trade-off between fat storage and jumping performance. Relative hind limb length also determined a small but significant percentage of variance (2.4%) in jumping performance—that is, the longer the hind limbs, the greater the jumping capacity. Juveniles had relatively shorter and less muscular hind limbs than adults (for a given body size), and their jumping performance was poorer. In our study population, the hind limbs of the frogs were very symmetrical, and we found no effect of fluctuating asymmetry on jumping performance. Therefore, our study provides evidence that jumping performance in frogs is not only affected by body size, but also by body mass and hind limb length, and differ between age classes.

**Key words:** body mass, fluctuating asymmetry, hind limb length, hind limb width, locomotor performance, morphometry

Predation is one of the main selective forces acting on individuals, because being preyed on implies the death of the individual and reduces its future fitness to zero (Abrams 2000). As a consequence, many morphological and behavioral adaptations have evolved in potential prey to avoid predation (Ruxton et al. 2004; Caro 2005; Stevens and Merilaita 2011; Cooper and Blumstein 2015). Indeed, several of those adaptations are implied in the escape behavior, which occurs when the encounter with the predator is unavoidable (Ydenberg and Dill 1986; Lima and Dill 1990; Cooper and Blumstein 2015). Escape capacity is strongly influenced by body morphology affecting locomotor performance (Irschick and Garland

2001), thereby morphological variables involved in locomotion typically are under selective pressure by predators (Langerhans et al. 2004; Losos et al. 2004; Calsbeek and Irschick 2007; Irschick and Meyers 2007; Irschick et al. 2008; Langerhans and Makowicz 2009; Blob et al. 2010; Ingley et al. 2016).

Locomotor performance may also be affected by the fluctuating asymmetry (Swaddle 2003). Increased levels of fluctuating asymmetry in locomotor appendices have been related to reduced locomotor performance in birds (Thomas 1993), lizards (Braña and Ji 2000; Martín and López 2001; López and Martín 2002), and mammals (Manning and Ockenden 1994). The fact that fluctuating

asymmetry lowers locomotor performance would influence the fitness of the individual, causing a selective pressure against the most asymmetric individuals (Møller 1997; Brown and Brown 1998; Galeotti et al. 2005). In addition, body mass may also affect locomotor performance, because the more mass to be moved, the more muscular force becomes necessary (Witter and Cuthill 1993). Indeed, heavier individuals, for a given body size, show reduced escape capacity in birds (Witter et al. 1994) and lizards (Zamora-Camacho et al. 2014).

Besides morphology and body mass, locomotor performance may vary among age classes or sexes if individuals belonging to a given demographic class differ in their susceptibility to predator attacks. For example, in lizards, males are typically more exposed to predators than females because they devote more time to activities of territory defense and mate searching, or because they are more conspicuous, and thereby males typically have higher maximal escape capacity (Lailvaux 2007).

When adult anurans moving or sunbathing on earth have an encounter with a predator, they typically try to escape by jumping away from the predator. Thereby, jumping performance may determine the capacity to fly from the predator and thus to survive. Jumping capacity in anurans is greater in individuals with longer hind limbs in relation to body size (Zug 1978; Tejedo et al. 2000; Choi et al. 2003; James et al. 2005; James and Wilson 2008; Gomes et al. 2009; Orizaola and Laurila 2009; Drakulić et al. 2016; Zamora-Camacho and Aragón, 2019a, 2019b). Yet, the exact causes why hind limb length affects jumping performance are not completely understood, as many mechanisms related to the characteristics of the musculoskeletal system may be responsible, such as limb length, the ratio of muscle mass to total mass, the proportion of muscle-fiber types, the thickness of the muscles or elastic storage (Biewener et al. 1981; Losos 1990; Dickinson et al. 2000). For example, regardless of hind limb length, more muscled individuals have better jumping capacity (Miller et al. 1993; Choi and Park 1996; Choi et al. 2003; James et al. 2005, 2007; James and Wilson 2008). Other morphological traits potentially influencing jumping performance, such as fluctuating asymmetry or body mass, have received little attention in anurans.

