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RESEARCH ARTICLE

Muscle preactivation and the limits of muscle power output during jumping in the Cuban tree frog *Osteopilus septentrionalis*

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ABSTRACT

Previous studies of jumping in frogs have found power outputs in excess of what is possible from direct application of muscle power and concluded that jumping requires the storage and release of elastic strain energy. Of course, the muscles must produce the work required and their power output should be consistent with known muscle properties if the total duration of muscle activity is known. Using the Cuban tree frog, *Osteopilus septentrionalis*, I measured jumping performance from kinematics and used EMG measurements of three major jumping muscles to determine the duration of muscle activity. Using the total mass of all the hindlimb muscles, muscle mass-specific work output up to 60 J kg^{-1} was recorded. Distributed over the duration of the jump, both average and peak muscle mass-specific power output increased approximately linearly with the work done, reaching values of over 750 and 2000 W kg^{-1}, respectively. However, the muscles were activated before the jump started. Both preactivation duration and EMG amplitude increased with increasing amounts of work performed. Assuming the muscles could produce work from EMG onset until toe-off, the average muscle mass-specific power over this longer interval also increased with work done, but only up to a work output of 36 J kg^{-1}. The mean power above this value of work was 281 W kg^{-1}, which is approximately 65% of the estimated maximum isotonic power. Several reasons are put forward for suggesting this power output, although within the known properties of the muscles, is nevertheless an impressive achievement.

KEY WORDS: Elastic energy storage, Muscle activation, Catapult mechanism, Work

INTRODUCTION

In animals undertaking a single jump without a rapid countermovement, skeletal muscle shortening is the only source of work to power jumping. However, during take-off, some jumping animals produce mechanical power exceeding the expected power-producing capacity of their skeletal muscles (Hall-Craggs, 1965; Bennet-Clark, 1975; 1977; Marsh, 1994; Aerts, 1998). This apparent contradiction has been explained by hypothesizing the use of elastic elements to temporally redistribute the work contributed by muscle. Elastic energy storage and release has been documented in diverse types of animal movement (Longo et al., 2019), but the emphasis here is on jumping.

The necessity of using stored elastic energy to power jumping was first recognized for insects, but this mechanism for enhancing jumping performance has been demonstrated in vertebrates as well and is likely to be quite general. Because of their small body size, insects have extremely brief take-off times during which work is applied to the center of mass, making elastic energy storage essential to explain their jumping performance (Bennet-Clark, 1977). In vertebrates, Marsh and John-Alder (1994) suggested that elastic energy storage also was required to explain the power output of small tree frogs during jumping. Confirming this finding, Peplowski and Marsh (1997) measured jumping distances of Cuban tree frogs, *Osteopilus septentrionalis*, and, based on jumping distance, estimated peak power output that exceeded the available muscle power by approximately 7-fold. The use of stored elastic energy has been shown to be necessary to explain the jumping performance of the bushbaby *Galago galago*, a specialized primate jumper (Hall-Craggs, 1965; Aerts, 1998). Even in large animals not specialized for jumping, such as humans, elastic energy storage also has been found to play a role in jumping (Bobbert, 2001; Kurokawa et al., 2001). Roberts and Marsh (2003) suggest that linking muscles to their loads through elastic structures may be a general way to enhance performance when the task is to accelerate an inertial load.

The temporal redistribution of muscle work output by elastic elements is particularly well developed in specialized jumpers, including frogs. In these animals, the amount of work done and the short time available during take-off suggests that the muscles must be active and begin shortening to stretch elastic elements considerably before any significant movement of the animal's center of mass is initiated. By analogy to human-designed devices, this system has been described as using a catapult mechanism (recently dubbed a latch-mediated spring-actuated system; Longo et al., 2019). Human designed catapults have a catch (or latch) to prevent movement of the mass to be accelerated while energy is being stored, and then a release mechanism to initiate the delivery of the energy to the mass. In jumping animals, the details of the catapult mechanism, including the mechanism of the catch, have been worked out for some jumping insects (Bennet-Clark and Lucey, 1967; Bennet-Clark, 1975), but are not known in detail for any vertebrate jumper. Marsh and John-Alder (1994) hypothesized that a changing mechanical advantage, as is seen in locusts (Bennet-Clark, 1975), could play a role. Modeling by Roberts and Marsh (2003) confirmed that in bullfrogs (*Rana catesbeiana*), the catch could be provided by the initially poor mechanical advantage of the muscles for transmitting force to the ground. Subsequently, using inverse dynamics calculations, this dynamic catch mechanism has been confirmed by Astley and Roberts (2014) in jumps of the smaller leopard frog (*Rana pipiens*). Whether this mechanism based on changing mechanical advantage during the jump is sufficient to explain performance in frogs with higher power outputs (Peplowski and Marsh, 1997) is not known, and further information on the performance of these animals is needed.

The goal of the present study was to uncover the limit of muscle power output during jumping by Cuban tree frogs,

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O. septentrionalis. The missing piece of information in revealing this limit is knowledge of the duration of muscle activity. In a series of jumps with increasing work and power output, I predicted that muscle activation would increase and the onset of activation would occur at longer times before the jump started, i.e. increasing preactivation. Because all of the work in the jump must come from the muscles, the power calculated using the duration from the onset of muscle activation until the frog leaves the ground should be consistent with the known properties of the muscles that power the jump. High-speed video was used to calculate work and document the distribution of power output during jumping. I also measured the electromyographic (EMG) activity of three of the major hindlimb muscles involved in powering the jump to determine the duration of muscle activity.

