

Explosive Jumping: Extreme Morphological and Physiological Specializations of Australian Rocket Frogs (*Litoria nasuta*)

Rob S. James^{1,*}

Robbie S. Wilson^{2,†}

¹Department of Biomolecular and Sport Sciences, Faculty of Health and Life Sciences, Coventry University, Coventry CV1 5FB, United Kingdom; ²School of Integrative Biology, University of Queensland, St. Lucia, Queensland 4072, Australia

Accepted 6/25/2007; Electronically Published 1/11/2008

ABSTRACT

Anuran jumping is an ideal system for examining the relationships between key morphological, physiological, and kinematic parameters. We used the Australian rocket frog (*Litoria nasuta*) as a model species to investigate extreme specialization of the vertebrate locomotor system for jumping. We measured the ground reaction forces applied during maximal jumps using a custom-designed force platform, which allowed us to calculate instantaneous measures of acceleration, velocity, power output, and total jump distance. We quantified the mechanical properties of the plantaris longus muscle using the work loop technique. We found that *L. nasuta* achieved the second-longest relative jumping distance for any anuran (55.2 body lengths for one individual) and the highest published anuran values for isolated net mean muscle power output measured using work loops (93.5 W kg⁻¹ muscle mass), hindlimb length to snout-vent length ratio (2.02), and relative hindlimb muscle mass (33% of body mass). *Litoria nasuta* also had a higher ratio of tibia length to snout-vent length than 19 related species. We found that the mean power output expended during the takeoff phase of jumping in the individual that jumped the farthest was about three times greater than our estimate of available muscle power output.

Introduction

Jumping performance is commonly used as a model to investigate relationships between morphology, skeletal muscle me-

chanics, and locomotor performance (Marsh 1994; Rome 2002; Toro et al. 2003; James et al. 2005, 2007). The relative simplicity of this locomotor system allows ballistics formulas to be used to calculate kinematic and kinetic variables of jump performance and to highlight the relationships between morphological, physiological, and kinematic variables. Jump distance can be calculated from takeoff velocity and takeoff angle, variables that can be readily determined using video analysis and force platform data (Eq. [1]). Jump distance is also found to be dependent on morphological and physiological variables (Eq. [2]; Marsh 1994). In theory, jump distance in geometrically similar animals is independent of body size (Hill 1950). However, considerable variation in body form between species results in variation in maximum jump distance with body size, typically yielding a between-species scaling exponent of $M_b^{0.20}$ (Marsh 1994; James et al. 2007). Anuran jumping is an ideal system for examining the relationships between these variables because the contractile properties of frog hindlimb muscle have been extensively studied, and there is large interspecific variation in jump performance that appears to be related to specialization in morphology (Zug 1978; Marsh 1994; James et al. 2007).

$$d = \frac{v^2 \sin 2\alpha}{g}, \quad (1)$$

where d is the horizontal displacement of the center of mass, essentially the distance jumped (e.g., m), v is takeoff velocity (e.g., m s⁻¹), α is takeoff angle, and g is the acceleration due to gravity (approximately 9.8 m s⁻²).

$$d = \left(\frac{\dot{W} \times L}{M_b} \right)^{2/3} \times \frac{\sin 2\alpha}{g}, \quad (2)$$

where d is the horizontal displacement of the center of mass, \dot{W} is the average power generated/required for the jump (the kinetic energy at takeoff divided by the time required to attain this energy), L is distance from the center of mass to the tip of the toes, and M_b is body mass of the animal (Marsh 1994).

Equation (2) suggests that any species capable of extreme jumping performance probably exhibits a number of morphological and/or physiological adaptations (Emerson 1985; James et al. 2007), such as increasing relative hindlimb length (to increase L and/or $L : M_b$ ratio), increasing the proportion of body mass devoted to skeletal muscle mass used to power jumping (to increase \dot{W} and/or $\dot{W} : M_b$ ratio), altering muscle mechanics to increase muscle power output (to increase \dot{W}), and

* E-mail: r.james@coventry.ac.uk.

† E-mail: r.wilson@uq.edu.au.

increasing elastic strain energy used to enhance muscle power output (to increase \dot{W}). Both relative hindlimb length (hindlimb length : body length) and relative hindlimb muscle mass (muscle mass : body mass) have been found to vary among species and to affect jump performance. Frog (Emerson 1978) and mammal species (Emerson 1985) that are jump specialists have relatively longer legs. Also, frog (Rand 1952; Zug 1972; Choi and Park 1996; Choi et al. 2003) and lizard species (Losos 1990; Toro et al. 2003) with relatively longer hindlimbs have generally been found to achieve greater jump distance and/or takeoff velocity. Hindlimb muscle mass in frogs (Emerson 1978) and mammals (Alexander et al. 1981) varies as a proportion of body mass, with jumping species having proportionally larger extensor muscles than nonjumping species. In addition, anurans with relatively greater thigh muscle mass have been found to achieve relatively higher jump takeoff velocity (expressed relative to body mass; Choi et al. 2003).

The total power available for a jump may also be improved by modifications in muscle contractile dynamics. It seems likely that during maximal jumping performance extensor muscles are maximally activated while shortening at an optimal velocity and while working over a range of sarcomere lengths for optimal power generation (Lutz and Rome 1994). However, the mechanical properties of frog skeletal muscle vary among species, among individuals within a species, and between muscles within an individual as a result of variation in muscle composition (Putnam and Bennett 1983; Marsh 1994). For example, there is significant variation in frog muscle fiber type proportions between different muscles within individual *Rana pipiens* (Lutz et al. 1998). The muscles used to power jumping are mainly (86%) composed of muscle fibers that contain only type 1 (the fastest and most powerful) myosin heavy chain, whereas in the nonjumping muscles, 29% of muscle fibers contained only type 1 myosin heavy chain (Lutz et al. 1998). Therefore, jumping performance could be maximized via an increased percentage of fast muscle fibers resulting in higher available muscle power output.

