

RESEARCH ARTICLE

What makes a great invader? Anatomical traits as predictors of locomotor performance and metabolic rate in an invasive frog

Pablo Padilla^{1,2,*}, Anthony Herrel^{2,3,4,5} and Mathieu Denoël¹

ABSTRACT

Invasive species are characterized by their ability to establish and spread in a new environment. In alien populations of anurans, dispersal and fitness-related traits such as endurance, burst performance and metabolism are key to their success. However, few studies have investigated inter-individual variation in these traits and more specifically have attempted to understand the drivers of variation in these traits. Associations of anatomical features may be excellent predictors of variation in performance and could be targets for selection or subject to trade-offs during invasions. In this study, we used marsh frogs (*Pelophylax ridibundus*), a species that has been introduced in many places outside its native range and which is now colonizing large areas of Western Europe. We first measured the inter-individual variation in resting metabolism, the time and distance they were able to jump until exhaustion, and their peak jump force, and then measured the mass of specific organs and lengths of body parts suspected to play a role in locomotion and metabolism. Among the 5000 bootstrap replicates on body size-corrected variables, our statistical models most often selected the stomach (75.42%), gonads (71.46%) and the kidneys (67.26%) as predictors of inter-individual variation in metabolism, and the gluteus maximus muscle (97.24%) mass was the most frequently selected predictor of jump force. However, endurance was poorly associated with the anatomical traits ($R^2_{\text{distance}}=0.42$, $R^2_{\text{time}}=0.37$). These findings suggest that selection on these predictors may lead to physiological changes that may affect the colonization, establishment and dispersal of these frogs.

KEY WORDS: Invasive species, *Pelophylax*, Metabolism, Morphology, Anatomy, Inter-individual variation

INTRODUCTION

One of the first steps of a biological invasion is the introduction of a non-native species to a new area (Sakai et al., 2001). While not all introductions are successful, some have led to widespread invasions across multiple regions (Compton et al., 2010; Ihlow et al., 2016; Zhou et al., 2021) and even continents (Kolbe et al., 2010; Tedeschi et al., 2022). To better understand these massive invasion events, previous studies have quantified physical performance traits (speed, endurance, jump force) as proxies of

dispersal capacity (Kolbe et al., 2010; Llewelyn et al., 2010; Lombaert et al., 2014; Louppe et al., 2017) and fitness-related traits (Dallas et al., 2021; Louppe et al., 2018; Romero-Báez et al., 2020; Young et al., 2022). However, studies often neglect the drivers of variation in performance (Arnold, 1983) and how variation in the propensity of an individual to become invasive may be driven by its anatomy and physiology (Bennett, 1987; Forsman and Wennersten, 2016).

Locomotor performance is composed of multiple traits among which maximal burst performance and endurance capacity are the most studied (Aerts et al., 2000; Herrel and Bonneaud, 2012). Burst performance reflects the capacity to quickly react and move a short distance and is often regarded as a proxy of an animal's capacity to escape predators or to catch prey, which can both be considered as a fitness-related trait. Endurance, in contrast, is the capacity to withstand fatigue while sustaining locomotion and is often viewed as a proxy of dispersal (Herrel and Bonneaud, 2012; Vanhooydonck et al., 2014). Indeed, animals that sustain locomotion longer will be able to spread further in the landscape (Gruber et al., 2017). Endurance is also a fitness-related trait because dispersing individuals may gain access to new locations with fewer or no competitors, granting them more resources for their growth or reproduction (Brown et al., 2013; Courant et al., 2019). Interestingly, both traits rely on the body size of the individuals (James et al., 2007; Mendoza et al., 2020) as well as the size of the limbs, limb muscles and bones (Nauwelaerts et al., 2007). Previous studies have obtained mixed results regarding the relationship between speed and endurance, with some showing trade-offs (Herrel and Bonneaud, 2012; Vanhooydonck et al., 2014) and others showing no or even positive trait relationships (Sorci et al., 1995; Vanhooydonck et al., 2007). This implies that the elements composing the limbs may differently impact the two types of locomotor performance (i.e. endurance and burst performance).

Metabolic rate, i.e. the energetic cost of an organism to fulfil its physiological needs, is another trait that is known to show variation in response to many ecological phenomena (Brown et al., 2004) and directly affects life history traits such as growth, reproduction, performance, behaviour and overall fitness (Burton et al., 2011; Metcalfe et al., 2016). In heterotrophs, metabolism is essentially the rate of ATP production, the fuel of most biological processes, by mitochondria during cellular respiration (Rolfé and Brown, 1997). At an inter-specific level, variation in metabolic rate is explained by variation in body mass (Andrews and Pough, 1985; Speakman, 2005; White and Seymour, 2004) but this relationship is less clear at the intra-specific level (Careau et al., 2008) and remains generally poorly investigated (Burton et al., 2011). Mass-corrected metabolic rate was shown to be explained by the size of some organs: whereas the liver was found to be a driver of metabolic rate in eels (Boldsen et al., 2013), the kidneys were the only organ to show any correlation with metabolic rate in leopard

¹Laboratory of Ecology and Conservation of Amphibians (LECA), Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, 4020 Liège, Belgium. ²UMR 7179 C.N.R.S./M.N.H.N., Département Adaptations du Vivant, 55 rue Buffon, 75005 Paris, France. ³Evolutionary Morphology of Vertebrates, Ghent University, B-9000 Ghent, Belgium. ⁴Department of Biology, University of Antwerp, Wilrijk 2610, Belgium. ⁵Naturhistorisches Museum Bern, 3005 Bern, Switzerland.