Moreover, in anurans, males are usually more exposed to predators when attracting mates (Tuttle and Ryan 1981), and hence higher escape capacity would be expected in males. However, sexual differences in jumping performance in anurans have been barely studied and the results have been mixed (Zug 1978). Herrel et al. (2012) specifically studied this topic in *Xenopus tropicalis*, finding that males have longer hind limbs and better escape capacity than females for a given body size (also see Zamora-Camacho 2018). Additionally, jumping performance augments with body size (Emerson 1978; Zug 1978; James and Wilson 2008; Gomes et al. 2009; Drakulić et al. 2016). Thereby, juvenile anurans, being smaller, have impaired capacity to escape from predators. In such a case, one possibility is that jumping performance is improved in juveniles, thus compensating for their reduced body size, by having longer or stronger hind limbs. Still, little is known on the ontogenetic variation in jumping performance in anurans (e.g., Wilson et al. 2000).

In this study, we examine the intrapopulation variability in jumping performance of juvenile and adult Iberian green frogs *Pelophylax perezi*. *Pelophylax perezi* is a medium-sized frog (snout-vent length [SVL] of 2.2–7.9 cm in our study area) closely linked to aquatic environments (Egea-Serrano 2014). These frogs are typically under a strong pressure by different predators such as snakes

(*Natrix maura* and *N. natrix*), herons (*Ardea cinerea*, *Bubulcus ibis*, and *Egretta garzetta*), water rails *Rallus aquaticus*, brown rats *Rattus norvegicus*, domestic cats *Felis silvestris catus*, and the conspecific frogs (cannibalism is frequent), among others (Egea-Serrano 2014). Due to this strong predation pressure, frogs present different antipredatory mechanisms (summarized in Egea-Serrano 2014). When basking on land, the main antipredatory strategy in juveniles and adults is crypsis, which allows them to spend more time sunning and feeding without being noticed by predators (Martín et al. 2005). However, when a predator approaches too closely, they normally jump into the water to escape (Martín et al. 2005, 2006). Frogs modulate their jumping performance according to risk conditions, and the distance jumped depends on several factors, such as the distance to the water body, or the presence of aquatic vegetation where they hide (Martín et al. 2005). Therefore, maximum jumping capacity may be vital for frog survival.

Here, we estimate jumping performance of *P. perezi* in order to examine how it is affected by different morphological and demographic variables. Concretely, we expect that larger frogs jump longer. However, although overall jumping capacity will be lower in juveniles—given their small size—it is possible that, once controlled by body size, jumping capacity will be greater in juveniles than in adults. We also predict greater jumping capacity in males, given that they are more exposed to predators when calling. Moreover, heavier frogs, for a given structural body size, are expected to have worse locomotor performance. Additionally, we examine the relationship between both hind limb length and muscle mass (estimated as hind limb width) and maximal jump distance. We predict that *P. perezi* with longer and more muscular hind limbs should jump farther. Finally, we test the effect of fluctuating asymmetry on jumping distance, expecting that more asymmetric individuals show worse locomotor performance.

## Materials and Methods

### General procedures

The fieldwork was conducted in the urban park Jardins de Mossèn Cinto Verdager (100 m a.s.l., 41°22'2.472"N, 2°9'54.481"E; Barcelona, Spain; Supplementary Figure S1A), between January and February of 2015. The study area consisted of 31 interconnected artificial ponds, built for ornamental purposes (Supplementary Figure S1B), inhabited naturally by a population of Iberian green frog *P. perezi*. This population is under a strong predation pressure, mainly by grey herons *A. cinerea*, gulls *Larus michahellis*, magpies *Pica pica*, domestic cats, brown rats, and conspecific frogs (G. Pascual, personal communication). We took advantage of the maintenance work of the park, during which the ponds were emptied, and captured by hand 712 individuals of *P. perezi*. In closed buckets with a layer of 2–3 cm of water, the frogs were transferred to a nearby facility, where the measurements were taken. The overall study was carried out under permit by the Barcelona city hall.