The August Krogh principle states that “for many problems there is an animal on which it can be most conveniently studied” (Krebs 1975, p. 221). In essence, by studying a frog that exhibits extreme jumping performance, we would more likely gain a better understanding of the relevant specializations used to enhance locomotor performance. Zug (1978) found that the Australian rocket frog (*Litoria nasuta*) achieved the second-best relative maximal jumping performance out of 83 anuran species tested, with a peak jump distance of 51.4 times its own body length (2.27 m). This relative measure of performance was exceeded only by *Acris gryllus*, which managed 61.7 times its own body length (1.81 m). A more up-to-date approach than the August Krogh principle would be to study a species with extreme jumping performance and to use a known phylogeny to relate relevant data gained for that species to other closely related species to better understand how such adaptations may have evolved. Therefore, our aims were to use adult male *L. nasuta* to do the following: study jump performance in detail

using a force platform to determine acceleration, takeoff velocity, and jump distance; calculate total power output used in the jump; determine the muscle mechanics of one of the primary jumping muscles; analyze gross morphology and amount of muscle devoted to jumping; estimate the likely contribution of elastic potential energy to jumping performance; compare measurements made on this extreme specialist to those made previously on other anurans. The likely mechanisms involved in the extreme jumping performance of the rocket frog will then be evaluated using an appropriate anuran phylogeny where possible.

Material and Methods

Animals

Fourteen calling male Australian striped rocket frogs (*Litoria nasuta* Gray, 1842) were collected in the areas surrounding Brisbane, Australia. All frogs were immediately returned to the University of Queensland and maintained in 30 × 30 × 30-cm aquariums containing freshwater and several shelters. Jumping performance of frogs was recorded within 24 h of capture, while muscle mechanics experiments were performed within 5 d of collection. All experiments were approved by the University of Queensland Animal Ethics and Experimentation Committee (SIB/821/05/UQ) and Queensland National Parks and Wildlife Permits (WISP03763706).

Jumping Performance

Maximum jumping performance was determined for eight *L. nasuta* between 2.7 and 8.8 g body mass at 25°C using a custom-designed force platform. The design of the force platform was based on that outlined by Katz and Gosline (1993) for measuring the jumping performance of locusts, and our design and validation of this equipment is described in detail by Wilson et al. (2000a). Ground reaction forces during takeoff were recorded at a frequency of 1,000 Hz using a Powerlab data acquisition system. The calculations used to determine the maximum jumping acceleration, maximum instantaneous power output during takeoff, average power output during takeoff, takeoff velocity, and jump distance from the force platform data are also described by Wilson et al. (2000a). Acceleration of the frog is proportional to the force applied on the force platform. For comparison, mean power output during takeoff was also calculated from velocity and morphology measurements using the equations outlined by Peplowski and Marsh (1997). Each individual *L. nasuta* was stimulated to jump from the platform at least five times, with the jump that produced the greatest ground reaction force for each individual used as a measure of maximum jumping performance.

Muscle Mechanics

Six *L. nasuta* (body mass range 4.89–5.05 g) were euthanized by pithing and transection of the spinal cord. Each frog was

then pinned out in cooled (5°C) aerated McKenzies Ringer solution (composition [mM]: NaCl, 111; KCl, 2.5; CaCl₂, 1.8; MgCl₂, 1.0; HEPES, 5.0; glucose, 10; pH 7.4 at 25°C) kept on a frozen metal plate. The plantaris longus (gastrocnemius) muscle was dissected from the right leg with a small piece of bone left at the end of both the proximal and distal tendons. This plantaris longus muscle preparation was then used for measurement of muscle mechanics. Plantaris longus was chosen because it is one of the main muscles that power jumping in frogs (Marsh 1994), representing 19.4% of total leg muscle mass of the individuals used in this study. Plantaris longus is a pennate muscle that originates from the aponeurosis covering the knee and inserts distally onto the plantar surface of the foot (Duellman and Trueb 1994). The primary action of plantaris longus is to extend the ankle.

The bone at either end of the plantaris longus muscle preparation was clamped via crocodile clips to a strain gauge (UF1, Pioden Controls, UK) at one end and a motor arm (V201, Ling Dynamics Systems, UK) attached to a linear variable displacement transformer (DFG 5.0, Solartron Metrology, UK) at the other. The muscle was then maintained at $25^{\circ} \pm 0.5^{\circ}\text{C}$ in circulating aerated McKenzie's frog Ringer solution. The preparation was stimulated via parallel platinum electrodes while held at constant length to generate a series of twitches. Stimulus amplitude, pulse width, and muscle length were adjusted to determine the stimulation parameters and muscle length corresponding to maximal isometric twitch force. Time from stimulus to peak twitch force and time from peak twitch force to 50% relaxation were measured. An isometric tetanic force response was then elicited by subjecting the muscle to a 150-ms train of stimulation. Stimulation frequency was then altered to determine maximal tetanic force. Time to 50% peak tetanic force and time from last stimulus to 50% tetanic force relaxation were measured. A rest period of 5 min was allowed between each tetanic response.