*Author for correspondence (pablo.padilla@uliege.be)

© P.P., 0000-0003-1278-5143; A.H., 0000-0003-0991-4434; M.D., 0000-0002-3586-8323

frogs (Steyermark et al., 2005). Yet, both organs were found to be correlated with metabolic rate within the same strain of laboratory mice (Konarzewski and Diamond, 1995). Finally, both the kidneys and the heart explained 50% of mass-corrected resting metabolic rate across 22 species of bird (Daan et al., 1990). These studies reveal that despite their small proportion of total body mass, some organs can be more metabolically active than others. Therefore, these organs could be subjected to allocation trade-offs (Konarzewski and Diamond, 1995). For example, reproductive periods may induce a high metabolic cost that in turn may be detrimental to the efficiency of other functions such as the immune system (Brokordt et al., 2019).

Here, we examined whether inter-individual variation in anatomical features predicts resting metabolism and locomotor performance in an invasive frog species, the marsh frog, *Pelophylax ridibundus*. Marsh frogs are one of the most invasive amphibians in Europe as a result of multiple events of introduction followed by a quick establishment and spread over entire regions and even countries (Bellati et al., 2023; Dufresnes et al., 2017, 2018; Holsbeek et al., 2010). They impact native populations through genetic processes (Holsbeek and Jooris, 2010) as well as predation (Pille et al., 2021, 2023). Previous studies have shown that marsh frogs have a high ecological flexibility (Denoël et al., 2022; Ivanova and Berzin, 2019) and show behavioural and physiological traits that favour their invasion (Padilla et al., 2023). Here, we aimed to link variation in physiology and performance to anatomical features to highlight which parts of the body may favour their invasion success. To do so, we: (1) evaluated resting metabolic rate, the time and distance jumped until exhaustion, and peak jump force; and (2) measured the mass of specific organs and/or length of relevant body parts. We expected reproductive organs to induce a high energetic cost, especially in females, and thus to be a good predictor of metabolism. Endurance is a matter of fatigue and of muscle oxygenation, and therefore we expected circulatory organs such as the heart and lungs to predict this performance trait. Lastly, because jumping in frogs is produced by the extensor muscles acting on the bones, we expected the biggest extensor muscle, the gastrocnemius muscle, as well as the length of the tibio-fibula and the astragalus–calcaneus to be best predictors of jump force.

MATERIALS AND METHODS

Study species

Frogs were caught in August 2020 and April 2021 by hand or with a dip net in five ponds across the southern part of Larzac plateau, France. Genetic analyses confirmed that the mtDNA haplotypes of these populations are assigned to the marsh frog, *Pelophylax ridibundus* (previously named *Rana ridibunda*) (Pallas, 1771), which is allochthonous in the studied area, with the origin of these lineages being more than 1000 km from the Larzac in South-Eastern Europe (Dufresnes et al., 2017). A previous survey carried out in the 1970s showed that *Pelophylax* were historically absent in the plateau at that time (Gabrion et al., 1978; M. Gabrion, personal communication). The environment where the frogs were sampled is a karst plateau with traditionally managed landscapes and ponds for watering cattle (Denoël and Lehmann, 2006; Durand-Tullou, 1959). Frogs were manipulated with nitrile gloves. All material was cleaned and disinfected between sampling different ponds to avoid translocation of organisms (e.g. pathogens).

Housing

Animals were transported to the MECADEV laboratory (Museum National d'Histoire Naturelle, Paris) in refrigerated boxes. In the

laboratory, they were isolated individually in 5 l plastic boxes (21.4×36.5×16.5 cm; water depth: 5 cm) with holes in the top. Boxes were elevated and inclined allowing animals to choose between a terrestrial and an aquatic side. Boxes were cleaned weekly. Two adult crickets, *Acheta sp.*, were given twice a week. Night and day periods were set to 12 h each (light starting at 08:00 h). All animals were PIT-tagged during capture in the field, allowing individual identification (biolog id, Bernay, France; 12 mm tag). The temperature during trials was the same as the room temperature, which was set at 24°C, a temperature close to the thermal preference of this population (Padilla et al., 2023). Frogs were kept a week without manipulation to allow them to acclimate to laboratory conditions before the onset of observations.