To measure jumping performance, we placed each individual in a bucket with water at ~22°C, so that all individuals had roughly the same body temperature to avoid confounding effects of body temperature on jumping performance (Navas et al. 1999; Mitchell and Bergmann 2016). Frog body temperature was assessed by inserting a 1-mm diameter thermocouple connected to a thermometer (model Hybok 14, accuracy 0.1°C) 8 mm inside the cloaca. Then, individuals were placed on absorbent paper in which the position of their urostyle was marked with a marker. One researcher induced each individual to jump by gently touching its hind limbs, and the

position of the urostyle was marked again after the jump. This process was repeated 5 times in each individual, and the longest jump of each frog was noted, considering this as the maximum jumping distance (in centimeter) of the individual, which we define here as jumping performance. The maximal jumping distance attained by an individual in laboratory standardized trials is the upper limit of its performance capacity (Irschick and Garland 2001; Irschick 2003; Irschick et al. 2005). In nature, individuals do not always escape predators at their maximal performance capacity (Husak 2006b) and, indeed, the distance jumped by *P. perezi* depends on a series of environmental factors, such as distance to water or the presence of vegetation (Martín et al. 2005). Nonetheless, locomotor performance measured in laboratory is typically considered a good indicator of field survival (e.g., Warner and Andrews 2002; Le Galliard et al. 2004; Husak 2006a). Hence, we assumed that, when attacked by a predator, the frog's maximal jumping capacity is an upper limit of its capacity to escape.

Once jumping performance was measured, individuals were anesthetized by immersion in lactate RingelVet with MS-222 diluted (1 g/L) (Mitchell 2009) until they lost responsiveness. Then, anesthetized individuals were weighed with a digital balance (Denver Instrument Company Model 100 A, 0.1 g accuracy). Before being weighed, frogs were dried with absorbent paper to minimize the possible error due to water excess. We recognized frog sex based on the presence of nuptial pads and vocal sacs in males (Egea-Serrano 2014). Individuals that weighed <11 g and lacked nuptial pads were considered juveniles. Subsequently, each individual was photographed on graph paper with a digital single-lens reflex camera (Nikon D80). Afterwards, each frog was placed individually in a bath of dechlorinated water until they had clearly regained consciousness and was in good condition. Finally, frogs were released in the same ponds as where they were captured. No individual suffered permanent damage as a consequence of the study.

With the photographs taken, we measured a total of 712 specimens, by using the software GNU Image Manipulation Program (GIMP) 2. We measured SVL, right and left femur length, maximum width of the right and left thighs, right and left tibiofibula length, maximum width of the right and left calves (Supplementary Figure S2). All measurements are expressed in centimeters. We calculated the average values between the 2 legs (left and right).

Fluctuating asymmetry was calculated using the formula:  $FA = |L - R|$  (Møller and Swaddle 1997), L being the value of the morphometric measurement of the left side and R that of the right side. We tested whether the data met the requirements to be considered true fluctuating asymmetry following Cuervo (2000) (see Supplementary Material A). The measurement error, which required checking that the presumed fluctuating asymmetry was not an artifact (Palmer and Strobeck 2003), was calculated using a subset of 10 randomly assigned individuals. In each, all the morphometric variables were measured 3 times, leaving a time period of 2–4 weeks between each measurement. The measurement error was calculated with a 1-way ANOVA, in which morphometric variables were the dependent variable and the id number of the individuals the independent variable (Senar 1999). The measurement error was very low for each variable, with repeatability consistently >98% (Supplementary Table S1). Fluctuating asymmetry was larger than measurement error in femur length, thigh width, and calf width, but not in tibiofibula length (Supplementary Material A).

### Statistical analysis

All statistical analyses were performed with the software R 3.5.1 (R Development Core Team 2015). First, we examined the dataset and

removed outliers detected graphically (Zuur et al. 2010). As all the data fulfilled the criteria of normality and homoscedasticity, for which we tested them both graphically and statistically (Zuur et al. 2010), we analyzed them with parametric statistics (Quinn and Keough 2002).

