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TOOLS AND TECHNIQUES

A Morphological Method to Approximate Jumping Performance in Anurans for Macroevolutionary Studies

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Abstract

Organismal performance is often key in understanding macroevolutionary patterns but characterizing performance across many species is challenging, as the disparate geographic locations of taxa often prohibit in vivo measures. In theory, however, inferences on the evolution of performance could be investigated using anatomical approximations of performance parameters, allowing for a wider range of species to be sampled. In this study, we use biological and physical principles to mathematically derive three size-standardized anatomical approximations for three different aspects of jumping performance at take-off in anurans: peak jumping velocity, energy, and power. We also describe several ways to parameterize these approximations using, for example, measurements of leg length, leg muscle mass, and body mass. We evaluate the efficacy of these approaches via comparison with direct size-standardized measures of jumping performance across 256 individuals from 51 anuran species. Using both phylogenetic and non-phylogenetic approaches, we find that two of the three anatomical approximations (velocity and energy) are highly correlated with in vivo measures, while a third (power) is not. This reveals that the former may serve as reliable estimates of those aspects of jumping performance, while the latter approximation does not capture all aspects of jumping power in anurans. We also report significant phylogenetic signal for the approximations, as found in in vivo measures. These analyses demonstrate the utility of anatomical approximations for use in macroevolutionary studies. Relative to in vivo laboratory methods, this new method allows for broad museum-based taxonomic surveys of jumping performance in anurans and possibly other jumping animals.

Keywords Anatomy · Frogs · Regression · Phylogenetic signal · Phylomorphospace

Introduction

Understanding the relationship between form and function is paramount in explaining how organisms perform tasks. Arnold (1983) expanded the theoretical context of research on form and function to include individual fitness by proposing the ecomorphological paradigm. This paradigm states that the association between morphology (form) and fitness is mediated by organismal performance (function). Arnold's pioneering work provided a framework for linking morphological variation with ecology and organismal performance (e.g. Pounds 1988; Losos 1990a; Patek et al. 2007; Kaliontzopoulou et al. 2012). Studies based on it have led to diverse insights into the evolutionary processes that have shaped patterns of phenotypic diversity (e.g. Losos 1990b; Kaliontzopoulou et al. 2015; Muñoz et al. 2017).

Anuran amphibians have long been a focus of understanding the relationship between anatomical form and organismal performance. These studies have largely focused on jumping and the degree to which anatomical traits relate to measures of jumping performance. Early work identified statistical associations between morphological measures such as leg length or body size, with performance measures such as jumping distance or acceleration (e.g. Rand and Rand 1966; Zug 1972, 1978; Emerson 1978). Notably, integrating biomechanical theory with functional morphology resulted in a series of analytical equations that relate different aspects

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of jumping performance, such as velocity or angle at take-off to peak jumping distance (Alexander 1968; Bennet-Clark 1977; Marsh 1994; Peplowski and Marsh 1997). Such measures have proven useful in characterizing variation in jumping performance within species (Marsh and John-Alder 1994; Wilson et al. 2000; James et al. 2007; for non-anuran examples see: Toro et al. 2003; Toro et al. 2004).

In recent years, researchers have used a cross-species approach to examine the evolutionary associations between anuran morphology, performance, and ecology from a phylogenetic perspective (e.g. Gomes et al. 2009; Moen et al. 2013; Citadini et al. 2018; Astley 2016; Moen 2019). For example, Gomes et al. (2009) showed that jumping distance in anurans depends on both microhabitat use and body size. Likewise, Moen et al. (2013) revealed that across continents, locomotor performance and morphology were associated with microhabitat use, a pattern implying evolutionary convergence of functional traits. Such studies have revealed much about the extent to which ecomorphological patterns are shaped by ecological and evolutionary factors. Nevertheless, the degree to which such trends are displayed across the broader diversity of anurans remains underexamined. One reason for this is that, as a clade, anurans are circumglobal and species are found in diverse ecological communities across six continents (Pyron and Wiens 2013; Moen et al. 2016). As such, obtaining comprehensive datasets of live-animal jumping performance across many species is logistically challenging.

An alternative approach to collecting in vivo measurements of performance is to develop anatomical estimates that may serve as approximations for jumping performance. For instance, Carroll et al. (2004) developed a biomechanical model for predicting suction feeding performance in centrarchid fishes, based on a series of anatomical parameters. They found a strong relationship ($r^2 = 0.71$) between in vivo suction feeding performance and that predicted by morphology across five species, implying that their model may serve as an approximation for suction performance in this group. The approach of Carroll et al. (2004) serves as a conceptual template for how anatomical approximations may be developed for other aspects of organismal performance.

Motivated by their example, we develop three size-standardized anatomical approximations for jumping performance to serve as estimates of three key aspects of jumping performance at take-off in anurans: peak velocity, energy, and power. Since these three performance measures commonly scale with body size (see e.g., Marsh and John-Alder 1994; Astley 2016), we account for this pattern by size-standardizing the approximations. Specifically, our concern is whether these may serve as reasonable approximations of the relative differences in jumping performance between species, and thus may serve as approximate measures of performance in macroevolutionary studies. Thus, we evaluate the efficacy of the size-standardized approximations by comparing them with direct size-standardized measures of performance using a dataset containing 256 individuals and 51 species of anurans that span a broad range of morphological, ecological, and geographic diversity. We also describe several ways to parameterize these anatomical approximations to obtain precise estimates of jumping performance. In developing these approximations, we provide a new avenue for obtaining estimates of jumping performance, and we set the stage for future broad-scale macroevolutionary studies of jumping performance in anurans and potentially other jumping animals.