Respirometry

Basal or standard metabolic rate, i.e. the oxygen uptake by an organism at rest and in a post-absorptive state (Blaxter, 1989), was measured. Fifty-five frogs (28 males, 27 females) were measured using a flow-through respirometer. Chambers were set inside an incubator (Aqualytic-LIEBHERR, TC 256 G/256L/2–40°C) to acclimate the animal to the desired temperature. A direct sampling method was used by pushing air with a pump (PP2 dual channel field pump, Sable Systems International) to a mass flow meter (FB8 flowbar-8 mass flow meter system, Sable Systems International) directly into eight chambers of 270 ml. The first chamber was left empty for baselining. Flow was then transmitted to a multiplexer (RM8 respirometry flow multiplexer, Sable Systems International) into a gas analyser (FMS field Metabolic System, Sable Systems International), allowing a sequenced recording of each chamber. Preliminary measurements were performed for 1 week to acclimate the frogs before the measurements and to find the optimal parameters for the set up. A flow rate between 150 and 250 ml min⁻¹ for larger animals (more than 20 g) resulted in CO₂ concentrations between 0.05 and 0.2 ml h⁻¹ which we considered as a good trade-off between distinguishing gas exchange and avoiding hypercapnia. Because of the semi-aquatic behaviour of these animals, we set the relative humidity to 80%. The recordings consisted of repeated sequences that started with 2 min of baseline recording in the empty chamber, followed by 5 min of recording for each chamber with an animal (35 min total), and ended with 3 min of baseline recording. Recordings were done continuously for a total of four sequences (approximately 3.5 h), to allow the incubator and frogs to reach the desired body temperature and the frogs to calm down, as indicated by a stable gas exchange (Fig. S1). Recordings were done at least 72 h after the frogs were fed to prevent an increase in metabolic rate due to digestion (Jobling, 1981). Animals were measured between 09:00 h and 19:00 h. Before each acquisition, the frogs were patted dry and weighed. After acquisition, animals were weighed, their box was cleaned, and they were put back inside with two adult crickets, which were instantly eaten, denoting a relatively low stress from the measurements.

Gas analysis followed Lighton's (2019) methods. O₂ and CO₂ received the same corrections: (i) a mathematical scrubbing transformation [(CO₂×barometric pressure)/(barometric pressure–water vapour)], (ii) a drift correction using baseline measurements, and (iii) division by 100 to get fractional concentrations. Flow rate was also corrected for its water vapor content {[flow rate×(barometric pressure–water vapour)]/barometric pressure}. Because O₂ was analysed last, we corrected its lag time response so that its decreases overlapped with CO₂ increases. Because the flowmeter was upstream, and the CO₂ was not removed from the excurrent air stream, we calculated \dot{V}_{O_2} using the

following equation:

$$\dot{V}_{O_2} = \frac{FR_I[(F_{IO_2} - F_{EO_2}) - F_{EO_2}(F_{ECO_2} - F_{ICO_2})]}{1 - F_{EO_2}}, \quad (1)$$

where FR denotes flow rate, F_{O_2} and F_{CO_2} are the fractional content of each gas, and 'I' and 'E' refer to incurrent and excurrent mass flow, respectively. The mean lowest 90 s pre-sampled from a 180 s stable recording of \dot{V}_{O_2} for each individual was extracted using ExpeData 1.9.27 software.

Terrestrial exertion

Thirty-eight frogs (26 males, 12 females) were used in the endurance trials. Animals were induced by hand to jump back and forth across a linear track (200×40×50 cm; graduated each 10 cm) with a humidified cork bottom providing grip. Endurance or exertion was measured as the time and distance moved until exhaustion (Herrel and Bonneaud, 2012; Padilla et al., 2023). Time was assessed using a stopwatch and distance was counted from the number of back and forth trips and the number of graduations on the bottom of the track. Frogs that were not able to directly recover a normal position after being turned on their back were considered exhausted. At the end of the trial, animals were returned to their maintenance boxes, fed, and left to rest for a week. We then repeated the trial a second time to use only the maximum time and distance for each individual.

Jump forces

Maximal jump forces were measured for 38 frogs (26 males, 12 females) using a piezo-electric force platform (20×10 cm, Kistler Squirrel force plate, ±0.1 N; Herrel et al., 2014; Padilla et al., 2023). The platform was connected to a charge amplifier (Kistler – Charge Amplifier type, 9865) and forces were recorded for 60 s at 500 Hz using BioWare software (version 5.4.3.0, 2011 Kistler group, Switzerland). A cork surface was glued to the force plate to provide a better grip for jumping. The frog was placed on the force plate and allowed to rest for a few seconds. Jumping was induced by a quick approach by the observer. Peak force data were extracted using Kistler BioWare software and the total resultant force (vector sum of the X-, Y- and Z-forces) was calculated. A trial consisted of three jumps and each trial was repeated three times. Only the jump with the highest total resultant force was retained for each individual.

Anatomy and morphometrics

Frogs were euthanised by placing them inside a plastic box (5 l) filled with water and MS-222, which was progressively added for 30 min (Close et al., 1996). To better preserve tissues, we fixed frogs in a solution of formaldehyde (5%) for 48 h. Individuals were then rinsed with tap water for 24 h and conserved in ethanol (70%).

Frogs were dissected and organs were removed from the abdomen including the heart, the lungs, the liver, the stomach, the intestines, the kidneys, the gonads and the bladder. We then selected and removed three of the biggest hindlimb muscles: the gastrocnemius, the gluteus maximus and the cruralis (Přikryl et al., 2009). All organs were then dried in an oven (BINDER E028-230 V) at 60°C for 48 h and then weighed using a precision scale (Ohaus PA64, readability: 0.0001 g). A calliper was used to measure the length to the nearest 0.05 cm of each bone involved in locomotion: the ilium, the femur, the tibio-fibula, the astragalus–calcaneus, the longest toe, the humerus, the radio-ulna, the carpus and the longest finger.

Statistical analysis

We first calculated the mean and s.e.m. of each variable (response and predictor variables; Table 1). To assess any allometric effects, we first visualised the correlations between body size (SVL) and each predictor (Fig. 1). Because of these strong allometric effects, we consequently created and used in all subsequent analyses a new set of body size-corrected variables, obtained from residuals of the linear model of each anatomical trait regressed on SVL. We then visually inspected, through principal component analysis, whether the sex of the animal or the locality of sampling differed in the residuals of the size-corrected anatomical variables. None of these factors seemed to affect inter-individual variation (Figs S2 and S3). We therefore decided to not include these factors in our subsequent analysis.