MATERIALS AND METHODS

Animals

Adult Cuban tree frogs, *Osteopilus septentrionalis* (Duméril & Bibron 1841), of mixed sex were obtained from commercial suppliers who collected them in Florida, USA. They were received in June or July and maintained up to 3 weeks before the experiments. Animals were kept in 38 l aquaria with a maximum of 3 frogs per aquarium. The floors of the aquaria were covered with moist paper towels and the frogs also had access to a bowl of clean water. Three times per week, the frogs were fed crickets that had been dusted with a mixture of vitamins and calcium carbonate. Frogs were housed in a room maintained at approximately 27°C with a light:dark cycle of 12 h:12 h. The Northeastern University Institutional Animal Care and Use Committee approved all experimental procedures.

Choice of muscles for EMG recordings

The small size of Cuban tree frogs places limits on the number of muscles to be instrumented. I choose three large pinnate muscles – plantaris, peroneus and cruralis – that based on their anatomy (Dunlap, 1960) and some previous EMG recordings (Olson and Marsh, 1998; Reynaga et al., 2019) likely are major contributors to powering the jump. Pinnate muscles and their associated tendons are expected to have major roles in elastic storage of energy. The plantaris, which is located posterior to the tibiofibula, is the major ankle extensor. Its origin at the knee is complex with one 'head' having a flexor moment, and one having little action at the knee (Dunlap, 1960). Recent work on the plantaris of the Cuban tree frog has demonstrated the tuning of force production and elasticity in this muscle, enhancing its role in elastic storage (Mendoza and Azizi, 2021). The peroneus, located anterior to the tibiofibula, is a knee extensor originating on the extensor surface of the knee and via a deep tendon that also exerts an extensor action (Dunlap, 1960). The insertion near the ankle is somewhat complex (Dunlap, 1960). The major portion of the insertion on the malleolus of the tibiofibula has no action at the ankle. A secondary insertion on the fibulare in the proximal foot probably exerts some moment about the ankle, but this moment is not in the flexion–extension axis. The cruralis is the largest knee extensor located in the thigh, originating near the hip joint capsule, and inserting by tendinous and fleshy attachments around the knee. When the hip is flexed, this muscle apparently also exerts a flexor moment around the hip joint (Lombard and Abbott, 1907).

EMG recordings

Bipolar-hook electrodes with 1 mm bare tips were fashioned from Teflon-coated silver wire with a bare diameter of 0.076 mm and coated diameter of 0.1 mm (Medwire AG3T, Sigmund Cohn Corp.). Silver wire was used instead of stainless steel because the flexibility of the silver allowed these small animals unrestricted movement of the joints in their hindlimbs. Prior to implantation of the EMG electrodes the animals were anesthetized with 1% tricaine methanosulfate (MS222) and maintained at a surgical depth of anesthesia by periodically applying to the skin a paper towel moistened with 1% MS222. Using a stainless-steel guide cannula, electrodes were routed from an incision on the back, near the midpoint of the coccyx, to two incisions, one on the anterior thigh allowing access to the cruralis muscle and one on the lateral side of the proximal shank allowing access to the plantaris and peroneus muscles. Electrodes were inserted approximately parallel to the fascicles using a chamfered 25-gauge hypodermic needle. At the exit site on the back, the electrodes and a ground wire were attached to a lightweight connector fashioned by gluing together two 4-pin connectors, which were then encased in epoxy. The finished electrode assembly had a mean (±s.e.m.) mass of 0.88±0.014 g. During recording, this connector was plugged to a mating connector on the end of an approximately 2 m long custom-made lightweight cable. The cable was fashioned by threading seven strands of 0.076 mm diameter, Teflon-coated stainless-steel wire through PE-50 tubing. Most of the mass of this cable was in the connector, which added an additional 0.9 g to the mass lifted by the frog. The extra mass of the electrode assembly and the cable was added to frog's mass for the calculations of work and power.

Signals from the EMG electrodes were conditioned using DAM-50 preamplifiers (World Precision Instruments, Sarasota, FL, USA) with hardware filters set to a band-pass of 10–3000 Hz. Preamplified signals were digitized at 4000 Hz with a 12-bit analog-to-digital (A–D) converter (GW Instruments, Charlestown, MA, USA) in a Macintosh computer running Superscope software (GW Instruments). Further analysis of the EMG signals was performed with the application Igor Pro (WaveMetrics, Lake Oswego, OR, USA). Signals were digitally filtered using a FIR bandpass filter set to 90–800 Hz. EMG onset and offset times were selected by eye and, after rectification, the mean amplitude and duration of the signals were calculated. The mean value of the rectified EMG was used because this value should represent the mean level of recruitment of the volume of muscle surrounding the recording electrode. The mean amplitude over the whole period of activation should reflect the ability to produce force and thus work during shortening (Roberts and Gabaldón, 2008). Following the kinematic analysis, the EMG onset and offset times were expressed relative to the start of the acceleration of the frog's center of mass.