The work loop technique was used to determine the power output of muscles during cyclical length changes (Josephson 1985). Each muscle preparation was subjected to a set of four sinusoidal length changes symmetrical about the length that was optimal for maximal twitch force production. The muscle stimulation parameters found to yield maximal isometric force were used (stimulation frequency, amplitude, and pulse width). Electrical stimulation and length changes were controlled via a D/A board and an in-house program produced using Testpoint software (CEC, Norton, MA). For each work loop cycle, muscle force was plotted against muscle length to generate a work loop, the area of which equated to the net work produced by the muscle during the cycle of length change (Josephson 1985). The net work produced was multiplied by the frequency of length change cycles to calculate net power output. The total strain of length change cycles was maintained at 0.10 throughout all experiments (i.e., $\pm 5\%$ of resting muscle length). The cycle frequency of length change was altered up and down within the range of 2–14 Hz to generate a power output–cycle frequency curve. During these length changes, the muscle was

subjected to phasic stimulation to generate an active work loop cycle. Every 5 min, the muscle was subjected to an additional set of four work loop cycles, with stimulation duration and stimulation phase parameters being altered until maximum net work was achieved at each cycle frequency. A set of control sinusoidal length change and stimulation parameters was imposed on the muscle every three to four sets of work loops to monitor variation in the muscle's ability to produce power/force. Any variation in power was found to be due to a matching change in ability to produce force. Therefore, the power produced by each preparation was corrected to the control run that yielded the highest power output by assuming that alterations in power-generating ability were linear over time. All preparations produced more than 70% of the initial power output throughout each experiment.

At the end of muscle mechanics experiments, the bones and tendons were removed and each plantaris longus muscle was blotted on absorbent paper to remove excess Ringer solution. Wet muscle mass was determined to the nearest 0.0001 g using an electronic balance. Mean muscle cross-sectional area was calculated from muscle length and mass, assuming a density of $1,060 \text{ kg m}^{-3}$ (Méndez and Keys 1960). Maximum isometric muscle stress was then calculated as maximum tetanic force divided by mean cross-sectional area (kN m^{-2}). Normalized muscle power output was initially calculated as power output divided by wet muscle mass (W kg^{-1}).

Morphological Measurements

External morphological measurements were made on all *L. nasuta*. Frog snout-vent length, femur length, tibiofibula length, and foot length were measured to the nearest 0.1 mm using electronic vernier calipers. Frog body mass (M_b) was measured to the nearest 0.01 g using an electronic balance. Following preparation of the plantaris longus for muscle mechanics, plantaris longus, cruralis, gracilis (major and minor combined), semimembranosus, and gluteus magnus muscles were all dissected from the left leg of the six frogs. These muscles were chosen as being indicative of the major muscles used to power jumping (Marsh 1994), and their masses were combined to estimate total jumping muscle mass. Wet muscle mass was determined for each muscle to the nearest 0.0001 g using an electronic balance after the muscle was blotted on absorbent paper to remove excess Ringer solution. All remaining muscles were removed from the left leg and blotted on absorbent paper to remove excess Ringer solution, and combined nonjumping muscle wet mass was determined as above.

The ratio between tibia length and snout-vent length was used as another measure to indicate relative limb length. Maximum tibia length to snout-vent length ratio data for *L. nasuta* and nineteen related species were taken from Tyler et al. (2000): *Litoria bicolor*, *Litoria caerulea*, *Litoria cavernicola*, *Litoria coplandi*, *Litoria inermis*, *Litoria meiriana*, *Litoria pallida*, *Litoria rothii*, *Litoria rubella*, *Litoria splendida*, *Litoria tornieri*, *Litoria wotjulumensis*, *Cyclorana cryptotis*, *Cyclorana cultripes*, *Litoria*

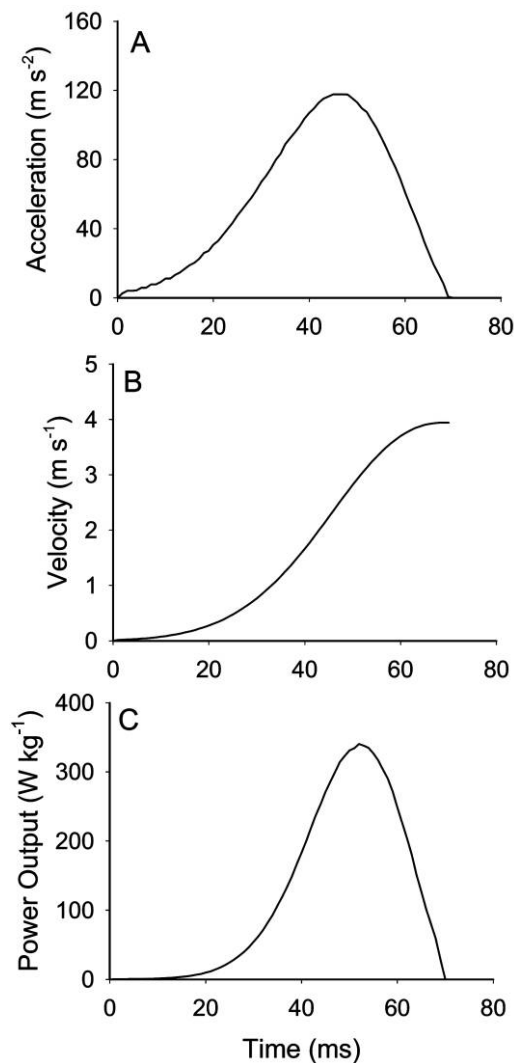


Figure 1. Changes in acceleration (A), velocity (B), and instantaneous body-mass-specific power output (C) during a typical maximum jump at 25°C for an Australian rocket frog (*Litoria nasuta*) measured using a force platform recording at 1,000 Hz.

dahlia, *Cyclorana longipes*, *Cyclorana maini*, *Cyclorana platycephalus*, *Cyclorana vagitus*.

