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HINDLIMB EXTENSOR MUSCLE FUNCTION DURING JUMPING AND SWIMMING IN THE TOAD (*BUFO MARINUS*)

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Summary

Many anurans use their hindlimbs to generate propulsive forces during both jumping and swimming. To investigate the musculoskeletal dynamics and motor output underlying locomotion in such physically different environments, we examined patterns of muscle strain and activity using sonomicrometry and electromyography, respectively, during jumping and swimming in the toad *Bufo marinus*. We measured strain and electromyographic (EMG) activity in four hindlimb muscles: the semimembranosus, a hip extensor; the plantaris, an ankle extensor; and the gluteus and cruralis, two knee extensors. During jumping, these four muscles are activated approximately simultaneously; however, joint extension appears to be temporally staggered, with the hip beginning to extend prior to or initially faster than the more distal knee and ankle joints. Mirroring this pattern, the gluteus and plantaris shorten quite slowly and over a small distance during the first half of limb extension during take-off, before beginning to shorten rapidly. The hip and knee extensors finish shortening near the point of take-off (when the feet leave the ground), while the ankle-extending plantaris, which exhibits the longest-duration EMG burst, on average, always completes its shortening after take-off (mean 26 ms). During swimming, activation of the four muscles is also nearly synchronous at the start of a propulsive stroke. The onset of fascicle shortening is temporally staggered, with the knee extensors beginning to shorten first, prior to the hip and ankle extensors. In addition, the knee extensors also often exhibit some degree of slow passive shortening prior to the onset of EMG activity. The offset of muscle shortening during swimming is also staggered, and to a much greater extent than during jumping. During swimming, the cruralis and gluteus finish shortening first, the semimembranosus finishes 30–60 ms later, and the plantaris, which again exhibits the longest EMG burst, finishes shortening last (mean 150 ms after the cruralis). Interestingly, much of this extended shortening in the plantaris occurs at a relatively slow velocity and may reflect passive ankle extension caused by fluid forces, associated with previously generated unsteady (accelerative) limb movements, acting on the foot. Average EMG burst intensity tends to be greater during jumping than during swimming in all muscles but the gluteus. However, EMG burst duration only changes between jumping and swimming in the cruralis (duration during jumping is nearly twice as long as during swimming). The cruralis is also the only muscle to exhibit substantially greater fractional shortening during jumping (mean 0.28) than during swimming (mean 0.20 active strain, 0.22 total strain). On the basis of these results, it appears that toad hindlimb function is altered between jumping and swimming. Moreover, these functional differences are influenced by passive effects associated with physical differences between the external environments, but are also actively mediated by shifts in the motor output and mechanical behavior of several muscles.

Key words: terrestrial, aquatic, locomotion, muscle, electromyography, jumping, swimming, sonomicrometry, toad, *Bufo marinus.*

Introduction

Vertebrate limbs are complex multi-jointed mechanical systems actuated by numerous muscles spanning one or more joints. The importance of many of these muscles is most obvious during locomotion, when they can function in a variety of ways. Perhaps most commonly, limb muscles can shorten while active, producing forces and generating positive mechanical work and power that can be used to produce propulsive thrust and to move skeletal elements of the limb relative to one another, to the body and to the external environment. Limb muscles can also lengthen while active as a result of the actions of antagonistic muscles or externally applied forces. Active lengthening enables muscles to generate high levels of force (Woledge et al., 1985), while allowing mechanical energy to be absorbed and dissipated by muscle fibers (if stretched beyond their short-range stiffness) or stored and potentially recovered in elastic elements such as tendons

and ligaments. Such active lengthening is often associated with decelerating or controlling the movement of body and limb elements during locomotion. A third possibility is that limb muscles can be active isometrically, again generating high levels of force, but generating no net work or power. In such a case, the muscles might actively stabilize a joint or allow for the storage of elastic strain energy in tendons (e.g. Biewener, 1998). Finally, muscles can undergo contractions in which different combinations of the scenarios described above can occur (i.e. a muscle that is pre-stretched or held isometric while active and then begins to shorten).

Although limb muscles always generate force when active, the amount of force produced per unit volume of muscle, the energetic cost of producing this force and the amount of work and power developed by any muscle can vary substantially depending both on its inherent structural and physiological properties and on its patterns of strain and stimulation (Goldspink, 1977; Josephson, 1999; Marsh, 1999). The diversity of sizes, shapes and fiber type distributions present among the limb muscles of an individual tetrapod is striking and yet, given this backdrop of architectural diversity, the functional repertoire of active limb muscles during locomotion is significantly less well characterized (Bertram and Marsh, 1998).

Multiple studies have examined electromyographic activity patterns in muscles acting at different joints throughout a limb during normal locomotion in a broad range of vertebrate taxa (e.g. Ashley-Ross, 1995; Gatesy, 1999; Goslow et al., 1981; Reilly, 1995). Such studies have been critical for determining the general patterns of use of individual limb muscles and, when combined with kinematic analyses, are also able to provide some insight into the mechanical behavior and function of specific limb muscles during locomotion. However, because most examinations of limb movements and muscle function have tended to focus on steady, unidirectional locomotion across a single uniform and flat environment, our understanding of how the musculoskeletal system might act to accommodate a broader range of limb use is somewhat limited (although see Full et al., 1998, for an example of invertebrate locomotion across a complex environment).