Kinematics and calculation of mechanical work and power

Prior to the recording sessions the animals were housed for several hours in individual containers with water in a controlled temperature cabinet at 28°C. This temperature was chosen because jumping performance in Cuban tree frogs has been shown to peak at approximately this value (Peplowski and Marsh, 1997). Animals were videoed at 500 Hz with a NAC Visual Systems HV-1000 recorder at 500 fields s^{-1}. Timing of the video fields relative to the EMG measurements was determined using a square-wave signal generated by the computer software and recorded on the video fields using a NAC wave inserter. Two different video setups were used. In both cases, the rooms were maintained at approximately 28°C. In the first setup, four animals were videoed in a lateral view jumping along a marked trackway. This group of animals was jumped in three sessions: two sessions on the day after the surgery,

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one in the morning and one in the afternoon after at least 4 h of recovery; and one session the following day. Accurately recording jumping performance with this setup required the frogs to jump nearly parallel to the plane of the recording. To allow a greater number of successful jumps to be recorded, the second setup employed an overhead-mounted camera and used a 45 deg mirror to record a simultaneous lateral view. These animals were recorded in four sessions: one the day after the implantation surgery, two the following day in the morning and afternoon; and one on the third day after surgery. In both groups, for jumps that were along the trackway, the jumping distance was also measured by noting the landing point on the marked trackway. For all the recording sessions, multiple jumps were attempted in sequence by returning the frog to the starting location. Between 1 and 4 successful jumps were recorded for each frog in each session.

Immediately following the jumping sequence, cloacal temperature was recorded using a fine wire thermocouple attached to Keithley thermocouple thermometer.

Following all the recording sessions, the frogs were euthanized and the mass of each of the instrumented muscles was measured as well as the total mass of all the hindlimb muscles. The forelimb muscles were not weighed. The forelimbs have been found to play a role in orienting the body during take-off in frog jumping (Wang et al., 2014) and play a major role in landing in some jumps (Nauwelaerts and Aerts, 2006). However, they leave the ground very early in take-off and thus would have a minimal contribution to power production in the jump (Fig. S1).

The video from the NAC HV-1000 recorder was converted to digital video (720×480 pixels) using an A–D converter (Canopus ADVC 55, Kobe, Japan). The digital video was then de-interlaced (JES Deinterlacer v. 3.8.4, Jan E. Schotsman) to recover the 500 Hz field rate of the NAC HV-1000 recorder. The de-interlaced frames were imported into ImageJ (Wayne Rasband, http://rsb.info.nih.gov/ij/). The imported video was first redimensioned to 720×528 pixels, which compensates for the rectangular aspect ratio of digital video pixels. The spatial resolution was 0.39 mm pixel^{-1} for the videos with a lateral view only, and 0.45 mm pixel^{-1} for the ones with both vertical and lateral views. The exit point of the EMG electrodes on the coccyx was used to approximate the center of mass and this point was tracked manually. For videos with simultaneous vertical and lateral views, both views were digitized to allow for correction of the horizontal position for the angle of the jump.

Kinematic data were imported into Igor Pro (WaveMetrics) for analysis. Vertical and horizontal position data for the center of mass were smoothed using a smoothing spline interpolation. Vertical (V_v) and horizontal (V_h) velocity in m s^{-1} were obtained by differentiating the smoothed displacement data and vertical (a_v) and horizontal (a_h) acceleration in m s^{-2} were obtained by differentiation of the velocity curves. Horizontal force (F_h) in N was calculated as $F_h=M_b a_h$; vertical force (F_v) in N was calculated as $F_v=M_b a_v+\boldsymbol{g}M_b$ (where M_b is body mass and $\boldsymbol{g}$ is gravitational acceleration). Resultant take-off velocity (V_{res}) was calculated as the vector sum of the vertical and horizontal velocity: $V_{res}=(V_h^2+V_v^2)^{0.5}$. Horizontal ($\dot{W}_{h,m}$) and vertical ($\dot{W}_{v,m}$) muscle mass-specific power were calculated as the product of force and velocity divided by total hind-limb muscle mass M_m for each individual. Total muscle mass-specific power output ($\dot{W}_m$) was calculated as the sum of $\dot{W}_h$ and $\dot{W}_v$ The start of the jump (time=0) was designated as the time when the horizontal or vertical acceleration consistently rose above zero. Muscle mass-specific work output (W_m) was calculated by integrating $\dot{W}_m$ with respect to time from the start of take-off until the time of toe-off. The take-off angle at toe-off (ø) relative to horizontal was calculated as the arctangent of V_h/V_v at toe-off. A graphical example of the calculations can be found in Fig. S1.

Statistics

Single and multiple least squares regressions were run in IBM SPSS for Macintosh. Analyses of covariance (ANCOVA) was performed using the general linear model in SPSS. A segmented regression analysis was performed in R using the package Segmented.