Methods

Anatomical Approximations for Jumping Performance

Here we derive approximations for several aspects of jumping performance during take-off, including peak velocity (Eq. 1), peak energy (Eq. 3), and peak power (Eq. 5). These approximations are derived through mathematical equivalencies between equations from physics and their analogous equations obtained from functional morphology (e.g. Peplowski and Marsh 1997). We then describe size-standardization using conventional methods. Specific morphological measures used as input variables to estimate our model parameters are discussed below. Presently, we do not consider the effects of limb positioning or power amplification on jumping, since these phenomena have not been yet been characterized across a sufficiently wide set of taxa to facilitate parameterization (see "Discussion" section).

Peak Jumping Velocity

We first note that many of our kinematic equations assume constant acceleration, which does not occur in frog jumping (Marsh and John-Alder 1994). Although variable acceleration influences the relationship between velocity and time, previous work has shown that acceleration profiles have similar shapes across individuals and species (e.g. Marsh and John-Alder 1994; James and Wilson 2008; Moen et al. 2013). Thus, for example, the relationship between any estimate of average jumping velocity (v_{avg}) and peak velocity (v_f) at take-off should be constant across individuals and species. We can leverage the comparative nature of our study to find an estimator of peak jumping velocity, because differences between mean velocity estimates under constant acceleration and under variable acceleration will be similar across species.

Under constant acceleration, the movement of an organism during a jump is described by the kinematic equation

$v_f^2 = v_i^2 + 2da$, where v_i is the initial velocity, d is the distance traveled by a body, and a is acceleration in the direction of motion during take-off. $v_i = 0$ for organisms at rest, before the start of a jump. One can substitute d with a morphology-based estimate, L_{com}, the distance from the tip of the toes to the center of mass of the organism (Peplowski and Marsh 1997). By solving for v_f and applying Newton's second law ($F = ma$, where m is body mass and F is muscle force), we arrive at an approximation for peak jumping velocity whose parameters can be estimated entirely from anatomical measurements:

$$v_{Appx.} = \sqrt{\frac{2L_{com}F_{PCSA}}{m}}. \quad (1)$$

Here, F_{PCSA} is an estimate of muscle force obtained from muscle physiological cross-sectional area (PCSA; see section on morphological measures below). Dimensional analysis of this anatomical approximation for velocity yields dimensions of length per time, as in actual velocity. Possible morphological measures that may be used as input variables for m, L_{com}, and F_{PCSA} are discussed below. Note that for interspecific datasets used in phylogenetic comparative studies, size is a confounding factor. Thus, as is implemented in some comparative biomechanical studies (e.g. Marsh and John-Alder 1994; Astley 2016), snout-vent length (SVL) may be used to size-standardize $v_{Appx.}$, yielding units of body lengths per time (if L_{com} and SVL share the same units).

Peak Jumping Energy

From classical physics, we know that kinetic energy (E) at take-off is:

$$E = \frac{1}{2}mv_f^2. \quad (2)$$

By using Eq. 1 to estimate v_f in Eq. 2, we arrive at an anatomical approximation for peak jumping energy as (Eq. 3):

$$E_{Appx.} = L_{com}F_{PCSA}. \quad (3)$$

Dimensional analysis of $E_{Appx.}$ yields dimensions of mass times length squared per time squared (i.e. the same dimensions as kinetic energy), and units of Joules (J) if estimated using SI units. We note that change in energy, or the product of force and distance, is the work done in performing a jump. Work and peak jumping energy are equivalent when initial energy is 0, as in an organism at rest prior to initiating a jump. As above, interspecific datasets used in phylogenetic comparative studies must account for differing body sizes among species. In this case, we use body mass (m) to size-standardize $E_{Appx.}$ (see Marsh and John-Alder 1994). We note that other standardizations are possible. For example, Astley (2016) standardized peak energy by muscle mass instead of body mass.

Peak Jumping Power

Jumping power, like acceleration, is also known to vary throughout the take-off phase (Marsh and John-Alder 1994). Similar to how average jumping velocity (v_{avg}) is a summary statistic of change in position, average power (P_{avg}) describes the average amount of mechanical work done per unit time during take-off. Average power (P_{avg}) from rest to take-off (see Eq. 15 in Marsh 1994) is peak kinetic energy (at take-off) divided by contact time:

$$P_{avg} = \frac{mv_f^2}{2t}. \quad (4)$$

To estimate peak power (P) at take-off, we may substitute Eq. 1 to estimate v_f and the relationship $t = \frac{2L_{com}}{v_f}$ (Marsh and John-Alder 1994) into Eq. 4, and double it ($P = 2P_{avg}$; Peplowski and Marsh 1997) to arrive at an anatomical approximation for peak jumping power (Eq. 5):

$$P_{Appx.} = \sqrt{\frac{2L_{com}F_{PCSA}^3}{m}}. \quad (5)$$

Dimensional analysis of $P_{Appx.}$ yields dimensions of mass times length squared per time cubed (as in actual power), and units of Watts (W) if estimated using SI units. As we did for peak jumping energy, we standardized $P_{Appx.}$ to size with body mass (m; see Marsh and John-Alder 1994). As above, an alternative standardization is given by Astley (2016), who standardized peak power by muscle mass.