In the wild, most animals encounter and move through complex environments with diverse physical characteristics (e.g. gradient, substratum compliance, texture and even medium). How do animals accommodate such physical diversity during locomotion? Are the dynamics of the locomotor system altered in response to an environmental shift and, if so, do such alterations reflect differential output by the motor system to the change in environment? Such questions are difficult to answer, but to begin doing so we must start investigating musculoskeletal dynamics during locomotion in more diverse environments (e.g. Biewener and Gillis, 1999). Aquatic and terrestrial environments differ dramatically with respect to many physical properties (Denny, 1993; Vogel, 1994), and yet a variety of animals can use their limbs to move about successfully both in water and on land. Such animals afford an excellent opportunity to address potential changes in motor output and musculoskeletal dynamics during locomotion in different environments.

In this study, we use the toad *Bufo marinus* to examine hindlimb muscle function during locomotion across land and through water. During both jumping and swimming, anurans are known to use their hindlimbs to generate most, if not all, of the propulsive forces. Recent work by Kamel et al. (1996) and Olson and Marsh (1998) suggests that certain anuran limb muscles might have somewhat different functional roles depending upon the external environment through which the animal is moving. We examine this idea explicitly by characterizing the patterns of length change and electrical activity in four hindlimb extensor muscles during toad jumping and swimming. Our goal is to address whether and how patterns of strain and activation in these muscles are modulated to accommodate locomotion in both aquatic and terrestrial environments.

Materials and methods

Animals

Data were collected from 18 toads (*Bufo marinus* L., 66–114 g, mean 83 g). The animals were obtained from Glades Herp in Florida, USA, in October 1998. Toads were housed in plastic bins (1–4 individuals per bin) and fed a diet of waxworms. Temperature within the holding room was kept at 20–24 °C, and a photoperiod of 12 h:12 h light:dark was maintained during the holding period. All experiments were performed between December 1998 and August 1999, and animal temperatures recorded shortly after locomotor trials using a cloacal thermometer ranged between 19 and 24 °C. Differences in temperatures among individuals had no significant effect on locomotor performance, as measured by average jump distance (on land) or average limb cycle frequency (in water).

Surgical procedures

Sonomicrometry crystals and bipolar electrodes were implanted unilaterally in four major hindlimb extensor muscles to transduce *in vivo* length changes and activity patterns during locomotion in different environments. Typically, the left limb was implanted, although the right limb was used in one individual to ensure contralateral similarity. The four muscles examined were the semimembranosus (a hip extensor), the gluteus magnus and cruralis (knee extensors) and the plantaris (an ankle extensor). All these muscles are bi-articular, but their major actions are at the joints described above (Calow and Alexander, 1973; Dunlap, 1960; Marsh, 1994). Because of the invasive nature of the surgery and implantations, recordings from only two of these four muscles were attempted from any single individual.

Animals were anesthetized by immersion in a buffered tricaine methanesulfonate solution (MS-222; 1.0 g l^{-1}). To reveal limb muscles for implantation, an incision through the skin was made either between the hip and the knee (for the semimembranosus, gluteus magnus or cruralis) or between the knee and the ankle (for the plantaris). To record electrical

activity from the muscles, bipolar electrodes (offset twist hook; Loeb and Gans, 1985) were constructed using fine insulated silver wire (0.1 mm diameter; California Fine Wire Co., USA). Electrode tips were bared of insulation (0.5 mm) and implanted into muscles of interest using a 21 gauge needle. Electrodes were sutured in place at the site of implantation using 6-0 silk.

To transduce muscle fascicle length changes, pairs of 1.0 mm sonomicrometry crystals (37 or 42 gauge wire leads were 1 m long; Sonometrics Corp.) were implanted into each muscle 7–13 mm (mean 9.75 mm) apart (for thorough reviews of the use of sonomicrometry to measure *in vivo* fascicle length changes during animal locomotion, see Biewener et al., 1998a; Olson and Marsh, 1998). Small holes were created in the muscles using the sharp tips of small stainless-steel forceps, and the crystals were placed into the holes and aligned to be facing one another. The holes were then sutured shut, and the lead wires from the crystals were sutured to the surface of the muscle using 6-0 silk to help prevent any dislocation during experiments. For the semimembranosus and gluteus, crystals were implanted 1–2 mm below the surface, along the longitudinal axis of the muscle. For the highly pinnate cruralis, initial implantations were also along the long axis of the muscle 1–2 mm below the surface. However, strain patterns observed from these initial implantations could not be replicated in subsequent recordings in which crystals were implanted along the trajectory of the fascicle axes. These implants were also placed along the midline of the muscle, 8–10 mm apart, but at different depths. The proximal crystal was implanted approximately 4 mm deep, near where the fibers insert onto the thin central tendon running along the ventral surface of the muscle. The distal crystal was implanted approximately 1.5 mm deep, near the origin of the broad aponeurosis that extends over the knee. Thus, strain data from the initial recordings were discarded (although electromyographic data were still used), and in all subsequent cruralis experiments sonomicrometry crystal implantations took into account the pinnate trajectory (15–20 °) of the muscle fibers. For the less pinnate plantaris, the crystals were also aligned along the midline of the muscle, at depths of 2–3 mm. While these implantations were not aligned exactly along the fascicle axes, the difference in trajectories was minimal (<10 °), so any resulting errors in absolute strain levels were probably small (a measurement error of less than 2 % given that cos10 °=0.985). In addition, implantations subsequently placed along the plantaris fascicle axes confirmed the strain patterns recorded using these initial implantations.