The phylogeny of the *Litoria*-*Cyclorana* clade was taken from Byrne et al. (2002). The Byrne et al. phylogeny indicates that *Litoria* is a paraphyletic genus but that *Litoria*-*Cyclorana* is a monophyletic group.

Statistical Analyses

Data were analyzed using the statistical program SPSS. A one-way repeated-measures ANOVA with Bonferroni post hoc tests was used to analyze the effects of cycle frequency on maximum normalized power output of the isolated muscle. Data represent means \pm SD. Significance was taken at the level of $P < 0.05$.

Results

Jumping Performance

A typical jump involved rapid acceleration resulting in a peak acceleration after half to two-thirds of the total contact time and then a rapid decay of acceleration to takeoff (Fig. 1A). The entire contact time (takeoff) of the jump from the point of first movement until eventual takeoff was between 54 and 81 ms (Table 1). Positive acceleration occurred throughout the entire phase of contact with the platform (Fig. 1A), which resulted in a maximum velocity at the instant of takeoff (Fig. 1B). Instantaneous power output took longer to develop than force, with maximum power attained after approximately three-quarters of the total contact time (Fig. 1C). Average calculated jump distance was 1.24 ± 0.20 m (Table 1). When converted to body lengths, maximum jump distance was 31.5 ± 4.9 body lengths and ranged from 19.7 to 55.2 body lengths. The average power output during takeoff (mean power output during the contact period of jumping) varied between individuals, with a range from 318 to 747 W kg^{-1} jumping muscle mass (Table 1).

Muscle Mechanics

Maximal isometric stress was 149 ± 16 and 390 ± 30 kN m^{-2} for twitch and tetanic responses, respectively. Time from peak twitch to half relaxation was rapid relative to time to peak activation, with ranges of 11.5–14.5 and 36–43 ms, respectively (Table 2). Time to half peak tetanus ranged from 34.5 to 41.5 ms, whereas time from last stimulus to half relaxation ranged from 58 to 80 ms (Table 2). Normalized muscle power output tended to alter with cycle frequency (Fig. 2). The maximum mean normalized muscle power output produced by individual frogs during a complete work loop cycle ranged from 81.1 to 102.3 W kg^{-1} and in each case was achieved at cycle frequencies between 8 and 12 Hz. However, there were no significant differences in maximum normalized power output across the cycle

Table 1: Maximum jumping performance of adult Australian rocket frogs (*Litoria nasuta*)

	Mean \pm SD	Range
Contact time (ms)	67 \pm 4	54–81
Maximum force (N)	.537 \pm .078	.238–.824
Maximum jump distance (m)	1.24 \pm .20	.71–2.17
Maximum velocity (m s^{-1})	3.40 \pm .26	2.59–4.52
Maximum acceleration (m s^{-2})	104 \pm 7	86–140
Maximum instantaneous power output (W kg^{-1} body mass)	264 \pm 39	169–455
Average power output during takeoff (W kg^{-1} jumping muscle mass)	435 \pm 147	318–747

Note. Time and force were measured using a custom-designed force platform recording at 1,000 Hz, whereas other variables were calculated from the force and time data ($N = 8$).

Table 2: Isometric twitch and tetanus kinetics of plantaris longus muscle from adult Australian rocket frogs (*Litoria nasuta*)

	Mean \pm SD
Time to peak twitch force (ms)	40.7 \pm 2.5
Time from peak twitch force to 50% relaxation (ms)	13.3 \pm 1.1
Time to 50% peak tetanic force (ms)	38.5 \pm 2.6
Last stimulus to 50% tetanic relaxation (ms)	67.4 \pm 8.9

Note. $N = 6$.

frequency range of 4–14 Hz (one-way ANOVA; $P > 0.05$; Fig. 2).

Morphological Measurements

Litoria nasuta possess hindlimbs that are more than twice as long as their total body length, ranging from 1.91 to 2.13 (2.02 ± 0.07 ; Table 3). Plantaris longus and cruralis muscles accounted for 61% of the total mass of the specific jumping muscles (Table 3). Total jumping muscle mass represented $22.2\% \pm 0.7\%$ of body mass (Table 3), while total hindlimb muscle mass represented 33.1% of body mass. *Litoria nasuta* had a higher ratio of tibia length to snout-vent length than 19 related species (Fig. 3).

Discussion

Jumping Performance

The maximum jump distance achieved by a rocket frog in this study was 2.17 m, which represents 96% of the best absolute jump distance recorded by Zug (1978) for this species. However, in relative terms, the best individual in this study jumped 55.2 times its own snout-vent length, which is 7% better than in Zug (1978). Therefore, we can be sure that our frogs were jumping relatively long distances compared with most other frog species, providing a suitable model species in which to investigate adaptation for extreme jumping performance. The mean maximum takeoff velocity for rocket frogs is 3.4 m s^{-1} (86.6 snout-vent lengths s^{-1}), at the high end of a range of previously measured takeoff velocities for 14 species of anurans ($1.2\text{--}3.4 \text{ m s}^{-1}$; Zug and Altig 1978; Marsh 1994; Wilson et al. 2000a; Choi et al. 2003), again reinforcing the evidence that this species is good at jumping.