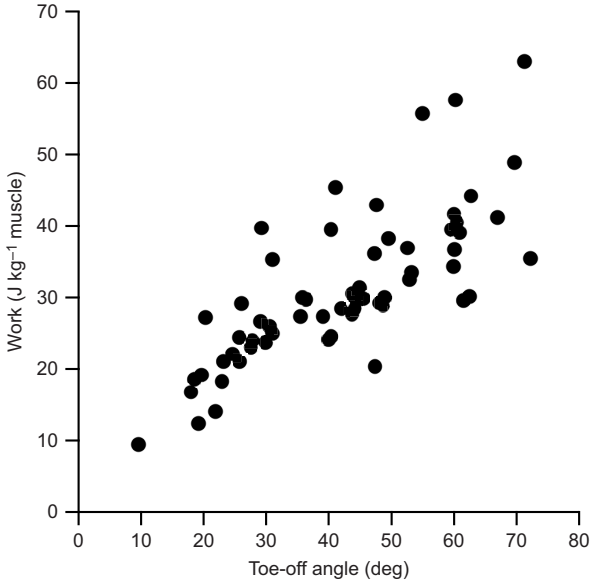

Fig. 1. Muscle mass-specific work in Cuban tree frogs as a function of the take-off angle at toe-off. n=61.

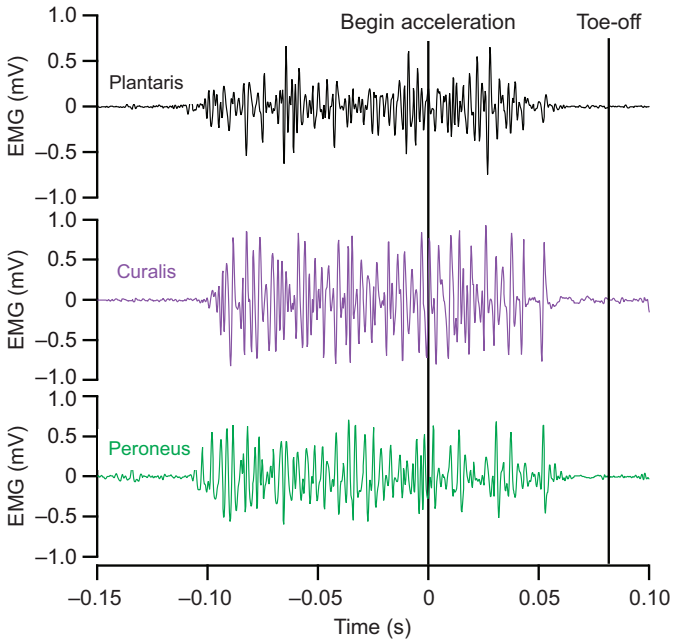

Fig. 2. Example EMG signals from a jump of a Cuban tree frog. This jump had a muscle mass-specific work output of 63 J kg^{-1}. The vertical line at zero time indicates the beginning of acceleration of the center of mass of the frog. The second vertical line indicates when the toes of the frog left the ground.

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RESULTS

Kinematic and EMG recordings were made on 8 animals. Measured on the day they were jumped, M_b (without the mass of the electrodes and cable) ranged from 14.3 to 24.6 g with a mean (±s.e.m.) of 18.4±1.3 g. Snout–vent length ranged from 63 to 79 mm with a mean of 71.3±1.7 mm. The mean total hindlimb muscle mass as a fraction of body mass was 0.198±0.008. The three muscles that were used for EMG measurements were 40.5±0.2% of the total hindlimb muscle mass. Immediately following the jumping sessions, cloacal temperature ranged from 27.4 to 28.5°C with a mean of 27.9±0.14°C.

A total of 61 jumps were successfully recorded. (The number of jumps attempted and successfully recorded for each animal is given in Table S1.) The muscle mass-specific work in these jumps varied from 9.5 to 63 J kg^{-1}. The highest work output occurred in jumps with large take-off angles at toe-off (Fig. 1).

The onset and offset times of the EMGs of all three muscles relative to the start of the jump were similar (Fig. 2). In subsequent analyses, the mean onset and offset times were used. Negative onset times indicate preactivation of the muscles before the acceleration of the center of mass begins. The duration of the EMGs did not correlate significantly with the work done in the jump (P=0.09) (Fig. 3). However, work increased significantly with both the duration of preactivation (Fig. 4) (linear regression: P<0.001; r^2=0.368) and the mean amplitude of the EMGs (Fig. 5) (linear regression: P<0.001; r^2=0.474). A multiple linear regression with both variables improved the correlation (P<0.001; r^2=0.622; Fig. 6).

Not unexpectedly, a portion of the variation in the relationship of work to EMG amplitude and duration of preactivation was due to variation among animals. This effect was quantified with an ANCOVA analysis using the general linear model in SPSS. In this analysis, EMG amplitude and preactivation duration (EMG onset) were covariates, and animal ID was entered as a random factor. The EMG amplitude and preactivation duration had highly significant effects on the work done (P<0.00001) and together accounted for 55% of the variation (sum of the partial η^2 values=0.547). The variation attributed to the individual animals was also significant (P<0.003, partial η^2 =0.297). Further ANCOVA results can be found in Table S2.

The average muscle mass-specific power during the take-off period (from the start of acceleration until toe-off) increased approximately linearly with the work done, reaching values of over 750 W kg^{-1} (Fig. 7). The peak power occurred late in the take-off (Fig. 8), averaged 2.8 times the average power, increased approximately linearly with the work done (Fig. 7) and reached values over 2000 W kg^{-1}.