When the implantations had been completed, skin incisions were sutured closed using 3-0 silk, except for a small hole in the skin at the hip or behind the knee (in the case of the plantaris) through which all wires emerged from the animal. External to the animal, the wires were bound together using suture at multiple sites (to prevent the limbs from becoming entangled among the wires during locomotor trials) and were soldered into four miniature female connectors (GF-6, Microtech).

Data recording and collection

Prior to surgery, each animal was recorded (Redlake Motionscope; 250 frames s^{-1}) performing three jumping and swimming trials. These recordings were used as an initial measure of locomotor performance to ensure that, after implantations, toads were still capable of jumping similar distances and swimming at similar speeds. After surgery, toads were typically allowed 15–20 h to recover before starting experiments. Before beginning locomotor trials, the female connectors conveying the wires from the animal were connected to matching male connectors (GM-6, Microtech) which, in turn, were each soldered to a lightweight cable 3 m long. These cables were bound together by heat-shrink-wrap and were connected to the recording equipment *via* paired male and female 25 position D subminiature solder-type connectors (Radioshack). EMG signals were run through Grass P511 preamplifiers, amplified 1000 times and filtered (60 Hz notch filter and 100–3000 Hz bandpass). Wires conveying length change information from the sonomicrometry crystals were connected to a Sonomicrometer unit (model 120-1001, Triton Technology Inc.), the output of which was monitored by an oscilloscope (Tektronix 2235A 100 MHz). Voltage signals from the EMG amplifiers and the Triton sonomicrometer system were digitized at 5000 Hz *via* a 12-bit A/D converter (Digidata 1200B system; Axon Instruments Inc.) and recorded onto a personal computer. Because of the phase delay introduced by filters within the Triton sonomicrometer, all length change data were offset 5 ms in time relative to the electromyographic (EMG) and imaging records. Voltages from the sonomicrometer were converted to distances (mm) using the relationship between voltage and distance (mm) given by the Triton system. To ensure that this relationship was accurate, we attached sonomicrometry crystals to the tips of digital calipers and placed them in a water bath. The distances between crystal surfaces, as measured by the calipers, were then compared with those calculated from the voltage/distance relationship given by the Triton sonomicrometer. For the crystals we were using, we obtained differences between these two methods of approximately 0.5 mm (i.e. sonomicrometer distance = caliper distance + 0.5 mm). Over all our implantations, the average distance between crystals was approximately 10 mm; thus, on average, this offset amounted to an underestimation of approximately 5 % of the actual distance between crystals. Because we were most interested in comparisons of relative strain (between hopping and swimming), rather than absolute changes in length, we felt it unnecessary to adjust our data for this offset.

Swimming trials were performed in a rectangular glass tank (30 cm×90 cm) filled with water to a depth of approximately 16 cm. The tank was lit from above with two 800 W Lowel Tota-lights. The cable conveying wires from the toad to the recording equipment was taped to a light stand approximately 0.5 m above the tank. Toads were placed manually at one end of the tank and released, at which point they voluntarily commenced swimming. Sequences in which toads swam in a single direction and performed a consistent series of at least three propulsive strokes were recorded onto a personal computer as audio-video interleaved (AVI) files using a

Table 1. *Number of sequences analyzed from each muscle in each environment for each individual*

	Semimembranosus		Gluteus		Cruralis		Plantaris	
Individual	Aquatic	Terrestrial	Aquatic	Terrestrial	Aquatic	Terrestrial	Aquatic	Terrestrial
1	5	4	5*	4*				
2	5	6	5**	6**	–	–	–	–
3	6	6	6	5	–	–	–	–
4	5	6	5	4	–	–	–	–
5	4	4	6	–	–	–	–	–
6	–	–	7	7	–	–	7	6
7	–	–	–	–	4**	2**	–	2
8	–	–	5	–	–	–	–	–
9	–	–	–	–	4**	–	4	6
10	–	–	–	–	6**	6**	6	3+3**
11	–	–	–	–	5**	5**	5	5
12	–	–	–	–	6**	5**	6	3+1**
13	–	–	–	–	5**	7**	–	–
14	–	–	–	–	4*	6	–	–
15	–	–	–	–	4**	7**	–	–
16	–	–	–	–	5	6	–	–
17	–	–	–	–	–	7	–	–
18	–	–	–	–	4	7	–	–

Numbers in columns represent the total number of sequences from which length change and electromyographic (EMG) data were obtained.

Cruralis data from individuals 7, 9, 10, 11, 12, 13 and 15 represent sequences in which the crystals were not aligned with the fascicle axes; hence, length change data were not used.

*Only length change data were obtained; **only EMG data were obtained.

Motionscope PCI high-speed digital imaging system (Redlake Imaging Corp.) with a frame rate of 250 frames s^{-1}. Swimming sequences were recorded from directly above the tank, providing a dorsal view of the animal as it swam. A plastic ruler was attached to the side of the tank at the water surface to provide scale when analyzing the video recordings. For each individual, 5–10 swimming sequences were recorded to obtain a range of limb-cycle frequencies.