Muscle Mechanics

The maximum mean power output generated by plantaris longus muscle from *Litoria nasuta* during work loops in this study was 32% higher than the next-highest value previously gained from an anuran muscle using the same experimental protocol (Fig. 4). Further analysis demonstrated that there was no relationship between frog body size and maximal power output for the data shown in Figure 4. The higher power output value

achieved in this study could be due to *L. nasuta* plantaris longus muscle differing in any of a range of intrinsic mechanical properties that affect work loop power output, including activation rate, relaxation rate, maximal shortening velocity, shortening deactivation, force enhancement, and maximal stress (Marsh 1990; Josephson 1993; James et al. 1996).

The maximum tetanic stress of 390 kN m^{-2} generated by *L. nasuta* plantaris longus muscle was high compared with a range of previously reported values for anuran whole skeletal muscle preparations ($148\text{--}372 \text{ kN m}^{-2}$; Marsh 1994; Altringham et al. 1996; Navas et al. 1999; Wilson et al. 2000b, 2002). This high stress may be due to the muscle containing a high density of relatively fast myofibrils or could (like many of the previous studies) be due to errors in the stress calculations caused by pennation of the muscle and the muscle fibers being shorter than the muscle length; however, any of these explanations would demonstrate an adaptation that could help maximize both muscle power output and jumping performance. The effects of pennation angle on muscle mechanics are complicated, and a detailed analysis of muscle architecture, including muscle fiber length being shorter than muscle length, would be required before conclusions could be made (Lieber and Fridén 2000).

In general, during work loop studies, the higher the optimal cycle frequency for power output is, the higher the maximal power output will be, since higher optimal cycle frequency is indicative of a muscle with faster muscle contraction kinetics that is more likely to produce higher power output (Josephson 1993; James et al. 1995). This relationship is a key feature of Figure 4, indicating that this is one of the main reasons for the high power output achieved by *L. nasuta* plantaris longus mus-

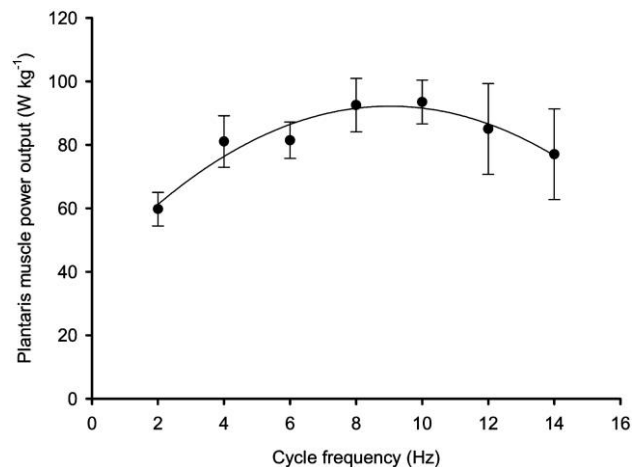


Figure 2. Relationship between cycle frequency and plantaris longus muscle normalized in vitro mean power output measured at 25°C using the work loop technique. Data represent mean \pm SD for all muscles tested. Each datum used to calculate these values represented power averaged across the whole work loop cycle (shortening and lengthening) for each individual ($n = 6$; Australian rocket frog *Litoria nasuta*). The line has been fitted to the data using a quadratic polynomial function.

Table 3: Morphological measurements of adult Australian rocket frogs (*Litoria nasuta*)

	Mean \pm SD	N
Body mass (g)	5.14 \pm 1.48	14
Plantaris longus muscle mass (g)	.172 \pm .011	6
Cruralis muscle mass (g)	.183 \pm .014	6
Gracilis (major and minor combined) muscle mass (g)	.0940 \pm .0100	6
Semimembranosus muscle mass (g)	.0574 \pm .0036	6
Gluteus magnus muscle mass (g)	.0722 \pm .0055	6
Snout-vent length (mm)	40.9 \pm 3.9	14
Femur (mm)	23.4 \pm 2.1	14
Tibiofibula (mm)	24.5 \pm 3.4	14
Foot (mm)	34.6 \pm 2.9	14

cle. It should be noted that part of the greater performance of *L. nasuta* plantaris longus muscle may be temperature related, since an increase in temperature from 20° to 25°C could itself enhance power output by affecting the intrinsic mechanical properties of muscle that limit work loop power output (Marsh 1994; James et al. 1995). However, the similar trends observed in Figure 4 at both 20° and 25°C indicate that the plantaris longus muscle of *L. nasuta* is still a relatively fast muscle. A high optimal cycle frequency for power output could be achieved in a muscle with high maximal shortening velocity, rapid activation, and rapid relaxation rates. The time to 50% twitch force relaxation of 13.3 ms measured for *L. nasuta* plantaris longus muscle was indeed on the fast end of a range of values previously reported for anuran skeletal muscle at test temperatures between 20° and 25°C (13–42 ms; John-Alder et al. 1989; Marsh 1994; Navas et al. 1999). However, the time to peak twitch force of 40.7 ms measured for *L. nasuta* plantaris longus muscle was on the slow end of a range of values previously reported for anuran skeletal muscle at test temperatures between 20° and 25°C (20–39 ms; John-Alder et al. 1989; Marsh 1994; Altringham et al. 1996; Navas et al. 1999; Wilson et al. 2000b). We do not know the maximal shortening velocity of *L. nasuta* plantaris longus muscle, but previous experimental modeling of work loops suggests that the value will be relatively high compared with other anurans (James et al. 1996; Lichtwark and Wilson 2005). However, previous studies also indicate that the intrinsic contractile properties measured in isometric and force velocity studies are not always good indicators of the likely power output that can be produced under work loop conditions (James et al. 1996; Caizzo and Baldwin 1997). The relatively high optimal cycle frequency for power output achieved in *L. nasuta* plantaris longus muscle also suggests that this muscle is of a relatively fast fiber type, probably containing more muscle fibers with type I myosin heavy chain and/or other fast muscle protein isoforms (such as myosin light chains) than other frog muscles (Lutz et al. 1998; Andruchova et al. 2006).