Morphological Measures Used in Approximations

Equations 1–5 provide mathematical derivations of approximations for peak jumping velocity, energy, and power based on anatomical measurements. Estimating jumping performance for an individual using the anatomical approximations above requires that specific morphological measures be used as input variables for m, L_{com}, and F_{PCSA}. Overall, we emphasize that the specific measurements chosen for study will affect the values and scaling of the approximations and hence their correspondence to in vivo measurements of performance. In most cases the exact values of the approximations will not be equivalent to those from observed performance estimates (see below for further discussion).

While a number of different measurement schemes can be envisioned (see Online Resources 1A and 1B), the following approach based on data available in Moen et al. (2013) and Astley (2016) was used in this study. 1: Mass (m): the body mass of the individual during the trial in which it produced

its peak jumping velocity (i.e. the trial whose performance data were published by Moen et al. 2013) or at the time of dissection (Astley 2016). See Online Resource 1A for discussion of using wet and preserved body masses as estimates of body mass. 2: L_{com}: Previous authors (Peplowski and Marsh 1997) have defined L_{com} as the distance from the tip of the toes to the center of mass of the organism. For the present study, we used total hind limb length (L_{hl}) as our measure, which will be shorter. 3: muscle force (*F):* For a single muscle, force is equivalent to its physiological cross-sectional area (*PCSA*) times the specific tension of the muscle: $F = PCSA * specifictension$ (Sacks and Roy 1982). See Online Resource 1B for additional alternative estimates of *F*. For a given value of *specific tension*, which has been shown to be relatively invariant among different types of muscle fibers, muscles, and vertebrates (see Online Resource 1C), *F* is thus proportional to *PCSA*: $F \propto PCSA$. Notably, Fukunaga et al. (1996) reported a strong correlation between muscle force exerted on the Achilles tendon in humans and both the *PCSA* and anatomical cross-sectional area (*ACSA*) of the ankle flexors. Furthermore, Hellam and Podolsky (1969) found that *F* is highly correlated to anatomical cross-sectional area (*ACSA*) in the semitendinosus muscle of the upper leg in *Rana pipiens*. ACSA may be used to approximate *PCSA*, but this does not account for muscle pinnation. Thus, this approximation will be most problematic for heavily pinnate muscles, such as the plantaris longus (Calow and Alexander 1973). However, all upper-leg muscles that contribute to the jump are parallel-fibered (Calow and Alexander 1973; Olson and Marsh 1998). Moreover, most gross architecture of anuran muscles (e.g. identities of muscles; whether a given muscle is parallel-fibered or pinnate) is congruent across highly divergent species (Duellman and Trueb 1986; Prikryl et al. 2009), and data on fine-scale architecture (e.g. angle of pinnation in the plantaris) is not available for most species. Therefore, following Marsh (1994), and assuming geometric isometry of muscles across species, we used the 2/3 root of muscle mass to estimate muscle force (*F*):

$$F \propto ACSA \propto m_{muscle}^{2/3}. \quad (6)$$

As we suggested before, all muscle fibers (i.e. the total cross-sectional area) from some extensor muscles in the upper and lower leg generate force during a jump (Hirano and Rome 1984; Lutz and Rome 1994). Therefore, following Eq. 6, we used the combined bilateral sum of muscle masses from the upper and lower portions of the hind limb to represent *F*. Although we use muscle mass to approximate muscle force (*F*), we recognize that alternative morphological measurements may provide improved representations of muscle force. For example, the muscles of the back and pelvis function in powering and controlling jumps (Richards et al. 2018). However, since back muscles make up < 0.5% of body mass (Emerson and De Jongh 1980), their contribution to jumping is small compared to leg muscle mass, which makes up 5–30% of body mass in anurans (Mendoza et al. 2020). Furthermore, Clemente and Richards (2013) found that plantaris muscle force scaled with body mass to the 0.94 power in *Xenopus laevis*, and not 2/3 as predicted above by geometric isometry. Thus, an improved estimate of the m_{muscle} exponent in Eq. 6 is possible for some species. However, across a broader set of body sizes and habitat utilization, the scaling of Marsh (1994) may be preferable (see additional discussion in Online Resources 1B and 1C). Future improvements of our approach should include more precise species-specific estimates of plantaris pinnation, specific tension of various muscles, and thus, force.

Data Collection

To determine the relationship between each anatomical approximation and in vivo measurements of jumping performance, we used the subset of data from Moen et al. (2013) and Astley (2016) that included necessary trait measurements (i.e. leg muscle mass) and species with more than one individual. Additionally, we did not include Astley's (2016) data on *Heterixalus alboguttatus* because these values showed unusually low performance for this genus; power values were more than an order of magnitude lower than our (DSM's) unpublished data on two other congeners and are inconsistent with all other published data on arboreal frogs of the same body size (Mendoza et al. 2020). Overall, our dataset comprised morphological measures and jumping performance from a total of 256 adult anurans from 51 species, which spanned a wide body-size range and were found in a variety of microhabitats.

The morphological measurements of Astley (2016) were collected from freshly dissected individuals. Morphological measurements from Moen et al. (2013) were collected on preserved specimens, with the exception of live body mass, which was the body-mass measurement made just prior to that individuals' highest velocity jump. The preserved length measurements included length of the femur, tibiofibula, tarsus, and foot. We obtained estimates of wet muscle mass for these individuals from Mendoza et al. (2020), who estimated wet muscle mass for the Moen et al. (2013) taxa as the product of live body mass and the live muscle-mass-to-body-mass ratio from a subset of taxa for which both types of data were available. These wet muscle mass estimates from Mendoza et al. (2020) came from the same individuals for which linear measurements were obtained (Moen et al. 2013). Although preservation is known to change the magnitude of original trait values through dehydration (Vervust et al. 2009; Shu et al. 2017; Sotola et al. 2019), the correlation between live and preserved body mass in anurans can be

quite high ($r > 0.98$; Shu et al. 2017). Similarly, in our study we found a correlation of $r > 0.99$ (see Online Resource 1A). Thus, we expect that future comparative studies that use only museum specimens (and thus preserved body and muscle mass) will give largely consistent results as with live or otherwise unpreserved measures (as we consider here).