Jumping trials were performed within a large fiberglass arena (0.75 m×1.3 m), lit from above by three 800 W lights. The toads were placed manually onto a flat surface at one end of the arena. This surface was covered with a thin sheet of cardboard onto which spray-mount adhesive was administered several days prior to experiments to provide the toads with sufficient traction to prevent slipping during jumps. Upon release, toads usually immediately jumped and were then quickly captured and placed into a small plastic container until ready to jump again. The dorsal and lateral views of jumps were recorded to computer at 250 frames s^{-1} using the Motionscope system. A mirror was placed at 45° along one side of the arena to allow both dorsal and ventral images to be recorded *via* a single camera placed above the jumping surface. A small 20 cm long box was placed along the side of the jumping surface for scale when analyzing the video recordings. For each individual, 5–10 jumping sequences were recorded so that a range of jump distances could be obtained. Because only single locomotor events (jumps) were being recorded on land and used for comparison with the repeated cyclical locomotor movements in water, several toads were also recorded jumping freely (i.e. multiple jumps in sequence were allowed) to ensure that muscle length change and activity were similar when toads performed multiple jumps *versus* the single jumps recorded for analysis.

Electromyographic and length-change data were synchronized with the video files using a voltage pulse that acted as the trigger to stop video recording and was recorded on its own channel along with the EMG and sonomicrometry signals. Experiments performed prior to locomotor trials using a light-emitting diode, also triggered by the voltage pulse and recorded onto video, revealed a 4 ms delay between the video system and A/D board, which was later accounted for during data analysis.

After all locomotor trials had been completed, the toads were killed by extended immersion in a buffered tricaine methanesulfonate solution (MS-222; 2.0 g l^{-1}). Dissections were then performed on the hindlimbs to locate and confirm the positions of EMG electrode tips and sonomicrometry crystals. In a subset of toads, the four hindlimb muscles of interest were excised and weighed to the nearest 10 mg on a Fisher balance.

Data analysis

Data were typically analyzed from 4–7 locomotor sequences per individual within each environment (see Table 1 for a complete description of the sequences used per individual). On land, jumps were chosen for analysis if they were straight (i.e. a trajectory no more than 25° from a line bisecting the mid-sagittal plane of the animal prior to take-off) with little or no

lateral rotation in mid-air. Although swimming sequences usually consisted of 3–5 propulsive cycles, only two cycles were typically performed completely within the field of view of the camera, so only these cycles were then used for subsequent analysis. Consecutive pairs of swimming cycles were used for analysis only if they were of similar duration (no more than 20 % difference in duration) and the data were then averaged over the two cycles. Cycles 2 and 3 or 3 and 4 in a sequence were usually analyzed.

For jumping sequences, the timing of five kinematic events was determined from the video files: (i) the onset of toad movement (which was readily identifiable, but does not necessarily coincide exactly with the onset of hindlimb extension, which would have required detailed three-dimensional kinematic analyses beyond the scope of this project to identify precisely); (ii) the onset of the aerial phase, as defined by the video field in which the toad's foot first left the ground completely; (iii) the end of the aerial phase, defined as the video field in which the first digit of the toad's forelimb comes back into contact with the ground; (iv) the time at which the hindlimbs first come back into contact with the ground, marking the end of the period in which the toad supports and decelerates its mass exclusively using its forelimbs; and (v) the time at which the toad's body completely comes to rest and stops moving. For swimming sequences, only two kinematic events were determined from the video files: the onset of hindlimb extension, and the onset of hindlimb retraction or flexion. A more detailed kinematic analysis of hindlimb movements during swimming in toads will be published in a future study.

In both jumping and swimming sequences, numerous variables quantifying the temporal sequence of muscle activation, deactivation and length change were determined. All such timing variables were measured with respect to time zero, which coincided with the toad's onset of movement on land and the onset of hindlimb extension during swimming. The onset of limb extension on land is often delayed by several milliseconds relative to the onset of body movement, but this latter variable was much easier to identify in the AVI video files. The onset, offset and duration of EMG activity and muscle fascicle shortening were measured for each muscle recorded during every locomotor sequence.

The magnitude of each EMG burst was assessed by calculating the average spike amplitude (intensity) of the rectified EMG signal. For each muscle, values of EMG intensity were then converted to relative values within each individual by dividing each intensity by the largest value recorded in that muscle for that individual. Thus, the largest intensity for a muscle in an individual was given a value of 1, and all other bursts in that muscle were assigned a value between 0 and 1.

Muscle fascicle strain and strain rate were also measured for each muscle in each locomotor trial. The time-dependent changes in muscle fascicle length during jumping and swimming were estimated on the basis of the changes in distance between the pairs of sonomicrometry crystals present in each muscle of interest. Any change in distance between crystals was assumed to represent a proportional change in length over the entire muscle fascicle. Because absolute length changes were not the focus of this study, small errors in distance measurements between crystals potentially introduced by small changes in muscle stiffness during contraction (2–3 %; Hatta et al., 1988) were not accounted for, but were likely to be quite small (Biewener et al., 1998b; Olson and Marsh, 1998).

Fascicle strain was measured as a fractional length change relative to the fascicle's resting length (L; defined as the average distance between crystals before and after jumps when the toad was not moving). Strain rate during shortening was calculated by dividing the total amount of shortening (after the onset of EMG activity) by the time elapsed during this period and is reported in muscle fascicle lengths s^{-1} ($L\,s^{-1}$).

Statistical analyses

To assess the general pattern of use of the four muscles during jumping and swimming, the overall mean and standard error of 10 variables characterizing the timing and degree of muscle activation and shortening were calculated for all terrestrial and aquatic sequences: (i) the time of onset of fascicle shortening, (ii) the time of offset of fascicle shortening, (iii) fascicle shortening duration, (iv) the fractional length change of the fascicle (strain), (v) the rate of fractional length change (strain rate), (vi) EMG onset time, (vii) EMG offset time, (viii) EMG duration, (ix) EMG–fascicle shortening lag, and (x) relative EMG intensity.