We analyzed the relationship between both SVL and body mass with jumping performance by using separate ordinary least square models, as both variables were strongly correlated ( $r = 0.99$ ,  $P < 0.01$ ) and hence including them in the same model would result in collinearity. Graphically, the relationship between these 2 variables with jumping performance appeared to be curvilinear, so we used quadratic models by including the terms  $SVL^2$  and (body mass)<sup>2</sup>, respectively. In these models, body temperature was included to control for a possible effect of body temperature on jumping performance (Navas et al. 1999). Additionally, we performed a Principal Component Analysis (PCA) with body mass, SVL, femur length, thigh width, tibiofibula length, and calf width, all variables log-transformed to guarantee linearity (Packard 2013). We retained the first 2 principal components because they correlated with jumping performance (see “Results” section), although it should be noted that the PC2 did not meet the Kaiser criterion, as its eigenvalue was <1 (Yeomans and Golder 1982; Supplementary Table S2). The PC1 strongly loaded for all the variables and may be considered an indicator of body size, whereas the PC2 loaded more for body mass than for the structural measurements, hence this one may be considered to be an indicator of body mass corrected by structural body size (Supplementary Table S2). Therefore, to distinguish the relative importance of structural body size and relative body mass on jumping performance, we examined the correlation between these 2 orthogonal principal components with jumping performance.

We also investigated the relationship between jumping performance and the different parts of the hind limb (femur length, thigh width, tibiofibula length, and calf width). The high correlation of these variables with SVL (see Supplementary Table S2) forced us to remove the effect of SVL to have a measurement of relative size of these parts, that is, a measurement of how the size of these parts deviated from that expected for a given SVL. For this, we extracted the residuals of the size of each hind limb part regressed on SVL (all variables log-transformed) in separate regressions. These residuals gave us a measurement of the size of each part, controlled by SVL. We tested the relationship between these residuals and jumping performance with a multiple regression model.

Looking for differences among age classes (juveniles vs. adults) and gender (males vs. females), we considered the variable age/sex classes, with 3 levels: juveniles, males, and females. We performed a linear model with jumping performance as the independent variable, and age/sex, SVL (log-transformed) and their interaction as predictors. We also examined how allometry of the different parts of the hind limb varied with SVL in each age/sex class. We used ordinary least squares regressions (Kilmer and Rodríguez 2017) with the logarithmic transformation of SVL, femur length, thigh width, tibiofibula length, and calf width in order to determine the value of its allometric exponent (Packard 2013). We calculated confidence intervals at 95% to test whether the allometric exponents were significantly higher or lower than 1. The allometric exponent of 1 implies isometry, while values <1 imply negative allometry (the trait grows slower than the body), and values >1 indicate positive allometry (the trait grows quicker than the body).

The effect of fluctuating asymmetry of the different parts of the hind limb on jumping performance was analyzed with partial Pearson product-moment correlations, controlling with SVL.

## Results

### Effect of body mass and body size on jumping performance

Jumping performance was higher with SVL, but at a certain SVL an asymptote was reached (Table 1; Figure 1A). Similarly, jumping performance improved with body mass also reaching an asymptote (Table 1; Figure 1B). The PC1 (which may be interpreted as an index of body size) was positively correlated with jumping performance ( $\beta = 0.693$ ,  $F_{1,709} = 723.13$ ,  $P < 0.001$ ), but PC2 (which may be interpreted as an index of body mass corrected by body size, see “Materials and Methods” section) was negatively correlated with jumping performance ( $\beta = -2.22$ ,  $F_{1,709} = 72.42$ ,  $P < 0.001$ ). The model including PC1 and PC2 explained 52.7% of variance in jumping performance (Adjusted  $R^2 = 0.527$ ), 48% was explained by PC1 and the remaining 4.7% by PC2. Femur and tibiofibula relative length (controlled for SVL) positively influenced jumping performance, whereas thigh and calf width had no significant effect (Table 2). Relative size of the parts of the hind limb explained little variance in jumping performance (adjusted  $R^2 = 0.024$ ).