Moen et al. (2013) and Astley (2016) obtained jumping performance from individual live frogs, including data used to calculate size-standardized peak velocity, energy, and power at take-off for this study. Both studies reported peak performance as the estimates collected from each individual's single fastest jump. Moen et al. (2013) took high-speed videos of jumping take-off for each frog, digitized and smoothed the distance-time plots, and calculated derivatives of these plots to estimate peak velocity and acceleration. Frogs were jumped over 3–5 sessions during the course of a week until performance was visually reduced (usually after 4–5 jumps) in each session (for additional details, see Moen et al. 2013). Astley (2016) collected performance data using either a force plate or high-speed videos, using methods similar to those found in Moen et al. (2013) to analyze data from high-speed videos. Frogs were jumped over a minimum of 5 sessions and with a period of 5 min rest between trials (for additional details, see Astley 2016). Finally, we calculated species means for each approximation and jumping performance measure and followed the size-standardization procedures described above to account for differences in mean body size across species.

Statistical Analysis

We evaluated the efficacy of each anatomical approximation using several procedures. First, since species means are not independent due to shared ancestry (Felsenstein 1985), we used phylogenetic generalized least squares (PGLS) regression (Martins and Hansen 1997; Blomberg et al. 2012; Adams 2014) to test for an association between each size-standardized approximation and its corresponding size-standardized measure of jumping performance. To obtain a time-scaled phylogeny, we drew 1000 replicate trees from a pseudo-posterior distribution of time-calibrated molecular phylogenies (Jetz and Pyron 2018) which included every species in the datasets of Moen et al. (2013) and Astley (2016). We calculated mean branch lengths for a consensus topology of these 1000 replicate trees. We then pruned this phylogeny for correspondence with the dataset using *geiger* version 2.0.7 (Harmon et al. 2008). See Online Resource 1D and 2 for further details on phylogeny generation for our analyses and access to the phylogeny. Additionally, we evaluated the relationship between the size-standardized approximations and measures of jumping performance using ordinary least squares (OLS) regression. See Online Resource 1E for details on OLS regressions. Finally, we used the Akaike Information Criterion (AIC) to evaluate the efficacy to predict in vivo jumping performance from the size-standardized approximations versus several linear anatomical measurements treated individually. These individual anatomical measurements included the input variables for each approximation. We standardized each individual variable using SVL or body mass, where appropriate, following previous studies linking morphology with jumping performance in anurans (e.g. Gomes et al. 2009; Astley 2016; Moen 2019). For example, we compared $v_{Appx.}$ with each one of its input variables including L_{com}, leg muscle mass, and body mass, after standardizing each by SVL.

For both the OLS and PGLS regressions, we report traditional regression statistics alongside standardized effect sizes (z-scores) obtained from empirical sampling distributions (sensu Collyer et al. 2015). We also calculated 95% confidence intervals and obtained the coefficient of determination (r^2) for these models, which is interpreted as a measure of the utility of the anatomical approximations. While we derived the approximations to have the same units as in vivo measurements of performance, our use of muscle mass to represent force changes the units and relative scale of the approximations (see Figs. 1–3), rendering the slope and intercept less useful as measures of utility than overall correspondence between approximated and observed performance. We implemented regressions and obtained

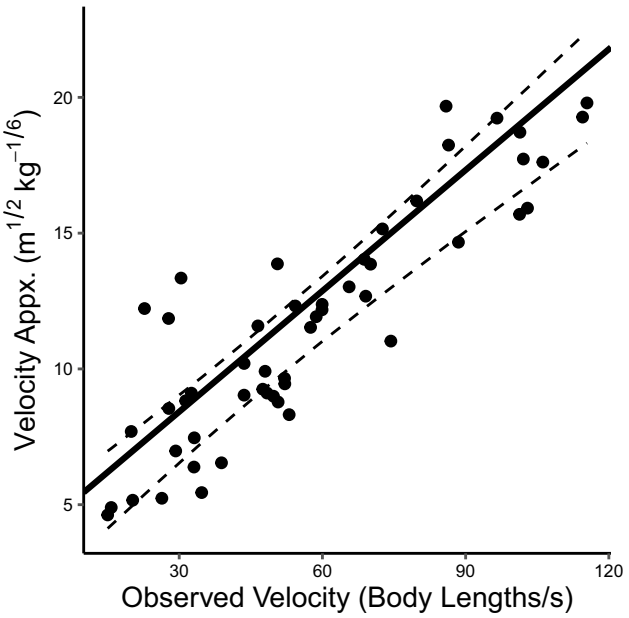

Fig. 1 Approximating peak jumping velocity. Points are species means. Dashed lines indicate upper and lower bounds of a 95% confidence interval for the phylogenetic generalized least squares regression line. In anurans, "body length" refers to snout-vent length (SVL). Statistics are as follows: $F = 141.35$, $r^2 = 0.74$, $P < 0.0001$, $z = 2.78$. Regression equation is: $Approximation = 3.972 + 0.148\frac{Velocity}{SVL}$, where SVL is snout-vent length

confidence intervals and effect sizes (z-scores) using RRPP, a procedure where residuals are randomized (i.e. permuted) to obtain model effects and evaluate model significance (for details see: Collyer et al. 2015; Adams and Collyer 2018a,b). We used the R-package *RRPP* version 0.6.0.9000 (Collyer and Adams 2018) to implement our regressions, using 10,000 permutations for tests of statistical significance. Further investigation comparing models based on individuals rather than mean values, and the effect of sex on our approximations, may be found in Online Resources 1F and 1G.