Morphology

We found that the length of the tibiofibula of rocket frogs was on average greater than the length of the femur, a characteristic that has previously been characterized as indicative of a species with good jumping performance (Zug 1972). We found a high hindlimb to snout-vent length ratio in rocket frogs (2.02) compared with a range of 1.07–1.94 from previous measurements made on 48 different frog species (Rand 1952; Rand and Rand 1966; Emerson 1978; Zug and Altig 1978; Choi et al. 2003; James et al. 2005). Relatively long hindlimbs should enable frogs to accelerate their center of mass over a relatively longer distance during takeoff to enhance takeoff velocity and jump distance. Frog species with longer hindlimbs generally achieve greater jump distance/takeoff velocity (Rand 1952; Zug 1972; Choi and Park 1996; Choi et al. 2003). Emerson (1978) found that those species that have the highest hindlimb to snout-vent length ratios primarily use jumping as their only land-based mode of locomotion, those species with the lowest ratios only walk, and those species with ratios inbetween use a combination of walking, jumping, or hopping. These findings suggest that in species where long jump performance confers a fitness advantage, selection for longer legs might be apparent. Interestingly, recent findings have demonstrated that rapid adaptive changes in cane toads have led to increases in relative hindlimb length and an associated increase in rate of movement during this species' invasion of Australia (Phillips et al. 2006). *Litoria nasuta* has a higher ratio of tibia length to snout-vent length than any of the 19 related species for which data are available (Fig. 3).

The five jumping muscles measured in this study accounted for a total of 22% of body mass, with the total hindlimb muscle mass accounting for 33% of body mass. These values are very high compared with a previous range of 12%–24% of body mass being composed of total hindlimb muscle mass, measured in 10 species of frogs (Marsh 1994; Peplowski and Marsh 1997; Wilson et al. 2000a; James et al. 2005). Frog species with relatively greater thigh muscle mass (expressed relative to body mass) generally achieve higher jump takeoff velocity (Choi and Park 1996; Choi et al. 2003), suggesting that for species where long jump performance confers a fitness benefit, selection may favor a greater proportion of body mass to be composed of jumping muscle.

Relationship between Muscle Mechanics and Jump Performance

In this study, the mean contact time for takeoff for rocket frogs was similar to the time plantaris longus muscle spent shortening during our in vitro work loop experiments at 8-Hz cycle frequency. The line fitted to the in vitro muscle power output–cycle frequency data (Fig. 2) indicates that at least 95% of maximal power output would be produced at any cycle frequency between approximately 6 and 12 Hz, with no significant difference found in maximal power output produced between 4 and 14 Hz. The mean in vitro muscle power output produced

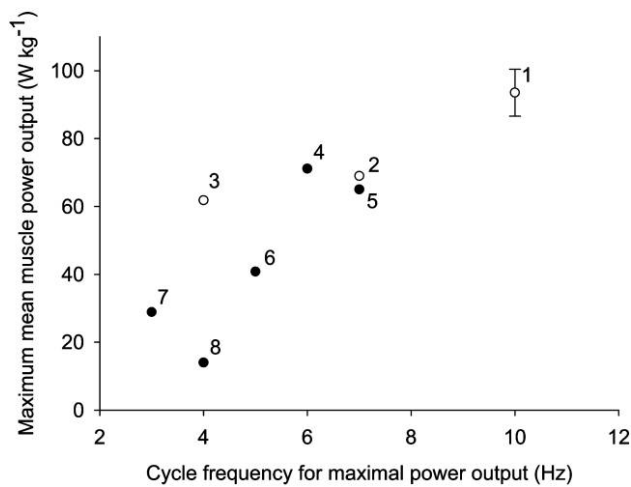


Figure 4. Comparison of maximal mean in vitro muscle power output between different anuran muscles and species. Each power output value is the maximal mean value determined for a muscle within a species and has been determined by using the work loop technique to deliver sinusoidal length change waveforms. In each case, strain rate was optimized via optimization of cycle frequency alone or, in some cases, also by optimizing strain. Filled circles and open circles represent measurements made at 20° and 25°C, respectively. In each case, data represent means for all muscles tested (with SD bars added for data point 1 from this study). Data were taken from the following studies: 1, plantaris longus muscle from *Litoria nasuta* (this study); 2, sartorius muscle from *Cyclorana alboguttata* (Symonds et al. 2007; the same species as *Litoria alboguttata* in Fig. 3); 3, ileofibularis muscle from *C. alboguttata* (Symonds et al. 2007; the same species as *L. alboguttata* in Fig. 3); 4, peroneus muscle from *Xenopus laevis* (Wilson et al. 2002); 5, sartorius muscle from *X. laevis* (Altringham et al. 1996); 6, plantaris longus muscle from *Bufo viridis* (Wilson et al. 2004); 7, sartorius muscle from *Bufo americanus* (Stevens 1993); 8, adductor magnus muscle from *X. laevis* (Altringham et al. 1996).