Because our dataset contains redundant and highly correlated predictors, which can drastically reduce the prediction accuracy of traditional multiple regression models as a result of overfitting and multicollinearity, we used a variable selection method using lasso regression (Zhou, 2013; Zou and Hastie, 2005) from the package glmnet v4.1-7 (Friedman et al., 2010). All variables were further centred and scaled before analyses. We used 10-fold cross-validation to choose the penalty term (lambda) of our lasso models. More specifically, we used the minimum cross-validation lambda, which is the location of lambda where the mean squared error was minimized (Kohavi, 1995). We replicated 5000 bootstraps for each lasso regression and counted in each replicate how many times each variable showed a coefficient higher than zero, which defines how often they were selected by the model.

To find the best predictors of resting metabolic rate (\dot{V}_{CO_2}) among anatomical traits (mass, SVL, heart, lungs, fat, kidneys, stomach, liver, intestine, gonads, bladder and the sum of all hindlimb muscles), we performed a lasso regression. Then, we performed two lasso regressions on endurance (distance and time until exhaustion)

Table 1. Descriptive statistics of raw performance and anatomical traits in invasive marsh frogs

	<i>n</i>	Mean±s.e.m.	Minimum	Maximum
\dot{V}_{O_2} (ml h ⁻¹)	55	1.78±0.16	0.02	5.93
Distance (cm)	38	2290±110	890	4350
Time (s)	38	162±7	70	256
Jump force (N)	38	0.782±0.072	0.12	1.94
Mass (g)	93	17.7±1.2	1.6	59.6
SVL (mm)	93	54.7±1.1	33.6	88.44
Ilium (mm)	93	16.5±0.4	9.1	26
Femur (mm)	93	23.9±0.6	10.6	39.4
Tibia (mm)	93	26.1±0.6	15.2	42.2
Foot (mm)	93	12.0±0.3	6.15	18.7
Toe (mm)	93	27.1±0.6	16	42.2
Humerus (mm)	93	13±0.36	6.65	21.6
Radius (mm)	93	9.37±0.26	4.05	16
Hand (mm)	93	3.07±0.09	1.65	6.65
Finger (mm)	93	11.6±0.3	6.35	19.45
Heart (mg)	93	16.4±1.5	2.5	65
Lungs (mg)	93	11.7±0.9	0.6	44.4
Fat (mg)	93	19.7±4	0	230
Kidneys (mg)	93	13.7±0.8	2.9	38.6
Stomach (mg)	93	62±3	12.4	162.4
Liver (mg)	93	102±10	7.8	606.7
Intestine (mg)	93	44.1±2.3	5.8	106.4
Gonads (mg)	93	149±42	0.6	2823
Bladder (mg)	93	3.75±0.29	0.3	16.7
Gastrocnemius (mg)	93	53.7±3.6	7.8	169.1
Gluteus maximus (mg)	93	22.1±1.5	3	62.1
Cruralis (mg)	93	72.6±4.9	9.5	229.6