### Morphology and jumping performance according to sex and age classes

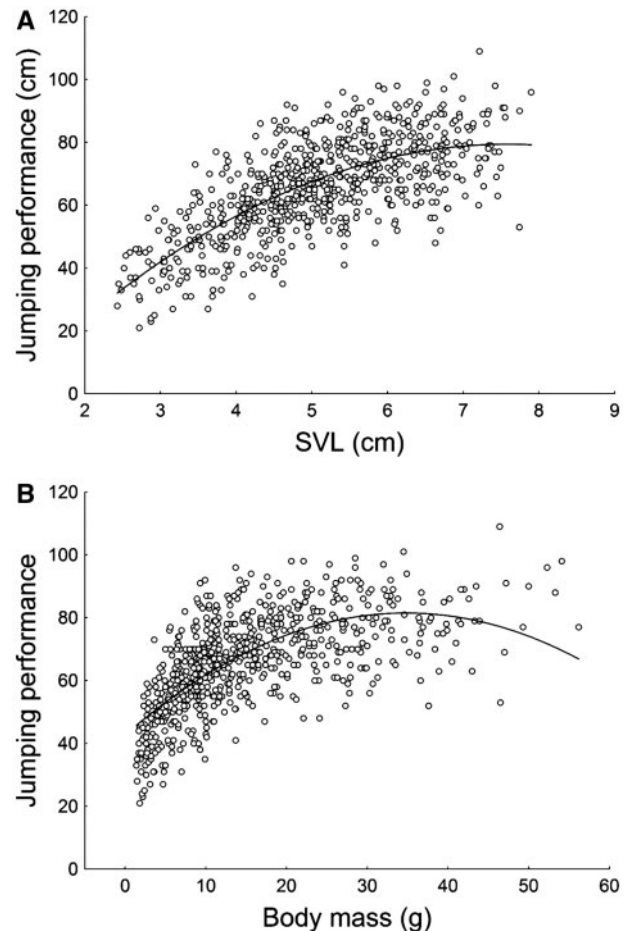
There was an interaction between SVL and sex/age group (Table 3), indicating that the slope of the relationship between SVL and jumping performance significantly differed among sex/age classes; specifically, jumping performance improved with increased SVL faster in juveniles than in mature individuals (males  $\beta = 0.43$ , females  $\beta = 0.32$ , and juveniles  $\beta = 0.64$ ; Figure 2). After controlling for SVL, males had slightly (but significantly, according to unequal N HSD post hoc tests,  $P < 0.001$ ) larger and wider hind limbs than did females, but the 2 sexes did not differ in jumping performance ( $P = 0.29$ ). Also, adults had larger and wider limbs and better jumping performance than did juveniles (always  $P < 0.001$ ; Tables 3 and 4). Females showed negative allometry with SVL for tibiofibula length, whereas males showed positive allometry for thigh and calf width, and juveniles showed positive allometry for calf width (Table 5).

**Table 1.** Models explaining the relationship between (Panel A) SVL and (Panel B) body mass with jumping performance of Iberian green frogs *P. perezi*

|                                      | df  | F-value | P-value | $\beta$ |
|--------------------------------------|-----|---------|---------|---------|
| (A) SVL Adjusted $R^2 = 0.509$       |     |         |         |         |
| Intercept                            | 1   | 7.89    | 0.005   |         |
| SVL                                  | 1   | 114.17  | <0.001  | 2.131   |
| SVL <sup>2</sup>                     | 1   | 53.02   | <0.001  | -1.442  |
| Temperature                          | 1   | 1.49    | 0.222   | 0.035   |
| Error                                | 708 |         |         |         |
| (B) Body mass Adjusted $R^2 = 0.460$ |     |         |         |         |
| Intercept                            | 1   | 10.81   | 0.001   |         |
| Body mass                            | 1   | 282.14  | <0.001  | 1.573   |
| (Body mass) <sup>2</sup>             | 1   | 121.34  | <0.001  | -1.008  |
| Temperature                          | 1   | 0.32    | 0.571   | 0.017   |
| Error                                | 708 |         |         |         |