Finally, to illustrate the utility of these anatomical approximations for evaluating evolutionary trends, we performed a series of phylogenetic signal analyses to determine if covariation in jumping performance (as estimated by size-standardized anatomical approximations) matches the evolutionary relationships among taxa, and the degree to which they might. To do this we estimated both multivariate phylogenetic signal K_{mult} (Adams 2004), for the dataset encompassing all three approximations, and the univariate estimate of phylogenetic signal K (Blomberg et al. 2003) for each of the three traits treated separately. We also estimated phylogenetic signal in the same manner for the in vivo measurements of peak jumping performance, for comparison. For each, we used 10,000 permutations to obtain model effects and evaluate significance. We then used principal components analysis and a phylomorphospace approach (by scaling each variable to unit variance) to investigate macroevolutionary trends and structure in our data (Sidlauskas 2008). Since the signs of all variable loadings and scores are arbitrary and only their relative sign patterns (contrasts) and relative magnitudes are meaningful (Jolliffe and Cadima 2016), we multiplied PC1 loadings and scores by -1 to enhance interpretability. Additionally, we labeled species by microhabitat using the data of Moen et al. (2013) and data found on AmphibiaWeb (2020). We analyzed phylogenetic signal and made phylomorphospace plots using *geomorph* version 3.3.1 (Adams et al. 2020).

Results

We found that size-standardized anatomical approximations for peak jumping velocity and energy, but not the approximation for jumping power, were highly and significantly correlated with jumping performance, regardless of whether we used ordinary least squares (OLS) or phylogenetic generalized least squares (PGLS) regression. PGLS regression yielded both high r^2values and effect sizes (empirically-derived z-scores) for peak jumping velocity (Fig. 1; $F=141.35$, $r^2=0.74$, $P<0.0001$, $z=2.78$) and peak jumping energy (Fig. 2; $F=118.18$, $r^2=0.71$, $P<0.0001$, $z=2.68$). We found a relatively lower r^2 for the approximation of peak jumping power (Fig. 3; $F=25.83$, $r^2=0.35$, $P<0.0001$,

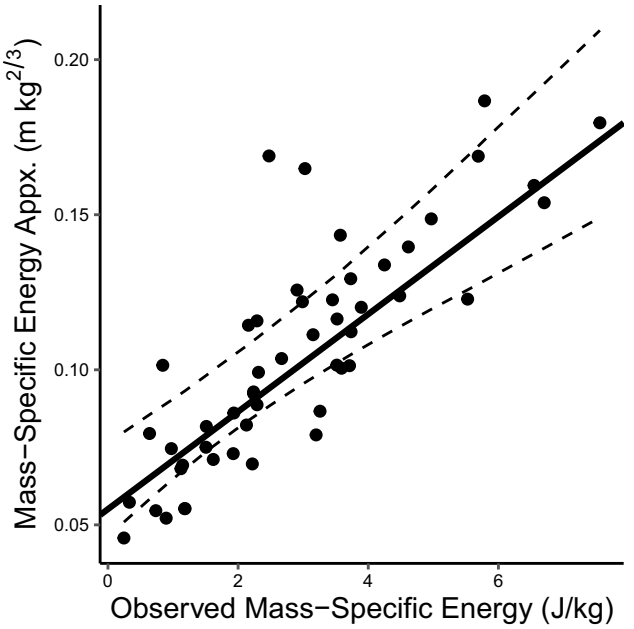

Fig. 2 Approximating peak jumping energy. Points are species means. Dashed lines indicate upper and lower bounds of a 95% confidence interval for the phylogenetic generalized least squares regression line. Statistics are as follows: $F=118.18$, $r^2=0.71$, $P<0.0001$, $z=2.68$. Regression equation is: *Approximation* = 0.055 + 0.016 *Mass–Specific Energy*

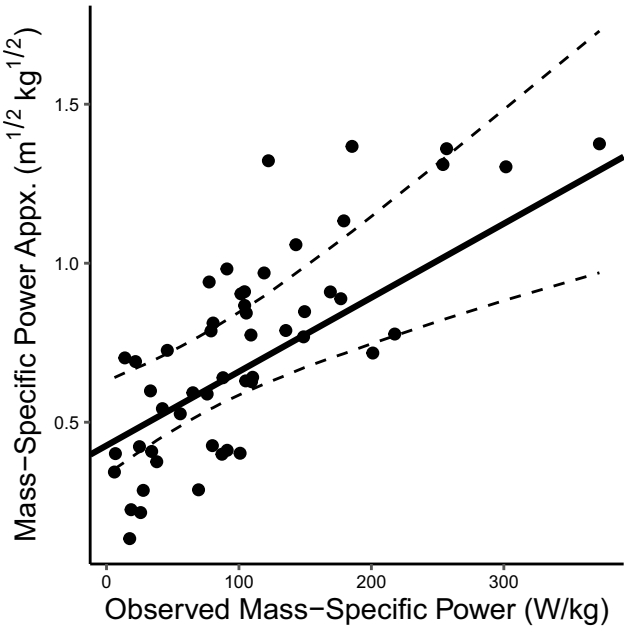

Fig. 3 Approximating peak jumping power. Points are species means. Dashed lines indicate upper and lower bounds of a 95% confidence interval for the phylogenetic generalized least squares regression line. Statistics are as follows: $F=25.83$, $r^2=0.35$, $P<0.0001$, $z=2.03$. Regression equation is: *Approximation* = 0.427 + 0.002 *Mass–Specific Power*