appears to be insufficient to power jumping in these frogs, never mind the much higher instantaneous power output value required at takeoff. While the muscle mechanics measurements made in this study are unlikely to have exactly replicated the activation patterns and length changes used by rocket frogs during jumping, the size of rocket frogs would exclude usage of the techniques necessary to determine realistic length change waveforms. Previous sonomicrometry measurements during jumping in semimembranosus muscle of *Bufo americanus* (Ahn et al. 2003) and plantaris longus muscle of jumping *Rana catesbeiana* combined with modeling of the frog plantaris longus muscle-tendon unit (Roberts and Marsh 2003) have demonstrated uncoupling of skeletal muscle length changes from whole-body movements during jumping. Rapid early shortening of plantaris longus muscle without movement of the frog causes stretching of tendons and consequent elastic energy storage, which subsequently enhances muscle power output during takeoff. Therefore, the apparent difference between skeletal muscle power output and jump power output can be resolved in rocket frogs if elastic energy storage causes a threefold enhancement of available muscle power output.

In conclusion, *L. nasuta* has the highest published anuran values for hindlimb length to snout-vent length ratio (2.02), hindlimb muscle mass as a percentage of body mass (33%), and isolated mean net muscle power output measured when using the work loop technique to deliver sinusoidal length changes (93.5 W kg⁻¹ muscle mass). When compared with 19 related species, *L. nasuta* has the highest ratio of tibia length to snout-vent length. Such specializations enable *L. nasuta* to be one of the most extreme vertebrate jumping specialists and suggest how this species has evolved to achieve the second-best recorded anuran jumping performance (when expressed relative to body size). However, it is clear that additional measurements need to be conducted on closely related species to enable further analysis in a phylogenetic context.

Acknowledgments

R.S.J. was supported by a Royal Society International Short Visit grant. We thank Professor Craig Franklin and Moreton Bay Research Station for lending us laboratory equipment. R.S.W. was supported by an Australian Research Council post-doctoral fellowship. We thank anonymous reviewers for their constructive comments.

Literature Cited

- Ahn A.N., R.J. Monti, and A.A. Biewener. 2003. In vivo and in vitro heterogeneity of segment length changes in the semimembranosus muscle of the toad. *J Physiol* 549:877–888.
- Alexander R.M., A.S. Jayes, G.M. Maloij, and E.M. Wathuta. 1981. Allometry of the leg muscles of mammals. *J Zool (Lond)* 198:293–313.
- Altringham J.D., T. Morris, R.S. James, and C.I. Smith. 1996. Scaling effects on muscle function in fast and slow muscles of *Xenopus laevis*. *Exp Biol Online* 1:1–8, doi:10.1007/s00898-996-0006-z.
- Andruchova O., G.M.M. Stephenson, O. Andruchov, D.G. Stephenson, and S. Galler. 2006. Myosin heavy chain isoform composition and stretch activation kinetics in single fibres of *Xenopus laevis* iliofibularis muscle. *J Physiol* 574:307–317.
- Byrne P.G., J.D. Roberts, and L.W. Simmons. 2002. Sperm competition selects for increased testes mass in Australian frogs. *J Evol Biol* 15:347–355.
- Caizzo V.J. and K.M. Baldwin. 1997. Determinants of work produced by skeletal muscle: potential limitations of activation and relaxation. *Am J Physiol* 273:C1049–C1056.
- Choi I.H. and K. Park. 1996. Variations in the take-off velocity of anuran amphibians: relation to morphology, muscle contractile function and enzyme activity. *Comp Biochem Physiol A* 113:393–400.
- Choi I.H., J.H. Shim, and R.E. Ricklefs. 2003. Morphometric relationships of take-off speed in anuran amphibians. *J Exp Zool* 299A:99–102.