A small amount of pre-lengthening occurred on occasion in all muscles during jumping, so one-way analyses of variance (ANOVAs) were run to determine whether this had any effects on each of the above variables (one set of ANOVAs was performed on the data from each muscle). If pre-lengthening had a significant effect on any of the variables (as was the case in the semimembranosus and plantaris), then, before running any additional ANOVAs, data were segregated as follows. First, for both the semimembranosus and plantaris, it was determined that pre-lengthening occurred in a minority of jumps (<50 % of the time). So for these two muscles, data used in further ANOVAs were only taken from jumps in which the muscle did not undergo pre-lengthening. For the other two muscles in which pre-lengthening, when it occurred, had no significant effect on any aspect of the shortening behavior (gluteus and cruralis), data from all jumps were used in further ANOVAs.

To determine the effect of environment on variables characterizing aspects of hindlimb muscle function (shortening duration, fascicle strain, fascicle strain rate, EMG burst duration, EMG–fascicle shortening lag and EMG burst intensity), two-way ANOVAs were used with individual and environment as random and fixed effects, respectively (one set of ANOVAs was performed on the data from each muscle). For each muscle, only data from individuals in which both swimming and jumping recordings were successfully obtained were used. To account for multiple ANOVAs, the sequential

Bonferroni technique was used within each ANOVA set to adjust the levels of significance (Rice, 1989).

Results

General movements during jumping and swimming

During both jumping and swimming, extension of the hindlimbs of *Bufo marinus* provides the propulsive power for locomotion (Figs 1, 2). On land, prior to jumping, toads typically sit with their hindlimbs bent underneath them with some of their weight supported by their forelimbs (Fig. 1). Power is generated as the hindlimb extensor muscles are activated and shorten, leading to a rapid extension of the hindlimbs which, in turn, propels the animal into the air (Fig. 1). In mid-air, the forelimbs are extended, and they re-establish contact with the ground before any other part of the animal (Fig. 1). The forelimbs act to support and decelerate the toad before its body is pivoted ventrally about the shoulder until the hindlimbs contact the ground (Fig. 1). At that point, the bulk of the body weight is transferred posteriorly to the hindlimbs, and the toad is ready to jump again (Fig. 1).

During a jump, the duration of the take-off phase (from when the animal begins to move until it takes off) is quite variable, ranging from 100 to 240 ms. Take-off duration decreases significantly as a function of jump distance ($P<0.0001$; Fig. 3A). In contrast, the duration of the aerial phase of the jump increases significantly with increasing jump distance ($P<0.0001$; Fig. 3B). Finally, the period during which the toad is supported exclusively by its forelimbs during landing is also highly variable (8–225 ms), but decreases significantly as a function of jump distance ($P=0.0002$; Fig. 3C). Jump distance in this study ranged between 17 and 54 cm.

In the water, toads also propel themselves *via* hindlimb extension. During swimming, the hindlimbs are cyclically extended through a propulsive stroke and then flexed and retracted back towards the body in a recovery stroke (Fig. 2). The forelimbs are either held along the side of the body during a swimming bout or are extended anteriorly. Occasionally, one forelimb is held against the body while the other is extended forwards. Swimming cycle durations in this study ranged between 375 and 725 ms.

Patterns of muscle activity and strain

Semimembranosus

The semimembranosus is a bi-articular muscle that crosses the hip and knee joints and acts largely in hip extension. The mass of this muscle averaged 0.68 g (7.2 % of limb mass or 1.6 % of body mass when the left and right muscles are considered) in the subset of toads measured ($N=7$).

During jumping, electrical activity within the semimembranosus typically began shortly before the onset of toad movement and lasted for an average of 91 ms (Figs 4, 5). On average, the muscle began to shorten 18 ms after the onset of EMG activity, and then shortened over a variable duration (80–175 ms; mean 127 ms). The magnitude and rate of fascicle strain during shortening averaged 0.25 and 1.99 $L\,s^{-1}$, respectively, but both showed considerable variation among toads and among jumps performed by the same toad.