$z = 2.03$). We present results for OLS regressions in Online Resource 1E. Model comparisons revealed that in the case of jumping velocity, the approximation outperformed other size-standardized variables including leg length ($\Delta AIC = 63$, $r^2 = 0.10$), leg muscle mass ($\Delta AIC = 63$, $r^2 = 0.10$), and body mass ($\Delta AIC = 56$, $r^2 = 0.23$). These relationships between velocity, leg length, and leg muscle mass are weaker than those found previously (Moen 2019), but the differences between studies disappear when we do not standardize velocity by body length (as in Moen 2019, results not shown). The approximation for jumping energy outperformed leg length ($\Delta AIC = 56$, $r^2 = 0.12$) and leg muscle mass ($\Delta AIC = 6$, $r^2 = 0.67$). Finally, we found that the jumping power approximation did not outperform all other variables, with the preferred variable being leg muscle mass ($\Delta AIC = 0$, $r^2 = 0.48$), followed by the approximation ($\Delta AIC = 12$, $r^2 = 0.35$), and leg length ($\Delta AIC = 33$, $r^2 = 0.00$). These latter results indicate that some aspects of jumping power are not captured well with this approximation.

Additionally, we found significant phylogenetic signal (though with traits showing less signal than expected under Brownian Motion) and strong effect sizes in our data for both the anatomical approximations and in vivo measures of jumping performance. In a multivariate context, the phylogenetic signal for the approximations of jumping performance was $K_{mult} = 0.71$ ($P < 0.0001$, $z = 5.36$). Likewise, univariate phylogenetic signal for the approximations showed significant phylogenetic signal and strong effect sizes (peak jumping velocity: $K = 0.58$, $P < 0.0049$, $z = 3.03$; energy: $K = 0.77$, $P < 0.0002$, $z = 4.45$; power: $K = 0.78$, $P < 0.0006$, $z = 4.38$). Analyses of phylogenetic signal for in vivo measurements of jumping performance yielded similar estimates ($K < 1$) for the multivariate set of traits ($K_{mult} = 0.72$, $P < 0.0012$, $z = 4.36$) and each trait individually: peak jumping velocity ($K = 0.82$, $P < 0.0003$, $z = 4.77$), energy ($K = 0.63$, $P < 0.0053$, $z = 3.09$), and power ($K = 0.76$, $P < 0.0048$, $z = 3.41$). These results emphasize the correspondence of macroevolutionary results between anatomical approximations and in vivo measurements of peak jumping performance. Investigation of these size-standardized variables using principal components demonstrated that most of the variation in the dataset (70.53%; Table 1) is encompassed by an axis which contrasts species with high values for all three aspects of peak jumping performance and species with low values for all three variables. Thus, PC1 of the resulting phylomorphospace (Fig. 4) represents an evolutionary contrast between strong and weak jumpers. For example, we found the best jumper in the dataset (the striped rocket frog *Litoria nasuta*) and the worst jumper (*Duttaphrynus melanostictus*) on opposite ends of PC1. Likewise, variation along PC2 was defined by 'inefficient' and 'efficient' jumpers who display contrasting levels of mass-specific energy, relative to their peak jumping velocity. For example, we found *Odorrana grahami* (a high mass-specific energy, low velocity jumper) and *Syncope bassleri* (a low mass-specific energy, high velocity jumper) on opposite ends of PC2.

Table 1 Eigenvectors of principal components analysis (variable loadings) based on approximations for peak jumping performance. Parentheses of PC axes indicate percent variance explained

	PC1 (70.53%)	PC2 (28.62%)	PC3 (0.86%)
$v_{Appx.}$	0.582	−0.564	−0.585
$E_{Appx.}$	0.442	0.823	−0.354
$P_{Appx.}$	0.682	−0.052	0.730

Discussion

In this study, we developed a series of size-standardized anatomical approximations for three estimates of jumping performance (peak velocity, energy, and power at take-off). We found strong, statistically significant relationships relating in vivo jumping performance of 51 species and the velocity and energy approximations based on their morphology. These approximations outperformed individual anatomical variables. Such results imply that our approach—based solely on anatomy—may serve as useful approximations of relative jumping performance in anurans. By contrast, we found the approximation for jumping power to be a poor predictor of in vivo jumping performance, implying that some aspects of anuran jumping power are not captured with this approximation (see below). We also characterized the phylogenetic signal of these size-standardized approximations of peak jumping performance and determined the principal evolutionary axes of variation in these traits, confirming the utility of these approximations in a phylogenetic comparative context. These findings demonstrate that anatomical approximations of anuran jumping performance can potentially further our understanding of macroevolution and the evolutionary shifts in these traits throughout the diversification of a lineage. In addition, given that our dataset encompassed a broad degree of taxonomic, morphological, and ecological diversity, the approximations presented here represent a new tool useful in comparative studies that can be used to obtain relative estimates of jumping performance. They can also be used to leverage data from museum specimens in studies of functional evolution. As such we argue that the results of this work can be used to learn about relative jumping performance, in a comparative context, by facilitating the incorporation of additional taxa where direct estimates of performance are not possible.