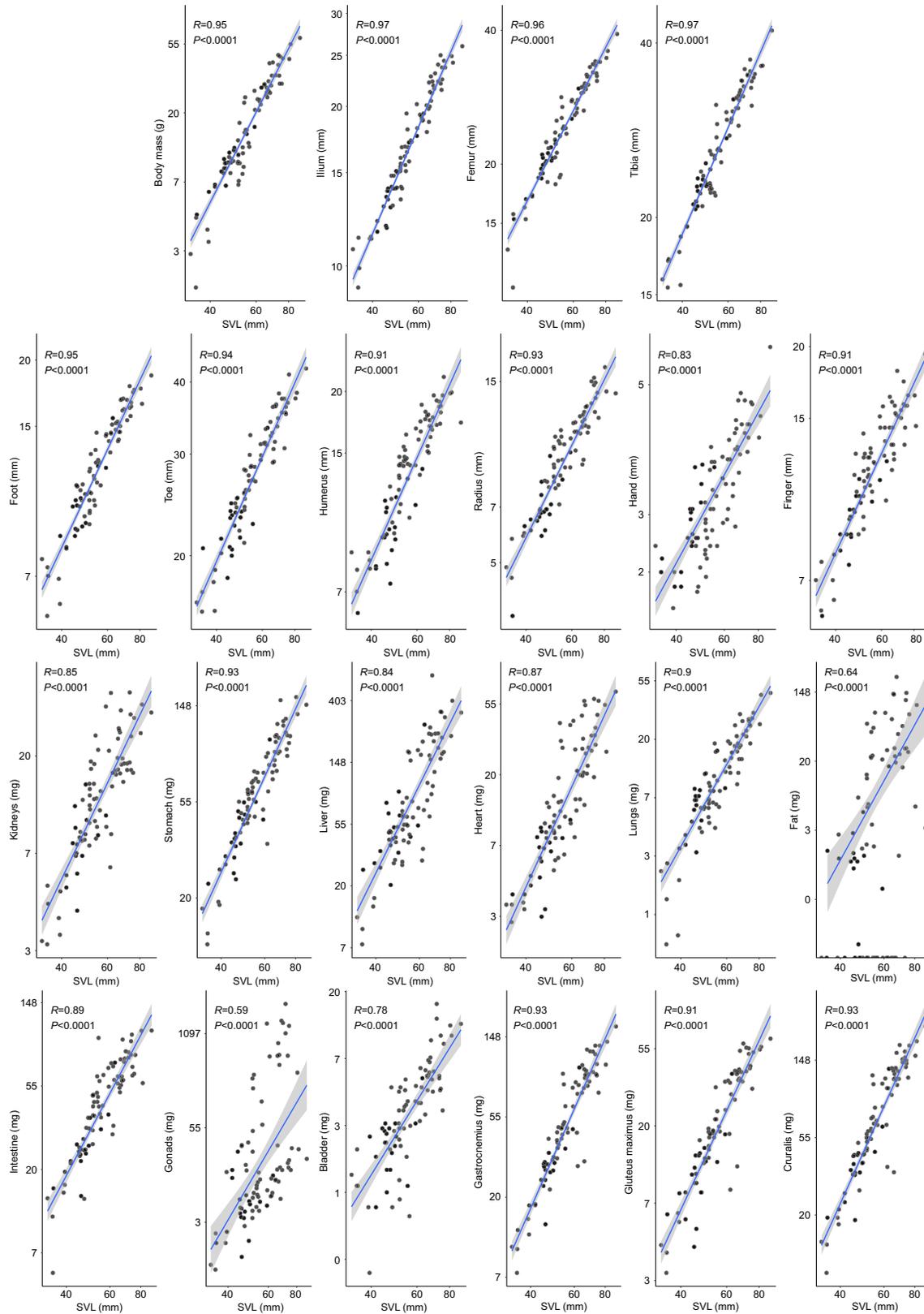


Fig. 1. Relationship of bone length and organ dry mass with body length (snout–vent length, SVL) in invasive marsh frogs. A log scale was used for each axis. A regression line (blue line) with a 95% confidence interval (grey shaded area) was plotted.

to find the best predictors among anatomical traits (ilium, femur, tibio-fibula, astragalus–calcaneus, toe, humerus, radio-ulna, carpus, finger, heart, lungs, gastrocnemius, gluteus maximus, cruralis).

Finally, a fourth lasso regression on jump force to find the best predictors among anatomical traits (ilium, femur, tibio-fibula, astragalus–calcaneus, toe, gastrocnemius, gluteus maximus,

cruralis) was run. The same tests were run again, without SVL and mass, on all residuals obtained from linear regressions of each response variable and each predictor with body size (SVL). All statistics were performed using R (<http://www.R-project.org/>).

RESULTS

Absolute predictors of performance

Bootstrap replicates of lasso regression showed that the studied anatomical variables explained on average half of the variation in metabolism ($R^2=0.50$, s.e.m.=0.0021). The mass of the kidneys [count (i.e. percentage of time selected among all bootstraps replicates)=81.68%] and gonads (count=80.68%), and SVL (count=71.72%) were the predictors that were most often selected by the model (Fig. 2A). Kidney mass (mean coefficient \pm s.e.m.=0.31 \pm 0.004), gonad mass (mean coefficient \pm s.e.m.=0.13 \pm 0.002) and SVL (mean coefficient \pm s.e.m.=0.26 \pm 0.006) showed a positive effect on resting metabolic rate (Fig. 2B).

Bootstrap replicates of lasso regression showed that the anatomical variables explained on average less than half of the variation in distance travelled until exhaustion ($R^2=0.42$, s.e.m.=0.0041). Predictors were not often selected by the model, with heart mass being the most selected (count=54.14%), followed by lung mass (count=49.22%) (Fig. 2A). Heart mass (mean coefficient \pm s.e.m.=−0.35 \pm 0.007) had a negative effect on the distance travelled until exhaustion, while lung mass (mean coefficient \pm s.e.m.=0.10 \pm 0.007) had a positive effect on this trait (Fig. 2B).

Bootstraps replicates of lasso regression showed that the anatomical variables explained on average less than half of the variation in the time spent moving until exhaustion ($R^2=0.37$, s.e.m.=0.0039). Again, predictors were not often selected; radius length was the most selected (count=55.96%, Fig. 2A) followed by heart mass (count=54.14%), lung mass (count=50.96%), toe length (count=49.80%) and hand length (count=49.22%). Radius length (mean coefficient \pm s.e.m.=0.06 \pm 0.004), lung mass (mean coefficient \pm s.e.m.=0.19 \pm 0.0063) and toe length (mean coefficient \pm s.e.m.=0.25 \pm 0.0074) showed a positive effect on the time spent moving until exhaustion while heart mass (mean coefficient \pm s.e.m.=−0.23 \pm 0.0082) and hand length (mean coefficient \pm s.e.m.=−0.13 \pm 0.0056) showed a negative effect on this measure of performance (Fig. 2B).

Bootstraps replicates of lasso regression showed that the anatomical variables explained on average more than 95% of the variation in jump force ($R^2=0.96$, s.e.m.=0.00026). Body mass (count=98.76%) and the mass of the gluteus maximus muscle (count=94.78%) were the predictors that were most often selected by the model (Fig. 2A). Both body mass (mean coefficient \pm s.e.m.=0.65 \pm 0.0047) and gluteus muscle mass (mean coefficient \pm s.e.m.=0.48 \pm 0.0046) had a positive influence on jump force (Fig. 2B).