A small degree of pre-lengthening prior to shortening of the

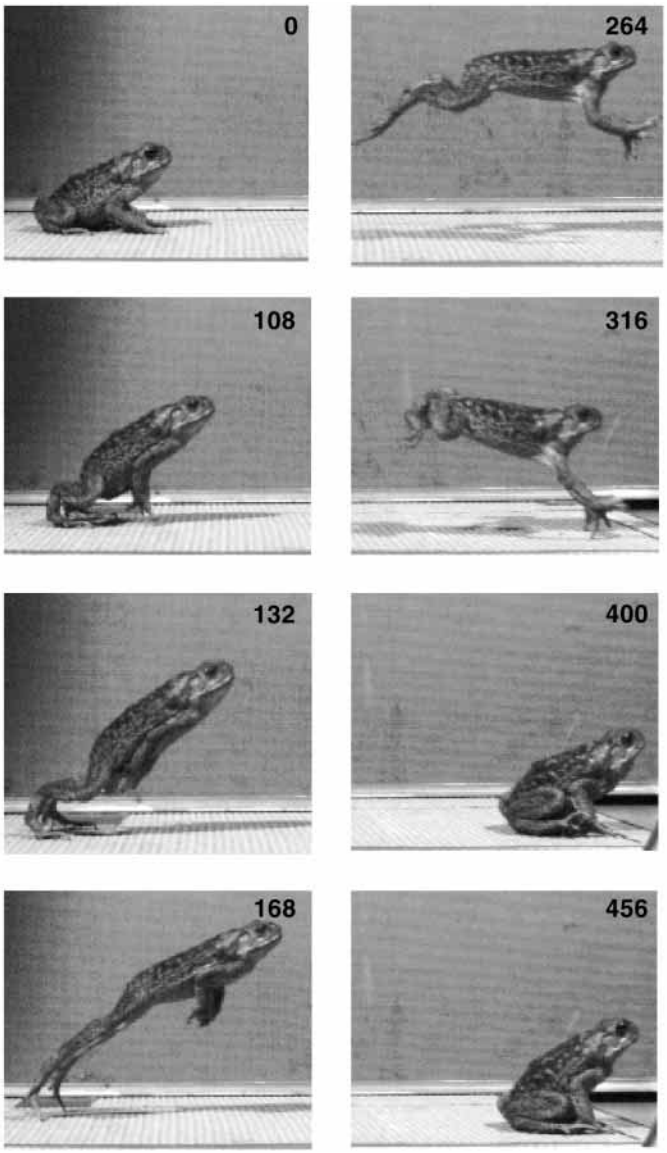

Fig. 1. A series of images of the lateral view of a 35 cm jump by a 75 g toad (*Bufo marinus*) prior to surgery to show the basic patterns of movement observed during a typical jump. The images are arranged chronologically in two columns, with time (in ms) represented in the upper right-hand corner of each image. Time 0 represents the video frame prior to the onset of toad movement. Time 168 represents the last frame in which the hindlimbs maintained contact with the ground and marks the onset of the aerial phase of the jump. Time 316 shows the first frame in which the forelimbs re-establish contact with the ground and marks the end of the aerial phase; at this point, the hindlimbs are nearly completely flexed and retracted; over the next 100 ms, the forelimbs decelerate and support the toad's body weight as the body rotates ventrally about the shoulder joint until the hindlimbs contact the ground. The jump is complete at time 456, and the toad is then ready to jump again.

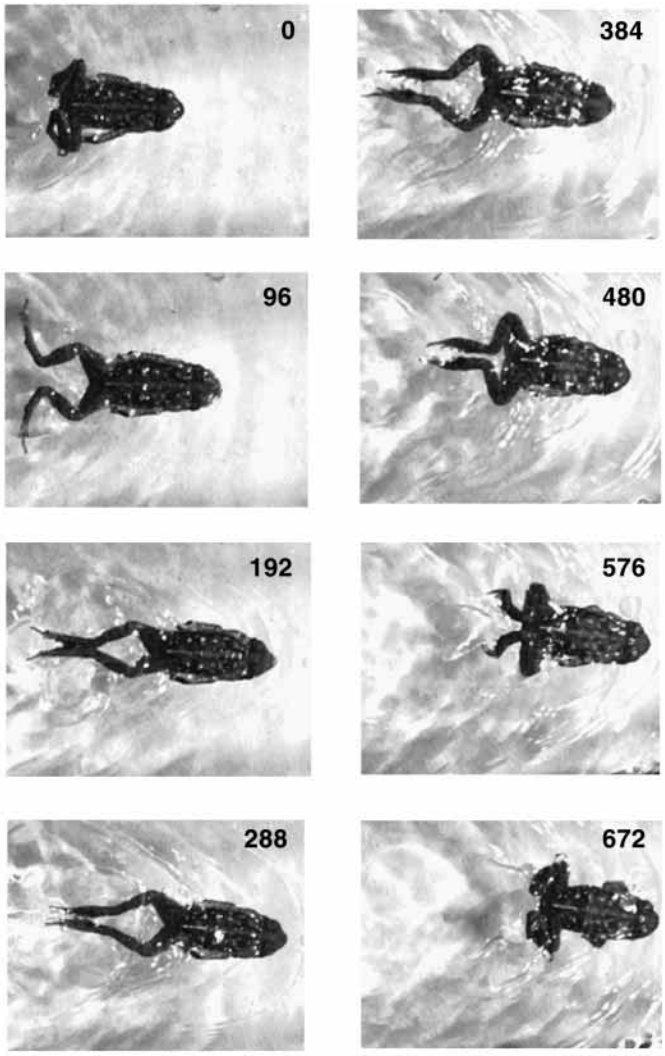

Fig. 2. A series of images of the dorsal view of one complete swimming cycle by a 67 g toad prior to surgery to show the basic movement patterns used during aquatic locomotion. Images are arranged chronologically in two columns, with time (in ms) represented in the upper right-hand corner of each image. Time 0 represents the video frame prior to the onset of hindlimb extension. The knee begins to extend first, followed by the hip and then the ankle; extension at all joints continues until time 192. Between times 192 and 288, the hip and knee begin to flex and retract, while the ankle continues extending. By time 576, the hip and knee joints have nearly returned to their original configurations as the ankle continues to flex and retract. At time 672, the ankle has returned to its original position, and the limb is ready to be extended again. The limb extension phase is defined as from the time the knee begins extending to the time when the ankle finishes extending (0–288 ms in this sequence).

semimembranosus occurred on occasion in several animals. Average strain during pre-lengthening was −0.02 (strain is defined here as positive during shortening and negative during lengthening). When it occurred, pre-lengthening began near the onset of toad movement and continued for 40–60 ms before the muscle began to shorten. As a result, the onset of muscle shortening was significantly delayed when pre-lengthening occurred (P<0.0001). In the two individuals that exhibited jumps both with and without pre-lengthening, EMG burst area, strain magnitude and strain rate tended to be lower when pre-lengthening took place.