### Fluctuating asymmetry and jumping performance

Individuals of our population were strongly symmetric, as suggested by the very low values of fluctuating asymmetry found for the different parts of the hind limb (mean  $\pm$  SE; femur length =  $0.077 \text{ cm} \pm 0.002$ ; thigh width =  $0.029 \text{ cm} \pm 0.001$ ; tibiofibula length =  $0.084 \text{ cm} \pm 0.003$ ; calf width =  $0.037 \text{ cm} \pm 0.001$ ). In fact, once we controlled for SVL, fluctuating asymmetry of no trait was significantly related to jumping performance (partial  $r < 0.2$ ,  $P > 0.5$ ).



**Figure 1.** Relationship between (A) SVL and (B) body mass, and jumping performance in the Iberian green frog *P. perezi*.

**Table 2.** Multiple regression model explaining the relationship between relative size of the different parts of the hind limb (residuals after controlling for SVL) and jumping performance in Iberian green frogs, *p. perezi*

|                    | df  | F-values | P-values | $\beta$ |
|--------------------|-----|----------|----------|---------|
| Intercept          | 1   | 13916.16 | <0.001   |         |
| Femur length       | 1   | 4.45     | 0.035    | 0.082   |
| Thigh width        | 1   | 0.31     | 0.580    | 0.031   |
| Tibiofibula length | 1   | 8.79     | 0.003    | 0.119   |
| Calf width         | 1   | 0.02     | 0.900    | -0.007  |
| error              | 707 |          |          |         |

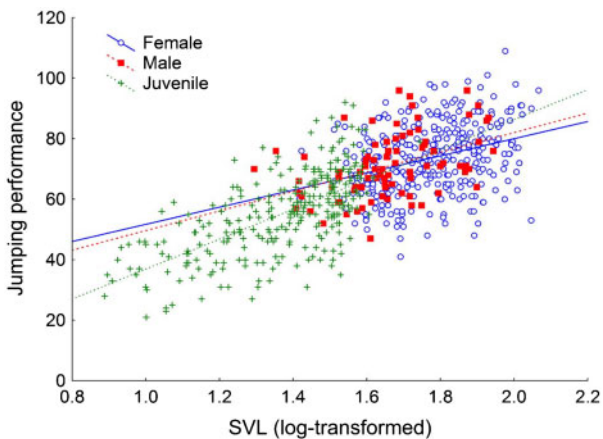
**Discussion**

Our study analyses the jumping performance of *P. perezi* frogs under controlled conditions, showing that the main determinant of

**Table 3.** Models explaining the relationship between age/sex, and jumping performance and relative size of the parts of the hind limb, controlling for SVL (log-transformed) and examining the interaction between age/sex and SVL, in Iberian green frogs, *P. perezi*

|                     | Intercept | Age/Sex | SVL     | Age/sex × SVL | error |
|---------------------|-----------|---------|---------|---------------|-------|
| df                  | 1         | 2       | 1       | 2             | 706   |
| Jumping performance | 2.84      | 8.42    | 119.39  | 7.42          |       |
| Femur length        | 500.70    | 100.04  | 3943.23 | 111.26        |       |
| Thigh width         | 444.338   | 86.81   | 2808.94 | 95.86         |       |
| Tibiofibula length  | 243.48    | 41.10   | 2655.29 | 46.51         |       |
| Calf width          | 532.62    | 92.63   | 2888.19 | 102.23        |       |

F-values are shown. Results were significant at  $P < 0.001$ , except for the intercept in the model of jumping performance, which was nonsignificant.



**Figure 2:** Relationship between SVL and jumping performance in juveniles (in green, crosses, and dotted line), males (in red, squares, and dashed line), and females (in blue, circles, and full line), of the Iberian green frog *P. perezi*.