Body size-corrected predictors of performance

Bootstrap replicates of lasso regression on the residual traits explained on average less variation in metabolism than analyses run on absolute variables ($R^2=0.28$, s.e.m.=0.0022). Stomach (count=75.42%), gonad (count=71.46%) and kidney mass (count=67.26%) were the predictors most often selected by the model (Fig. 3A). Stomach mass (mean coefficient \pm s.e.m.=−0.43 \pm 0.0052) showed a negative effect on metabolic rate, while gonad (mean coefficient \pm s.e.m.=0.12 \pm 0.002) and kidney mass (mean coefficient \pm s.e.m.=0.24 \pm 0.0037) showed a positive one (Fig. 3B).

Bootstrap replicates of lasso regression on the residuals of body size explained on average the same amount of variation in distance

travelled until exhaustion ($R^2=0.43$, s.e.m.=0.0037). The mass of the heart (count=54.12%) was the predictor most often selected by the model (Fig. 3A). Heart mass (mean coefficient \pm s.e.m.=−0.31 \pm 0.0067) showed a negative effect on the distance travelled until exhaustion (Fig. 3B).

Bootstrap replicates of lasso regression on the residuals of body size explained on average the same amount of variation in the time spent moving until exhaustion ($R^2=0.37$, s.e.m.=0.0037). The length of the radius (count=54.56%) was the predictor most often selected by the model (Fig. 3A) and showed a positive effect (mean coefficient \pm s.e.m.=0.35 \pm 0.0075) on the time spent moving until exhaustion (Fig. 3B).

Bootstrap replicates of lasso regression on the residuals of body size explained on average less variation in burst jumping performance ($R^2=0.67$, s.e.m.=0.0017). The mass of the gluteus maximus muscle (count=97.24%) was most often selected by the model (Fig. 3A) and showed a positive effect (mean coefficient \pm s.e.m.=0.54 \pm 0.0037) on jump force (Fig. 3B).

DISCUSSION

Anatomical traits predicted inter-individual variation of metabolism and burst jumping performance rather well, but were rather poor predictors of endurance (Figs 1 and 2). Metabolism seems to be mostly explained by body size differences in contrast to endurance and jump force (Figs 2 and 3). These results suggest that specific organs may contribute differently to the performance of different physiological and behavioural traits that could make marsh frogs great invaders. Ultimately, organs and body parts are expected to be the targets of selection as they can give invaders an advantage during their establishment and dispersal in new areas.

Kidneys, the main predictor of energy consumption

Despite the well-known body size effect on metabolism (Andrews and Pough, 1985; Speakman, 2005), kidney mass was the best predictor of inter-individual variation in metabolism (Fig. 2A), with bigger kidneys increasing the basal metabolic rate (Fig. 2B). When correcting for size, the model explained less variation, but predictors remained similarly selected (Fig. 3), with the mass of the stomach, kidneys and gonads being top predictors of metabolism. Steyermark et al. (2005) observed that it was the increase in size-corrected kidney mass that resulted in an increase in the standard metabolic rate of male leopard frogs (*Rana pipiens*). They argued that electrolyte absorption occurring in the kidneys may explain this high energetic cost. Indeed, the kidneys are responsible for homeostasis but also for the resorption of nutrients, the secretion of hormones and the extraction of waste produced by metabolism into the urine (Ogobuiro and Tuma, 2023). Because frogs were fasted before the observations, the absorption of nutrients and the extraction of waste were probably low during the recordings. We can therefore assume that homeostasis or the secretion of hormones might be associated with kidney size, leading to an increase in metabolic rate.

Reproduction, and more specifically gametogenesis, is also known to be a biological process with a high energetic investment (Facey and Grossman, 1990). This expectation was confirmed by our results as gonad mass was an important positive predictor of metabolism either relative to the size of the individual (Fig. 2) or when corrected for its body size (Fig. 3). The mass of the stomach was also often selected in both models, with a higher occurrence in the body size-corrected model (Figs 2 and 3). These results suggest that a relatively larger stomach may, instead of requiring a higher metabolic cost, allow a more efficient one.