During swimming, EMG activity began shortly before the onset of hindlimb extension (Figs 4, 5). Periods of EMG activity exhibited an average duration of 90 ms and an average burst intensity slightly, but not significantly, lower than that observed during jumping. Muscle shortening did not begin until well after the onset of EMG activity (mean delay 44 ms; Fig. 5), and this delay was significantly greater than that recorded during jumping (Table 2). Shortening continued for a variable duration (72–155 ms, mean 115 ms) which, on average, did not differ from that during jumping. Strain and strain rate also varied among individuals and swimming sequences, averaging 0.20 and 1.74 $L\,s^{-1}$, respectively, but were also not significantly different from those recorded during jumping.

Gluteus magnus

The gluteus is a bi-articular muscle that extends across the hip and knee joints but, unlike the semimembranosus, it acts largely in knee extension. It is the smallest of the muscles examined in this study, averaging only 0.38 g (approximately 3.8 % of limb mass or 0.9 % of body mass when both muscles are considered) in the subset of toads measured (N=7).

Simultaneous recordings of both EMGs and strain were available from only three individuals during jumping. Electrical activity within the muscle typically began just before the onset of movement (Figs 4, 5) and lasted an average of 120 ms. During jumping, the time between the onset of EMG activity and when the muscle began to shorten (EMG–shortening lag) was quite variable (15–100 ms; mean 36 ms), as was the duration of shortening (100–200 ms; mean 130 ms). Muscle fascicle strain and strain rate also varied considerably within and among individuals, but averaged 0.20 and 1.65 $L\,s^{-1}$, respectively. Interestingly, the rate of strain in the gluteus during jumping was not constant and was typically much lower during the first half of the shortening phase than during the second half (Fig. 4).

A small degree of pre-lengthening (mean strain −0.01) occurred during approximately half the jumps. The presence of pre-lengthening, however, did not have a significant effect on any of the variables characterizing muscle length change or EMG activity.

During swimming, EMG activity was observed in the gluteus shortly before the limb began to extend and continued for an average of 100 ms (Figs 4, 5). The duration and intensity of EMG bursts during swimming were not significantly different from those observed during jumping (Table 2). Gluteus muscle fascicles often began to shorten slowly before the onset of EMG activity (mean −68 ms; Fig. 4). Both fascicle strain and rate of strain during this period were typically low (mean 0.03 and 0.50 $L\,s^{-1}$, respectively). Shortly after the onset of EMG activity (mean 17 ms), a distinct increase in the

Table 2. *Two-way ANOVA comparing muscle function during jumping and swimming*

Muscle	Variable	Environment	Individual	Environment×individual
Semimembranosus		d.f.=1, 3	d.f.=3, 30	d.f.=3, 30
	Shortening duration	0.01	11.00**	1.32
	Strain	0.5	37.52**	6.69**
	Strain rate	0.26	34.38**	10.41**
	EMG burst duration	0.38	9.87**	2.781
	EMG–shortening lag	27.29**	6.37**	1.67
	EMG intensity	3.29	‡	‡
Gluteus		d.f.=1, 2–3	d.f.=2–3, 27 or 36	d.f.=2–3, 27 or 36
	Shortening duration	0.17	1.72	4.02
	Strain	0.67	16.31**	17.56**
	Strain rate	0.24	26.03**	8.38**
	EMG burst duration	0.44	44.64**	3.67
	EMG–shortening lag	1.93	0.34	4.43
	EMG intensity	0.66	‡	‡
Cruralis		d.f.=1, 3 or 7	d.f.=2 or 7, 26 or 63	d.f.=2 or 7, 26 or 63
	Shortening duration	3.15	12.18**	8.60**
	Strain	10.28*	5.71**	5.41**
	Strain rate	0.42	5.61**	10.69**
	EMG burst duration	70.22**	5.04**	2.39
	EMG–shortening lag	§	§	§
	EMG intensity	17.84*	‡	‡
Plantaris		d.f.=1, 3–4	d.f.=3–4, 30 or 40	d.f.=3–4, 30 or 40
	Shortening duration	7.18	28.00**	9.70**
	Strain	3.75	16.13**	3.83
	Strain rate	16.62*	28.26**	3.03
	EMG burst duration	0.38	14.54**	9.99**
	EMG–shortening lag	0.99	17.82**	3.82
	EMG intensity	29.81**	‡	‡

Table entries are *F* values.

For all muscles, strain rate refers to the average rate of strain after the onset of electromyographic (EMG) activity until the muscle has finished shortening.

For the gluteus and cruralis, strain refers to total strain (active+passive), although qualitative results are similar if active strain is used.

*Consistent trend, (F>10.0), but made insignificant at P<0.05 using the sequential Bonferroni method; **significant at P<0.05 using the sequential Bonferroni method described by Rice (1989).

‡EMG intensity was not compared among individuals.

§Simultaneous EMG and length change data were only recorded from two individuals, so an ANOVA was not performed.

shortening velocity was apparent (Fig. 4). We interpret this increase in shortening velocity as being caused by the activation and contraction of the muscle. Hence, we refer to this period of higher shortening velocity as active shortening and to the period of shortening prior to this as passive shortening. Active shortening duration averaged 110 ms, while fascicle strain and strain rate during this period averaged 0.16 and 1.49 $L\,s^{-1}$, respectively. None of these variables differed significantly between jumping and swimming in this muscle (Table 2). In addition, the total strain observed during swimming (active+passive strain) also did not differ significantly from that observed during jumping.