**Table 4.** Mean values ( $\pm$ SE) of jumping performance and the different parts of the hind limb, controlling for SVL (log-transformed), for male, female, and juvenile Iberian green frogs *P. perezi*

|                     | Males ( $n = 81$ ) | Females ( $n = 342$ ) | Juveniles ( $n = 289$ ) |
|---------------------|--------------------|-----------------------|-------------------------|
| Jumping performance | 69.02 $\pm$ 1.30   | 68.66 $\pm$ 0.97      | 66.48 $\pm$ 1.02        |
| Femur length        | 2.39 $\pm$ 0.01    | 2.37 $\pm$ 0.01       | 2.30 $\pm$ 0.01         |
| Thigh width         | 1.10 $\pm$ 0.01    | 1.09 $\pm$ 0.01       | 1.05 $\pm$ 0.01         |
| Tibiofibula length  | 2.34 $\pm$ 0.02    | 2.33 $\pm$ 0.01       | 2.28 $\pm$ 0.01         |
| Calf width          | 0.79 $\pm$ 0.01    | 0.78 $\pm$ 0.005      | 0.75 $\pm$ 0.005        |

All measurements are in centimeters.

**Table 5.** Allometric exponents of each morphometric variable with their confidence intervals at 95% (in bold), allometric exponents that significantly differed from 1

|                    | Males ( $n = 81$ )         | Females ( $n = 342$ )      | Juveniles ( $n = 289$ )    |
|--------------------|----------------------------|----------------------------|----------------------------|
| Femur length       | 1.011 (0.900–1.122)        | 0.938 (0.870–1.006)        | 1.006 (0.963–1.049)        |
| Thigh width        | <b>1.153 (1.019–1.287)</b> | 0.971 (0.883–1.059)        | 1.039 (0.987–1.091)        |
| Tibiofibula length | 0.887 (0.748–1.026)        | <b>0.877 (0.802–0.952)</b> | 1.008 (0.957–1.059)        |
| Calf width         | <b>1.191 (1.047–1.335)</b> | 1.037 (0.947–1.127)        | <b>1.130 (1.073–1.187)</b> |

jumping capacity (almost 50% of the variance) was body size: larger frogs jumped farther (see also Martín et al. 2005). Increased jumping performance with body size has repeatedly been reported (e.g., Zug 1978; Gomes et al. 2009; Orizaola and Laurila 2009) and is an expected result as larger individuals, with longer muscular fibers, need less energy per mass unit for their movement (Taylor et al. 1982). However, for very large individuals, jumping performance was constant with increased body size, probably owing to biophysical limits. Similar findings have been reported in other studies with anurans (Wilson et al. 2000). For this reason, in juveniles, jumping performance increased with body size at a steeper function than in adults. This implies that jumping as an antipredator strategy is not as effective in juveniles as in adults. In fact, the antipredator behavior may vary with body size (Dial et al. 2008): in this frog, small individuals rely more on crypsis, whereas large frogs use jumping to escape more frequently (Martín et al. 2005). Therefore, in order to decrease predation risk, small frogs could be selected to grow as fast as possible, although this is not free of costs. For example, rapid growth may increase oxidative stress or reduce telomere length (Burraco et al. 2017) and have consequences later in life (Metcalfe and Monaghan 2001). In our study population, hind limbs were shorter and narrower in juveniles than in adults (once body size was statistically controlled for). This could seem puzzling, given that juvenile small body size implies smaller jumping capacity and thereby selection for longer hind limbs in juveniles could be expected. However, given that jumping is of lower antipredator value in juveniles than in adults, juveniles may be selected to rely on crypsis instead of investing in hind limb growth.

Regarding sexual differences, we expected enhanced jumping performance in males, given that they are more exposed to predators. However, although males had larger and wider hind limbs than females, sexes did not differ in jumping performance. By contrast, Herrel et al. (2012) found that males of the frog *X. tropicalis* had larger hind limbs than females for a body size given, and this implied enhanced locomotor capacity with respect to females. Therefore, our findings suggest that, in the Iberian green frog, sexual differences in hind limb morphology are not related to differences in jumping performance. More muscular and longer hind limbs in males may be related to sexual behaviors such as the amplexus, in which

males use hind limbs to push competitors for accessing to the female.