In contrast, power output calculated from the onset of the EMG until toe-off leveled off at higher levels of work (Fig. 7). A segmented linear regression analysis indicated a break at a work output of 36 J kg^{-1}. The regression above this value was not significant. The mean value for this measure of power in jumps with work greater than 36 J kg^{-1} was 281±11 W kg^{-1}.

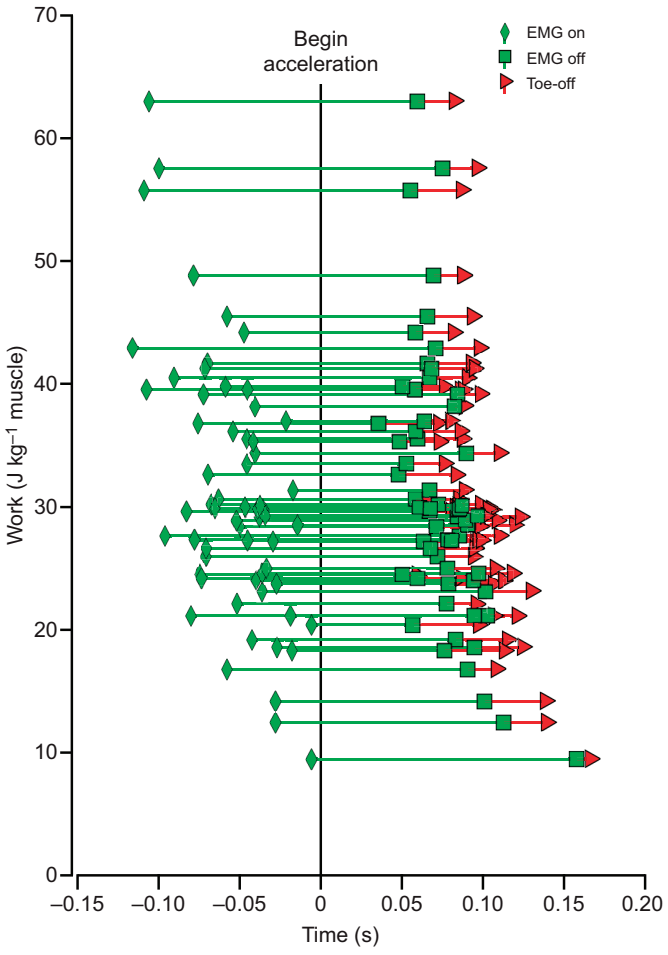

Fig. 3. Muscle mass-specific work for individual jumps of Cuban tree frogs. Plotted for each jump are: the time of EMG onset (green diamonds); the time of EMG offset (green squares); and the time the that toes left the ground (red triangles). The vertical line at zero time indicates the beginning of acceleration of the center of mass of the frog (n=61).

DISCUSSION

Preactivation

The data presented here show that three of the major muscles involved in powering the jump were activated before the start the

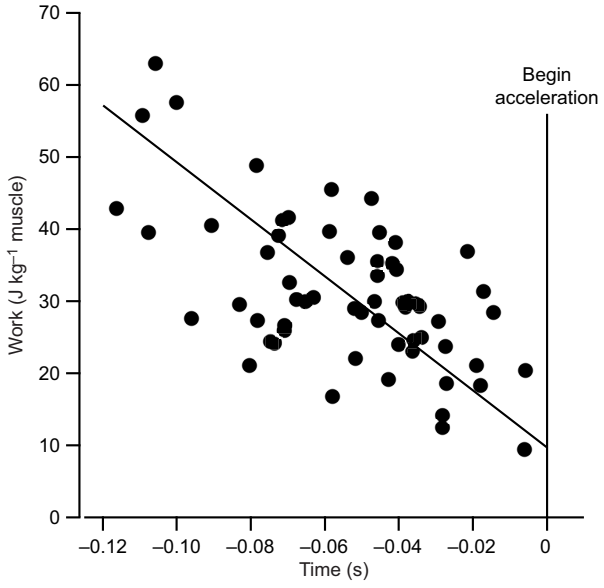

Fig. 4. Muscle mass-specific work in jumping Cuban tree frogs as a function of EMG onset time relative to the beginning of the acceleration of the frog's center of mass. Based on regression analysis, the relationship of work to mean EMG amplitude is highly significant (n=61; P<0.001; r^2=0.368). The solid line through the data is the reduced major axis regression line.