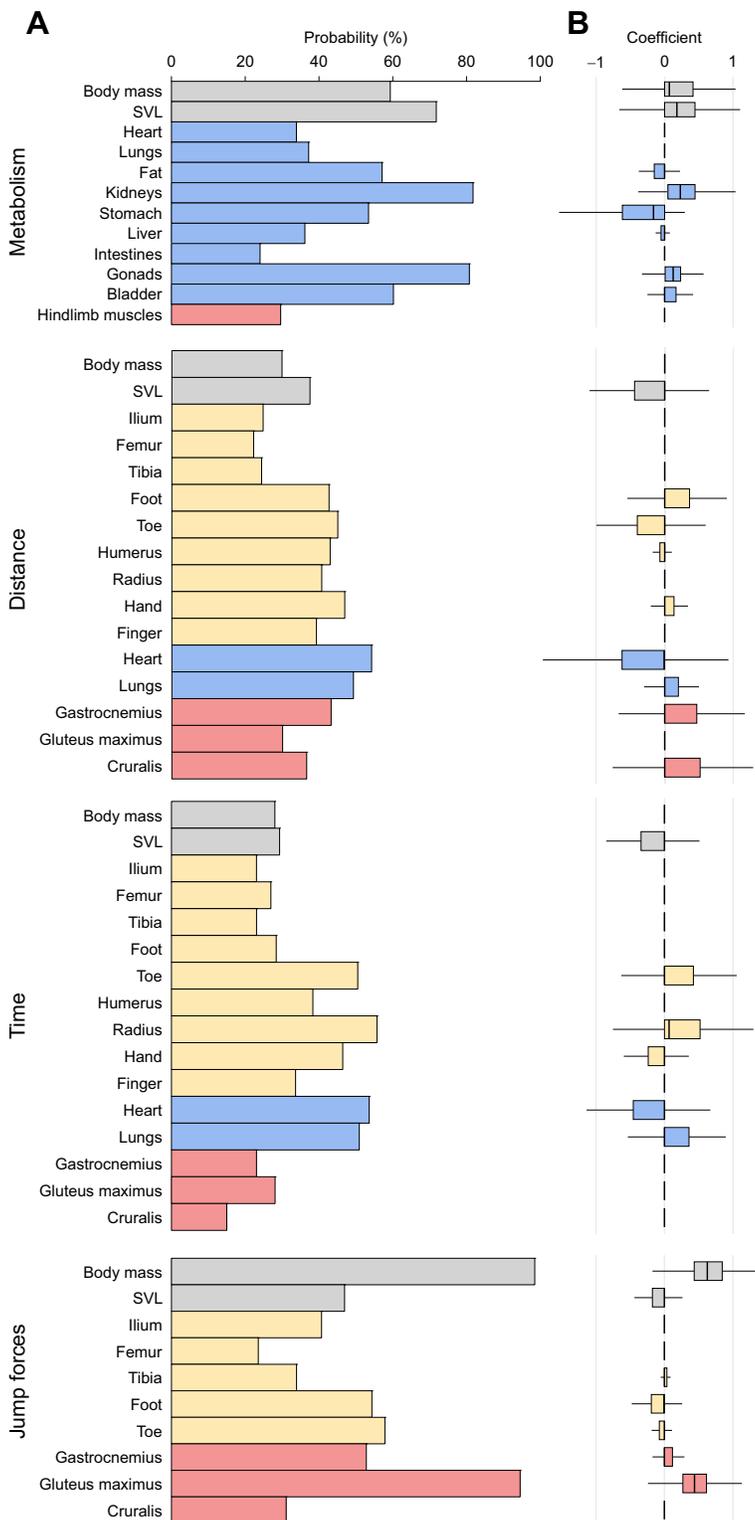


Fig. 2. Results of the lasso models with 5000 bootstrap replicates for each performance on anatomical predictors. Performance was measured as metabolism (quantified by oxygen consumption), distance travelled until exhaustion, time in movement until exhaustion and force produced during jumping. (A) Probability of selected predictors after all replicates. (B) Boxplot of average coefficients after all replicates (bar: median; boxes: first and third quartiles; whiskers: largest value not more than 1.5 times the inter-quartile range). Body size variables are represented in grey, viscera in blue, bone section lengths in beige, and muscle masses in red.

Endurance may not solely rely on anatomical traits

Inter-individual variation in endurance in marsh frogs was not clearly associated with any single anatomical trait and, compared with other physical performance traits, less variation was predicted by the lasso models. Therefore, the confidence in the selected predictors is limited, which suggests that future research needs to look for other predictors. Moreover, in both the distance travelled and the time spent moving until exhaustion, the raw model and the

one on the residuals of body size explained almost the same amount of variation, indicating that body size is not a great predictor of endurance. Despite this, heart size was retrieved as one of the most common predictors of endurance capacity (distance travelled and time until exhaustion) in addition to measures of the limbs (tibia for distance to exhaustion; radius and femur for time to exhaustion). Because differences in size-corrected hindlimb length were previously observed in expanding