- Duellman W.R. and L. Trueb. 1994. *Biology of Amphibians*. Johns Hopkins University Press, Baltimore.
- Emerson S.B. 1978. Allometry and jumping in frogs: helping the twain to meet. *Evolution* 32:551–564.
- . 1985. Jumping and leaping. Pp. 58–72 in M.E. Hildebrand, D.M. Bramble, K.F. Liem, and D.B. Wake, eds. *Functional Vertebrate Morphology*. Harvard University Press, Cambridge, MA.
- Hill A.V. 1950. The dimensions of animals and their muscular dynamics. *Sci Prog* 38:209–230.
- James R.S., J.D. Altringham, and D.F. Goldspink. 1995. The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. *J Exp Biol* 198:491–502.
- James R.S., C.A. Navas, and A. Herrel. 2007. How important are skeletal muscle mechanics in setting limits on jumping performance? *J Exp Biol* 210:923–933.
- James R.S., R.S. Wilson, J.E. de Carvalho, T. Kohlsdorf, F.R. Gomes, and C.A. Navas. 2005. Inter-individual differences in leg muscle mass and pyruvate kinase activity correlate with inter-individual differences in jumping performance of *Hyla multilineata*. *Physiol Biochem Zool* 78:857–867.
- James R.S., I.S. Young, V.M. Cox, D.F. Goldspink, and J.D. Altringham. 1996. Isometric and isotonic muscle properties as determinants of work loop muscle power output. *Pflug Arch Eur J Physiol* 432:767–774.
- John-Alder H.B., M.C. Barnhart, and A.F. Bennett. 1989. Thermal sensitivity of swimming performance and muscle contraction in the northern and southern populations of tree frogs (*Hyla crucifer*). *J Exp Biol* 142:357–372.
- Josephson R.K. 1985. Mechanical power output from striated muscle during cyclic contraction. *J Exp Biol* 114:493–512.
- . 1993. Contraction dynamics and power output of skeletal muscle. *Annu Rev Physiol* 55:527–546.
- Katz S.L. and J.M. Gosline. 1993. Ontogenetic scaling of jump performance in the African desert locust (*Schistocerca gregaria*). *J Exp Biol* 177:81–111.
- Krebs H.A. 1975. The August Krogh principle. *J Exp Zool* 194:221–226.
- Lichtwark G.A. and A.M. Wilson. 2005. A modified Hill model that predicts muscle power output and efficiency during sinusoidal length changes. *J Exp Biol* 208:2831–2843.
- Lieber R.L. and J. Fridén. 2000. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* 23:1647–1666.
- Losos J.B. 1990. The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* 44:1189–1203.
- Lutz G.J., S. Bremner, N. Lajevardi, R.L. Lieber, and L.C. Rome. 1998. Quantitative analysis of muscle fibre type and myosin heavy chain distribution in the frog hindlimb: implications for locomotor design. *J Muscle Res Cell Motil* 19:717–731.
- Lutz G.J. and L.C. Rome. 1994. Built for jumping: the design of the frog muscular system. *Science* 263:370–372.
- Marsh R.L. 1990. Deactivation rate and shortening velocity as determinants of contractile frequency. *Am J Physiol* 259:R223–R230.
- . 1994. Jumping ability of anurans. Pp. 51–111 in J.H. Jones, ed. *Comparative Vertebrate Exercise Physiology*. Academic Press, San Diego, CA.
- Méndez J. and A. Keys. 1960. Density and composition of mammalian muscle. *Metabolism* 9:184–188.
- Navas C.A., R.S. James, J.M. Wakeling, K.M. Kemp, and I.A. Johnston. 1999. An integrative study of the temperature dependence of whole animal and muscle performance during jumping and swimming in the frog *Rana temporaria*. *J Comp Physiol B* 169:588–596.
- Peplowski M.M. and R.L. Marsh. 1997. Work and power output in the hindlimb muscles of Cuban tree frogs *Osteopilus septentrionalis* during jumping. *J Exp Biol* 200:2861–2870.
- Phillips B.L., G.P. Brown, J.K. Webb, and R. Shine. 2006. Invasion and the evolution of speed in toads. *Nature* 439:803.
- Putnam R.W. and A.F. Bennett. 1983. Histochemical, enzymatic, and contractile properties of skeletal muscles of three anuran amphibians. *Am J Physiol* 244:R558–R567.
- Rand A.S. 1952. Jumping ability of certain anurans, with notes on endurance. *Copeia* 1952:15–20.
- Rand A.S. and P.J. Rand. 1966. The relation of size and distance jumped in *Bufo marinus*. *Herpetologica* 22:206–213.
- Roberts T.J. and R.L. Marsh. 2003. Probing the limits to muscle-powered accelerations: lessons from jumping bullfrogs. *J Exp Biol* 206:2567–2580.
- Rome L.C. 2002. The design of vertebrate muscular systems: comparative and integrative approaches. *Clin Orthop Relat Res* 403(suppl.):S59–S76.
- Stevens E.D. 1993. Relation between work and power calculated from force-velocity curves to that done during oscillatory work. *J Muscle Res Cell Motil* 14:518–526.
- Symonds B.L., R.S. James, and C.E. Franklin. 2007. Getting the jump on skeletal muscle disuse atrophy: preservation of contractile performance in aestivating *Cyclorana alboguttata* (Günther, 1867). *J Exp Biol* 210:825–835.
- Toro E., A. Herrel, B. Vanhooydonck, and D.J. Irschick. 2003. A biomechanical analysis of intra- and interspecific scaling of jumping and morphology in Caribbean *Anolis* lizards. *J Exp Biol* 206:2641–2652.
- Tyler M.J., L.A. Smith, and R.E. Johnstone. 2000. *Frogs of Western Australia*. Western Australian Museum, Perth.
- Wilson R.S., C.E. Franklin, and R.S. James. 2000a. Allometric scaling relationships of jumping performance in the striped marsh frog, *Limnodynastes peronii*. *J Exp Biol* 203:1937–1946.
- Wilson R.S., R.S. James, and I.A. Johnston. 2000b. Thermal acclimation of locomotor performance in tadpoles and adults of the aquatic frog, *Xenopus laevis*. *J Comp Physiol B* 170:117–124.
- Wilson R.S., R.S. James, T. Kohlsdorf, and V.M. Cox. 2004. Inter-individual variation of isolated muscle performance and structure in the toad *Bufo viridis*. *J Comp Physiol B* 174:453–459.
- Wilson R.S., R.S. James, and R. Van Damme. 2002. Trade-offs

- between speed and endurance in the frog *Xenopus laevis*: a multi-level approach. *J Exp Biol* 205:1145–1152.
- Zug G.R. 1972. Anuran locomotion: structure and function. 1. Preliminary observations on relation between jumping and osteometrics of appendicular and postaxial skeleton. *Copeia* 1972:613–624.
- . 1978. Anuran locomotion: structure and function. 2. Jumping performance of semiaquatic, terrestrial, and arboreal frogs. *Smithson Contrib Zool* 276:1–31.
- Zug G.R. and R. Altig. 1978. Anuran locomotion—structure and function: the jumping forces of frogs. *J Wash Acad Sci* 68:144–147.