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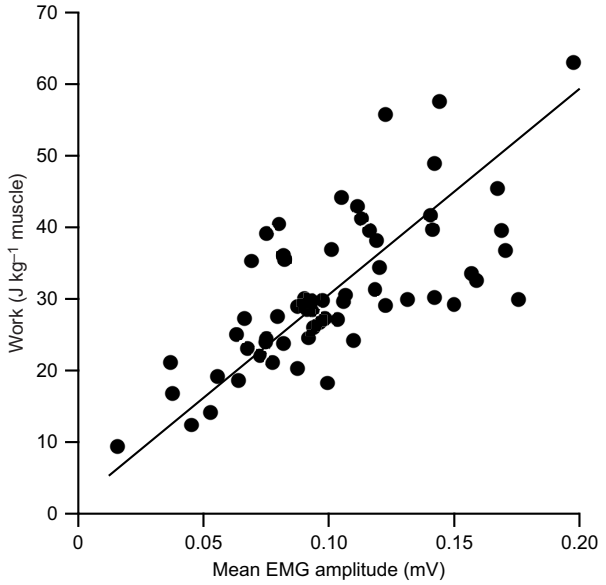

Fig. 5. Muscle mass-specific work in jumping Cuban tree frogs as a function of mean EMG amplitude. Based on regression analysis, the relationship of work to mean EMG amplitude is highly significant (n=61; P<0.001; r^2=0.474). The solid line through the data is the reduced major axis regression line.

movement of the center of mass in the jump (Fig. 2). The amount of preactivation increased with increasing jumping performance as measured by the work done (Figs 3, 4 and 8). The mean amplitude of the EMG also increased with increasing work (Figs 5 and 8). Limited comparable data exist on EMGs measured in frog jumping. Kamel et al. (1996) reported that in the leopard frog (*Rana pipiens*), the onset of the EMG occurred approximately 30–40 ms before movement. Olson and Marsh (1998) reported that the semimembranosus muscle in bullfrogs (*Rana catesbeiana*) was activated 38 ms before the start of movement and that preactivation was not significantly correlated with jumping distance. The jumping distances in both Kamel et al. (1996) and Olson and Marsh (1998) were considerably less than the maximal distances reported for these species in other studies. Kamel et al. (1996) do not report jumping distance, but the companion paper by Peters et al. (1996) reports the mean distance as 29 cm. This species has been reported to jump 1.1 m (Rand, 1952). Olson and Marsh (1998) recorded bullfrog jumps of up to 1.2 m, which agrees with other laboratory measures, but this species can jump over 2 m under the right conditions (Astley et al., 2013). Recently, Reynaga et al. (2019) also found considerable preactivation of the plantaris in Cuban tree frogs averaged across all the jumps they measured. However, they used the onset of ankle extension as their measure for the start of the jump and ankle extension is delayed relative to the extension of more proximal joints.

Jumping performance

The jumping performance Cuban tree frogs recorded here is comparable to or exceeds previous studies of this species. Zug (1978) recorded a maximum jumping distance of 1.2 m at an air temperature of 23°C. Peplowski and Marsh (1997) examined the effects of temperature and recorded a number of jumps in the range of 1.2–1.4 m at temperatures between 22 and 30°C. Based on jumping distance, Peplowski and Marsh (1997) calculated muscle mass-specific work output of 45–50 J kg^{-1} in the longest jumps. Using analysis of jumping kinematics, the current study recorded muscle mass-specific work as high as 60 J kg^{-1} in jumps with high take-off angles (Fig. 1). In the subset of jumps recorded that were along the trackway so that jumping distance could be recorded, work and average power calculated from jumping distance and kinematics were closely correlated (r^2=0.957) (Fig. S2). However, Peplowski and Marsh (1997) underestimated the peak power during take-off. To estimate peak power from jumping distance, they followed the simple assumption of constant acceleration resulting in the prediction that peak power will be twice the average power (Bennet-Clark, 1977). They estimated a peak muscle mass-specific power of over 1644 W kg^{-1} in the longest jump. However, the calculations done in the present study and in Roberts et al. (2011) and Reynaga et al. (2019) show that acceleration is not constant. Peak power averaged 2.8 times average power across all the jumps

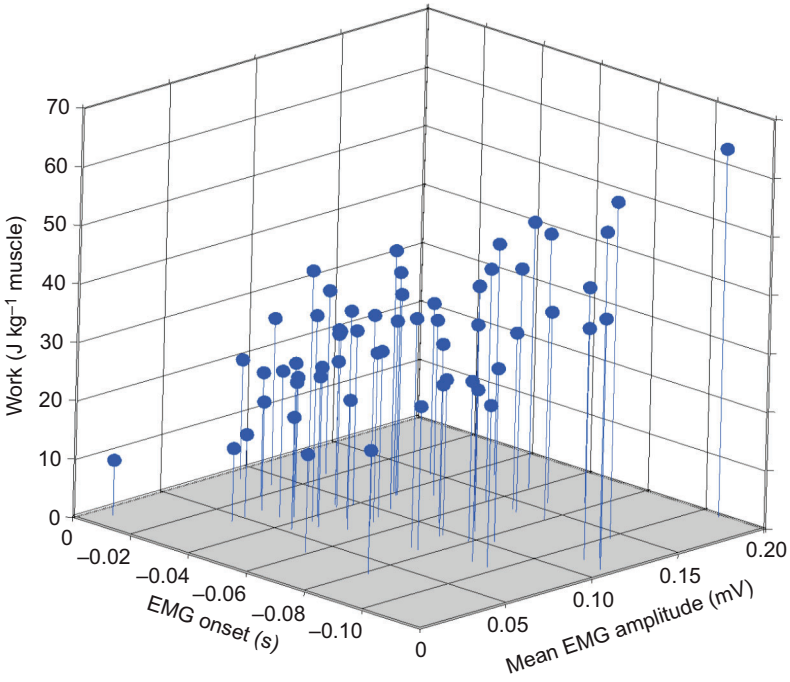

Fig. 6. Muscle mass-specific work in jumping Cuban tree frogs as a function of mean EMG amplitude and EMG onset time. Based on multiple regression analysis, the relationship of work to mean EMG amplitude and EMG onset is highly significant (n=61; P<0.001; r^2=0.622).