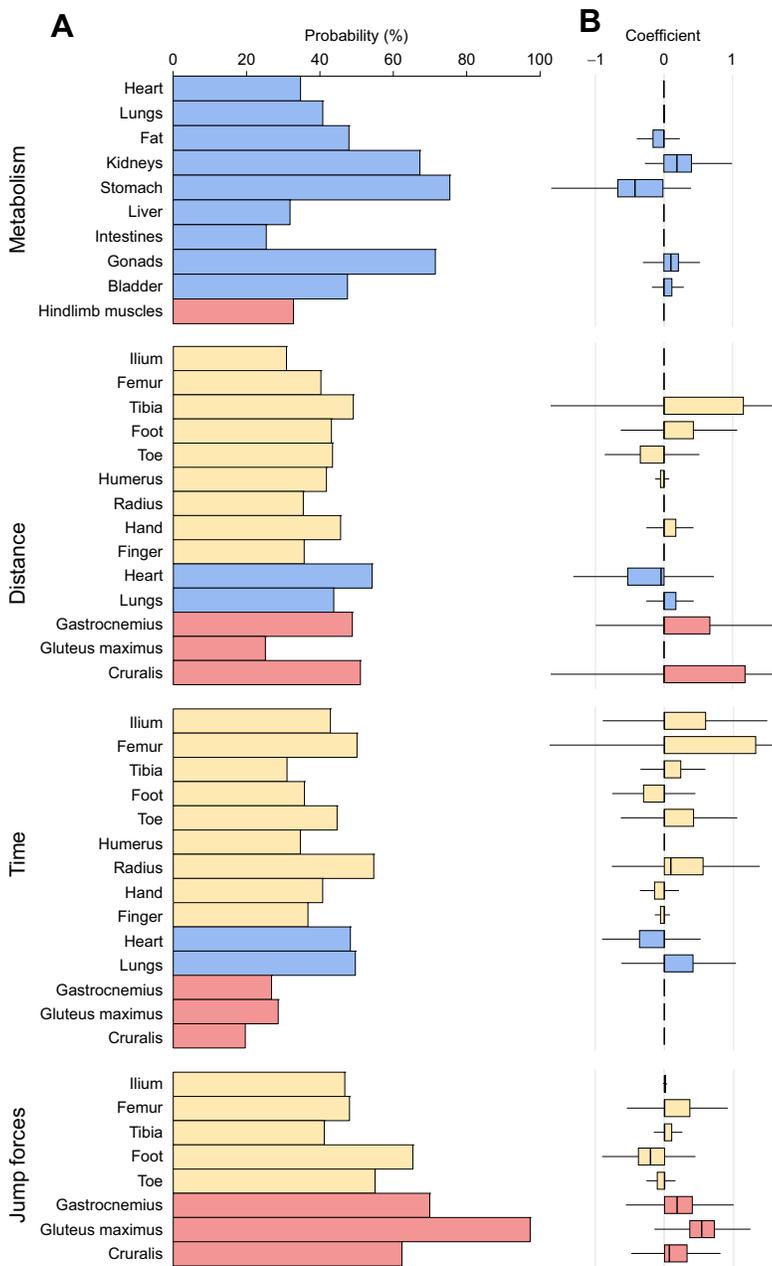


Fig. 3. Results of the lasso regression models with 5000 bootstrap replicates for each residual of performance and predictors on body size (SVL). (A) Probability of selected predictors after all replicates. (B) Boxplot of average coefficients after all replicates (bar: median; boxes: first and third quartiles; whiskers: largest value not more than 1.5 times the inter-quartile range). Viscera variables are represented in blue, bone section lengths in beige, and muscle masses in red.

populations of other invasive anuran species that also showed greater endurance capacity, we were expecting bone length to be a highly selected predictor of endurance. Indeed, longer legs and longer femurs were respectively observed in invasive expanding populations of cane toads, *Rhinella marina*, in Australia (Phillips et al., 2006) and African clawed frogs, *Xenopus laevis*, in France (Loupe et al., 2017). This phenomenon was also recently assessed in an expanded population of native green treefrogs, *Hyla cinerea*, in Southern Illinois (USA), where size-corrected femur length was longer than that of frogs from the historical range (Edwards et al., 2023). The fact that bone length was not often selected as a predictor of endurance in the current study suggests that morphological changes may not appear first. Therefore, other predictors, such as heart rate, \dot{V}_{O_2} , $\dot{V}_{O_{2,max}}$ and, more importantly, behaviour could be key to our understanding of anuran dispersal.

Large gluteal muscles facilitate powerful leaps

Inter-individual variation of jump force was well explained by variation in anatomical traits (Figs 2 and 3). The inter-individual variation in jump force was mostly predicted by body mass and the mass of the gluteus maximus muscle. Bigger individuals or individuals with bigger gluteus muscles were able to produce greater jump force. The model on residuals of body size also revealed that the gluteus muscle was undoubtedly the best predictor even when correcting for individual size. The gluteus muscle standing out as a better predictor than the other leg extensors is an interesting result. In fact, in all individuals, the gastrocnemius and the cruralis muscles were bigger than the gluteus (Table 1). These two bigger leg extensors undoubtedly produce force during jumping, as shown for another frog species, *Hyla multilineata* (James et al., 2005). However, it is the variation of the hip extensor muscle, the gluteus maximus, that principally predicts

inter-individual variation in jump performance. Interestingly, investigations of contractile properties of the gastrocnemius muscle of another expanding invasive frog (*X. laevis*) were not able to find differences between individuals in the core versus those on the range front (Padilla et al., 2020). The current study suggests that the gluteus maximus muscle may be a better target for physiological comparative studies of expanding populations of invasive anurans.

Conclusions

The present study reveals that multiple anatomical traits, such as kidney mass, gluteus maximus muscle mass and body mass, are excellent predictors of invasion-related locomotor and whole-body metabolic traits. We consequently expect that colonization, establishment and/or dispersal can be favoured by inter-individual variation in the size of these organs in the studied species and possibly other anurans. Indeed, selection on these traits may act as an important evolutionary driver of invasion potential, as observed in the current study.

Interestingly, almost half of the inter-individual variation of metabolism and endurance remains unexplained. Individual ‘personality’ is a predictor that we did not investigate and that is often lacking in these types of studies (Careau et al., 2008). Indeed, proactive individuals may be expending energy at a higher rate than reactive individuals (Yuan et al., 2018), which may potentially explain this gap in our predictions. As previously shown by Louppe et al. (2018), metabolic rate changes rapidly during the expansion phase in another species of invasive frog (*X. laevis*) and the optimization of energy expenditure may play a part in their success. Therefore, integrative studies investigating metabolic rate, locomotor performance, anatomical drivers and personality traits would be a great addition for future studies on expanding populations of invasive species.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.P., A.H., M.D.; Methodology: P.P.; Validation: A.H., M.D.; Formal analysis: P.P.; Investigation: P.P.; Data curation: P.P.; Writing - original draft: P.P.; Writing - review & editing: P.P., A.H., M.D.; Visualization: P.P.; Supervision: A.H., M.D.; Funding acquisition: P.P., A.H., M.D.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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