As happened with body size, jumping performance increased asymptotically with body mass. Although body mass increases with structural body size (following a cubic relationship), it also depends on fat reserves accumulated. Fat reserves may be of great importance to survive, but also may negatively affect locomotor performance. Our findings suggest that heavier individuals had worse jumping performance than lighter ones, which is consistent with what happen in other animal groups (Witter et al. 1994; Zamora-Camacho et al. 2014). This implies a trade-off between fat storage and locomotor performance (Witter and Cuthill 1993). Increased body mass for a given structural body size might also be the result of increased muscle mass, but even in this case, the weight of greater muscle mass may not be offset by the strength gained (Swaddle and Biewener 2000).

In addition to body size and body mass, relative length of both femur and tibiofibula had a positive effect on jumping performance. Individuals with longer hind limbs, which are the main extremities involved in jumping (Gillis and Biewener 2000), showed greater jumping performance, which has been repeatedly reported in other anurans species (Zug 1972; Zug 1978; Tejedo et al. 2000; Choi et al. 2003; James et al. 2005; James and Wilson 2008; Gomes et al. 2009; Orizaola and Laurila 2009; Drakulić et al. 2016; Zamora-Camacho and Aragón 2019a, 2019b). However, the underlying mechanism remains unclear. Distal leg segments have been associated with enhanced jumping capacity (Aerts 1998), but we found that the complete hind limb was related to jumping performance (although the effect size of tibiofibula length was slightly higher than of femur length). Similarly, Toro et al. (2004) found that distal segments of the hind limb were not related with greater jumping performance in *Anolis* lizards. Longer overall hind limbs would allow frogs to lengthen the distance through which the propulsive force acts and the time that the force acts on the supporting surface (Gray 1968), which would allow them to jump farther based on a phenomenon similar to a leverage effect (Wang et al. 2014). Longer limbs might also imply longer muscles, which would result in great jumping force (Alexander 2000; James et al. 2005; but see Olberding and Deban 2018).

Muscle mass has been reported to have a positive influence on jumping performance (Miller et al. 1993; Choi and Park 1996; Choi et al. 2003; James et al. 2005), as greater muscle mass would allow anurans to augment the power applied on jumping (Moo et al. 2017). Considering this, we expected that individuals with wider legs would have better jumping performance because the force exerted by muscles is proportional to its transversal section (Goldspink 1977). Nevertheless, we found no effect of hind limb width, neither of the thigh nor of the calf, on jumping performance. This result might indicate that hind limb width is not a good proxy of muscle mass, for example, if width is also influenced by fat storages. Moreover, some authors argue that studies should focus only on the influence of hind limb muscles that are actually involved in jump, such as the plantaris muscle, instead of the whole hind limb muscle mass (James et al. 2005; Olberding and Deban 2018).

Another factor that could affect jumping performance is fluctuating asymmetry. We expected that higher fluctuating asymmetry would negatively influence jumping performance. However, none of the fluctuating asymmetries calculated affected jumping performance. These results do not match with others studies that have shown a negative relationship between fluctuating asymmetry and locomotor performance in different species such as birds (Thomas

1993), mammals (Manning and Ockenden 1994), or lizards (Martín and López 2001), but match with findings reported for insects, fluctuating asymmetry not affecting locomotor performance in house fly *Musca domestica*, yellow dung fly *Scathophaga stercoraria*, and house cricket *Acheta domesticus* (Swaddle 1997; Pears et al. 2019). The absence of an effect of fluctuating asymmetry on jumping performance could mirror the large number of predators in our study area. Predators could trigger a strong selective pressure against the most asymmetric individuals (Fuller and Houle 2002), reducing fluctuating asymmetry in the population (Vershinin et al. 2007). This could explain the low fluctuating asymmetry in our study system, where frogs were almost symmetrical in comparison with other frog populations (see Söderman et al. 2007; Burghlelea et al. 2013).

In conclusion, our study shows that body size is the main determinant of jumping performance in *P. perezii* frogs. Besides body size, other morphological traits influence jumping performance in this species, although at much lesser extension. Concretely, hind limb length relative to body size positively affects jumping performance, whereas body mass has a negative effect.

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## Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

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