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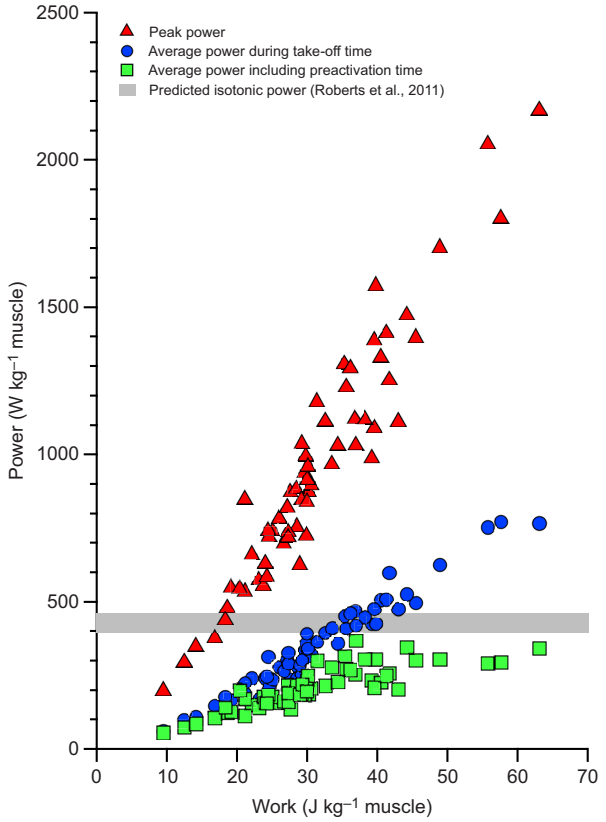

Fig. 7. Muscle mass-specific power output during jumping of Cuban tree frogs as a function of muscle mass-specific work output. The muscle mass-specific peak power during take-off is indicated by red triangles; the power output averaged over the take-off duration is indicated by blue circles; and the power output averaged over the time from EMG onset until toe-off is indicated by green squares. The gray bar indicates the isotonic power estimated from data on the Cuban tree frog plantaris muscle (Roberts et al., 2011) adjusted for temperature (Peplowski and Marsh, 1997) (n=61).

recorded here. In the most powerful jumps, the muscle mass-specific power exceeded 2000 W kg^{-1}, vastly greater than that available by direct application of muscle power (Figs 7 and 8).

Muscle work

Values for muscle mass-specific jumping work recorded in Cuban tree frogs are high, but within expectations based on the length–tension properties of frog muscle. Peplowski and Marsh (1997) calculated that if the muscles shortened symmetrically around optimal length with 50% strain and a force of 50% of maximal isometric force they would produce approximately 70 J kg^{-1}. Using fixed-end contractions of the plantaris muscle–tendon complex of Cuban tree frogs, Mendoza and Azizi (2021) found that the fascicles shortened, producing a mean work of 53 J kg^{-1}. This value is an estimate of the energy stored during the preactivation phase, and it may underestimate the total work capacity if the muscle continues to shorten and produce work during the release phase of the jump. In the present study, the highest value recorded for muscle mass-specific work output during jumping was approximately 60 J kg^{-1}. However, this value likely underestimates the mass-specific work done by some of the hindlimb muscles, because it was calculated based on the total mass of the hindlimb muscles, and some muscles likely do not contribute to powering the jump. In ranid frogs, the muscles that, based on anatomy, can contribute to the jump make up approximately 83% of the total hindlimb muscle mass (Marsh, 1994). If this value is similar in Cuban tree frogs, the maximum values for muscle mass-specific work would be approximately 70 J kg^{-1}. Although within the performance that is theoretically possible, this work output is impressive given the diverse architecture and anatomical positions of the muscles.