One key benefit of the approach used here is that the approximations are flexible; they may be estimated using a variety of different morphological measures and do not

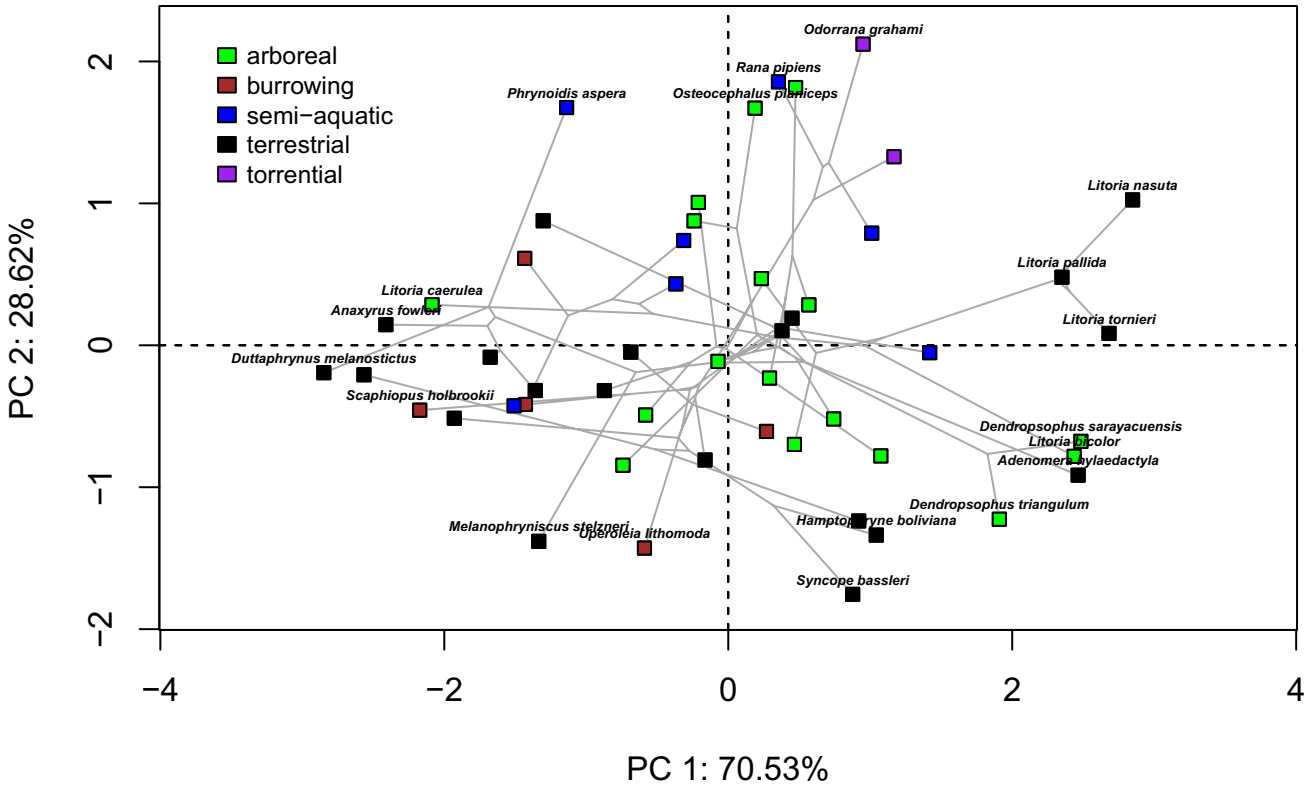

Fig. 4 Phylomorphospace of size-standardized peak jumping performance approximations. Variables in analysis included each approximation for peak jumping velocity, energy, and power. Variables were scaled to unit variance prior to analysis. Large colored squares are species. See Table 1 for variable loadings and variation explained by each axis (Color figure online)

require procedures that may damage museum specimens. For example, non-invasive measurements of the external dimensions of leg muscles may be used to estimate the cross-sectional area of those muscles to be used as approximations of muscle force, given the allometric principles described in Eq. 6. This is especially important in cases where leg muscle mass cannot be obtained (e.g. endangered species, rare museum specimens). It should be emphasized that the anatomical approximations are only in the same absolute units as the traditional measures of jumping performance when actual estimates of muscle force are used, rather than the allometric scaling approximations based on mass that we used here. For this reason, direct comparisons of the values of the approximations to estimates obtained using in vivo laboratory methods are not advised, given that they do not scale equally (e.g. compare axes of Figs. 1–3).

Our results demonstrate the efficacy of anatomical approximations of jumping performance. However, we recognize that our approach does not capture some aspects of the biomechanics of jumping. For instance, power amplification (Marsh and John-Alder 1994; Astley and Roberts 2012) and limb positioning (Kargo and Rome 2002; Nauwelaerts and Aerts 2006) may contribute to jumping ability in certain anuran species. Moreover, smaller species may use power amplification to a greater extent than do larger species (Sutton et al. 2019; Mendoza et al. 2020). Thus, power amplification and/or limb positioning across taxa of differing body sizes could affect variation in jumping performance at the macroevolutionary scale (such as in burrowing species; Mendoza et al. 2020). As others have discussed, percent muscle mass has been shown to play a large role in powering a jump (James and Wilson 2008; Astley 2016; Moen 2019). Specifically, muscle mass of the hamstring (semimembranosus) and calf (plantaris) muscles in anurans has been shown to correlate with power amplification (Roberts and Marsh 2003; Astley and Roberts 2012; Astley 2016). Thus, our result that the proportion of muscle mass relative to body mass outperformed the power approximation may imply that our approximation does not capture relevant variables involved in power amplification, offering little advantage over prior approaches based on relative muscle mass alone. Further research should seek improvements to the power approximation, perhaps including aspects of power amplification that may partially decouple power from F_{PCSA} (e.g. material properties of elastic elements, muscle power). However, at present we cannot incorporate these effects into the anatomical approximations presented here, as one must first develop reliable biomechanical models used to predict

the degree of power amplification found across a wide range of anuran species. Nonetheless, as we elucidate the extent to which different species use power amplification (Mendoza et al. 2020), refinements to the approximations that accommodate such effects could be developed.