The limits of *in vivo* muscle power

Considering the duration over which the muscles were likely producing force, the power output recorded in this study was within the known capacity of frog muscle. Marsh and John-Alder (1994) suggested that power output during frog jumping that exceeds that available by direct application of muscle power could be explained if the work was redistributed in time by energy storage in elastic elements before the jump as was known to occur in locust jumping (Godden, 1975; Bennet-Clark, 1975). Data on EMG timing presented here allow the calculation of average power output over the full duration that the muscles could be producing power. I have taken this duration as the time from the onset of the EMG until toe-off. This measure of power increased with increasing work done up to 36 J kg^{-1} and leveled off at a mean value of 281 W kg^{-1} (Fig. 7). How does this value compare with the maximum power available from the muscles during isotonic contractions? Peplowski and Marsh (1997) provided data from the sartorius muscle of Cuban tree frogs over a range of temperatures. At the body temperature of the frogs in the present study, their data predict maximum isotonic power of 260 W kg^{-1}. However, the power output of the sartorius likely does not provide the best data for comparison with *in vivo* jumping power. Multiple fast fiber types are present in frogs (Lännergren, 1987), and Lutz et al. (1998) found that the sartorius in *R. pipiens* has a lower proportion of the fastest fiber types compared with muscles that are expected to power the jump. Roberts et al. (2011) reported a mean maximum isotonic power of the plantaris muscle in Cuban tree frogs of 313 W kg^{-1} at 'room temperature'. If room temperature is taken to be between 20 and 22°C, and the temperature dependence is the same as that for the sartorius muscle (Peplowski and Marsh, 1997), the power from the plantaris muscle would be between 397 and 461 W kg^{-1} at 28°C. Using this range of values, the *in vivo* muscle mass-specific power from EMG onset until toe-off would be between 61% and 71% of the maximum isotonic power. Although consistent with the known properties of the plantaris muscle, a muscle power output of approximately 65% of the maximum isotonic power is still an impressive value for a number of reasons. First, as mentioned previously for maximum work, this value was calculated using the total hindlimb muscle mass. Second, the muscles that are active in the jump have diverse architectures and anatomical positions, and, based on other species, likely have diverse length trajectories during jumping (Olson and Marsh, 1998; Roberts and Marsh, 2003; Azizi and Roberts, 2010). Third, I used the entire duration of possible muscle force production from the EMG onset until the frog left the ground, but undoubtedly this duration includes periods of low force production after onset and before toe-off. Finally, bear in mind that the maximal power during an *in vitro* isotonic contraction is achieved only briefly as the muscle shortens through its optimal length, whereas *in vivo* the muscles must shorten substantially to produce the values of work performed.

Implications of the results for the catch mechanism

The results of this study have implications for the nature of the catch mechanism involved in the prestorage of elastic energy in jumping

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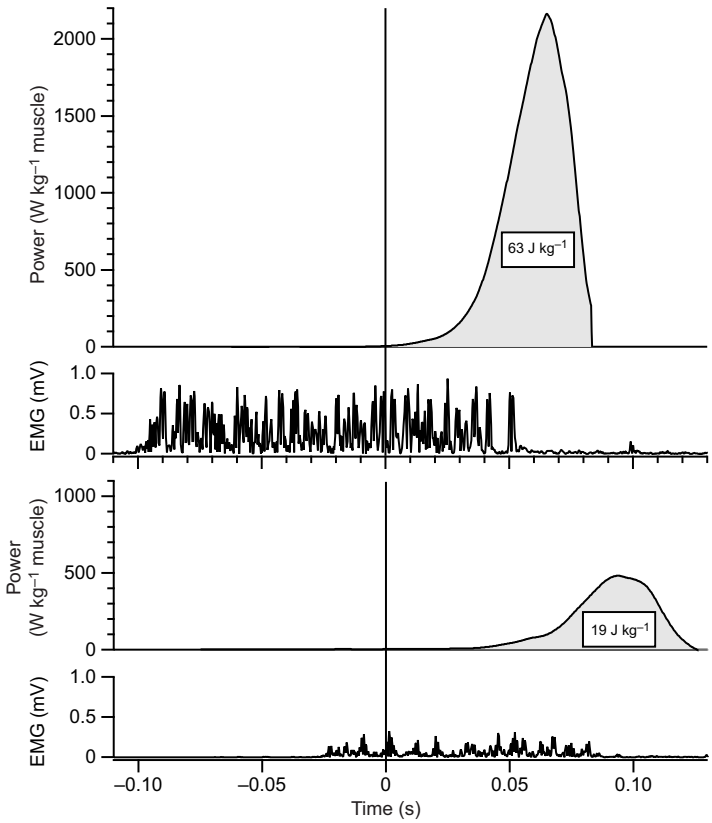

Fig. 8. Example calculations of the distribution of muscle mass-specific power output during jumping of Cuban tree frogs and the corresponding rectified EMG signals from the cruralis muscle. (A) A high-power jump. (B) A low-power jump. The shaded area under the power curves is the work done (as indicated). The vertical lines indicate the start of acceleration at zero time, after which power output is positive. (The power output in the low-power jump begins at time zero, but it remains at a very low level for an additional 50 ms.)

frogs. Previous work focusing on energy release at the ankle has suggested that the catch mechanism is a 'dynamic catch', 'based on the balance and orientation of forces throughout the limb rather than an anatomical catch' (Astley and Roberts, 2014). However, the details of the balance of forces at all the leg joints needs more attention. I found that in the highest performance jumps, at least three of the major muscles involved in powering the jump are activated more than 100 ms before any detectable acceleration of the center of mass of the frog (Figs 2–4, 8). In these jumps, the activation is approximately constant from onset to offset (Figs 2 and 8). In my opinion, this suggests that in the prestorage phase joint movements are prevented by mechanisms operating at multiple joints in the hindlimb. Given the diverse muscles that could contribute to powering the jump, particularly in the thigh, these mechanisms are likely to be complex. However, likely contributors are the actions of two-joint muscles including the cruralis and the plantaris. Strikingly, the duration of preactivation (and thus the prestorage duration) increased with increasing muscle activation. This correlation suggests the intriguing possibility of an automatic linkage between high muscle force production and setting the catch.

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Data availability
Data are available from the Brown Digital Repository: https://doi.org/10.26300/xnf4-jt93.

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