An additional caveat with the approximations presented here is that they are not guaranteed to yield reliable values in situations when morphology does not match behavioral performance. For example, some walking or swimming species may jump poorly due to behavioral differences but retain ancestral hindlimb proportions characteristic of primarily jumping close relatives. This may lead to imprecise estimates of jumping performance in those taxa because the anatomical approximations do not reflect behavior. Thus, a strong knowledge of the natural history of the species under investigation remains useful for interpreting patterns obtained from these estimates. We also note that while primary locomotor mode might change, some of these species retain the ability to jump well (e.g. the "running" frog *Kassina*; Porro et al. 2017; Richards et al. 2017). That said, we generally advise that anatomical approximations of jumping performance be applied only to species whose primary mode of locomotion is jumping.

We did not address here whether the approximations are capable of predicting jumping performance within species. Given our results using interspecific data, we anticipate that the relationship between form and function captured by the approximations is also reflected at the intraspecific level. However, the influence of scale (range of values) on the relationship between the approximations and jumping performance found in an intraspecific dataset may yield novel results, especially given that some studies have shown a reduced fit between morphology and performance within species (Emerson 1978; Wilson et al. 2000) as compared to across them (Emerson 1978; Gomes et al. 2009; Astley 2016; Moen 2019).

Additionally, sexual size dimorphism is prevalent in anurans (> 90% of species; De Lisle and Rowe 2013; Han and Fu 2013; Nali et al. 2014). Yet little is known about patterns of sexual shape dimorphism in anurans and its relationship to jumping. While there is some evidence that jumping performance (Moen et al. 2013) and hindlimb morphology (Petrović et al. 2017) may be similar between sexes across some taxa, this has yet to be examined thoroughly in an allometric context. This, then, raises the question as to whether morphology and jumping performance have evolved differently in male and female anurans. For example, in anurans, sexual dimorphism related to locomotor traits has been described within and between species (Hudson et al. 2016; Petrović et al. 2017). Furthermore, in lizards, it has been shown that morphological patterns evolve as a function of distinctive selective pressures associated with sex-specific habitat use (Kaliontzopoulou et al. 2015). If such trends are also present in anurans, selection for jumping performance and its associated traits (e.g. body mass, leg length) may also differ relative to allometry. As such, the evolution of sexual size and shape dimorphism, and how jumping performance relates to shape in an allometric context, is an important area of future research. One trait of particular relevance in anurans is mass, which in females may be greatly influenced by the amount of eggs being carried. Studies in lizards have found a negative relationship between increased loads and jumping take-off speeds (Kuo et al. 2011) or sprinting speed (Sinervo et al. 1991; Shine 2003). Since mass is explicitly incorporated in our approximations, at least some of the effect of an increased load exhibited by egg-carrying females should be reflected in the approximation values. Yet, this represents an area of further development in these anatomical approximations.

Finally, a particularly exciting avenue of future research concerns whether the approximations developed here can predict jumping performance in other jumping organisms, including mammals, lizards, arachnids, and insects. The same equations from functional morphology have been used independently to estimate jumping performance in both anurans (Marsh 1994; Marsh and John-Alder 1994; James et al. 2007) and *Anolis* lizards (Pounds 1988; Losos 1990a,b; Toro et al. 2003, 2004). All of these studies are ultimately based on the work of Alexander (1968), which provided the foundation of the ballistics equations used in these other studies, implying that the approximations derived here may be applicable across a wide variety of jumping organisms. We hypothesize that our approximations should be useful in estimating relative differences in jumping performance in all jumping vertebrates, given that most vertebrates are large enough that air resistance will not greatly affect jumping performance (Vogel 2005). One area of future research concerns whether the anatomical approximations presented here are useful in arthropods, which use a combination of muscle extensors and hydraulic pressure to produce force during a jump (Ellis 1944; Dillon 1952).

Conclusion

Estimating jumping performance using traditional in vivo techniques for frogs that live all around the world is logistically challenging. To bypass some of these challenges, we provide an alternative way of estimating relative jumping performance via anatomical approximation. By combining equations from physics and functional morphology, we derive size-standardized anatomical approximations that are able to predict jumping performance with high precision using only morphological data. These approximations are designed to be flexible through the use of input data such as muscle mass or diameter, which would

minimize destructive sampling of museum specimens. Using this new approach, on one hand our results echo previous studies: there is a strong relationship between percent muscle mass and peak jumping power, highlighting the role of power amplification at macroevolutionary scales. On the other hand, we demonstrate the efficacy of estimates for peak jumping velocity and energy, which implies that they may be useful in further elucidating the associations between morphology, performance, and ecology in anurans.

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Data Availability Time-scaled phylogenetic tree used in analyses may be found in the Online Resource.

Code Availability Scripts for all analyses in this study can be found at github.com/bhjuarez/approximations-for-jumping.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts or competing interests.

Informed Consent The authors all consent to publication.

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