Cruralis

The cruralis is a bi-articular muscle that extends across the hip and knee joints and acts largely in knee extension. It has a complex pinnate architecture and is the largest of the muscles studied (it is the largest muscle in the hindlimb), averaging 0.99 g (10.3 % of limb mass or 2.4 % of body mass when both muscles are considered) in the subset of toads measured (N=7).

Simultaneous recordings of both EMGs and strain were available from only three individuals during jumping and two individuals during swimming. During jumping, EMG activity in the cruralis began just after the onset of toad movement and continued for 107 ms, on average (Figs 4, 5). The muscle fascicles typically began to shorten approximately 15 ms after the onset of EMG activity and shortened for an average of 138 ms (range 94–200 ms). Fascicle strain and strain rate varied among individuals, and among hops within individuals, but averaged 0.28 and 2.07 $L\,s^{-1}$, respectively.

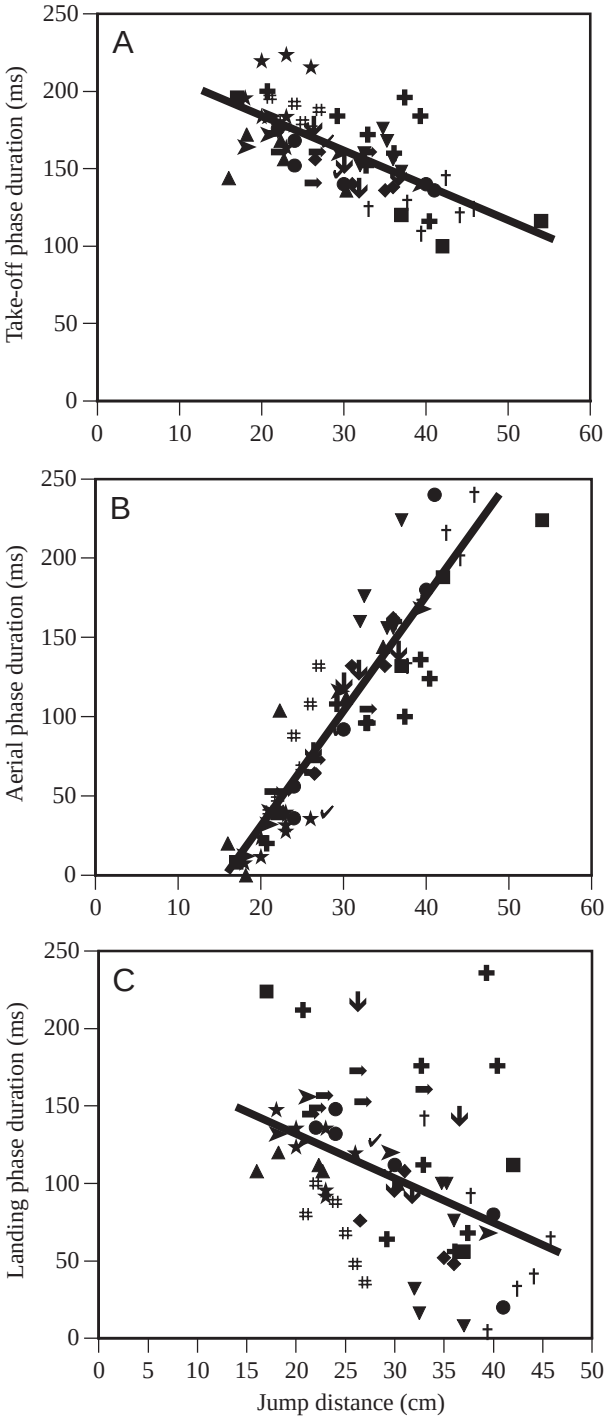

Fig. 3. Three graphs relating the duration of different phases during a jump to jump distance; the data represent 68 jumps from 13 individuals. (A) The duration of the take-off phase during a toad jump decreases significantly as a function of jump distance (y=–2.18x+225; r^2=0.44; P<0.0001). (B) The duration of the aerial phase increases significantly with jump distance (y=7.15x–112; r^2=0.83; P<0.0001). In one case, there was no aerial phase to a jump (duration 0), meaning that, by the time the toad's feet had left the ground, its forelimbs had already re-established contact. (C) The duration of the landing phase decreases significantly with jump distance (y=–2.94x+193; r^2=0.19; P=0.0002). Graphs include data from one individual in which muscle recordings were unsuccessful.

On a few occasions, a small degree of pre-lengthening occurred at the onset of toad movement, prior to muscle shortening. Lengthening strain during this period averaged –0.01 and took place over a period of 20–30 ms. Jumps in which pre-lengthening was observed did not differ from other jumps in terms of any of the variables characterizing muscle strain or activity.

During swimming, EMG activity in the cruralis muscle began shortly before the onset of hindlimb extension and continued for an average of 52 ms, a duration significantly shorter than that observed during hopping (Table 2). In addition, the relative intensity of these bursts averaged less than 50 % of that observed in the muscle during jumping (Fig. 5). As was the case with the gluteus (with which it converges onto a broad aponeurosis on the anterior surface of the knee), cruralis fascicles sometimes began shortening 40–50 ms before the onset of EMG activity (Fig. 4). During this time, strain and strain rate were both low (mean strain 0.02, strain rate 0.39 $L\,s^{-1}$) and, shortly after EMG activity began (mean 11 ms), a distinct increase in strain rate was observed (Fig. 4). In these cases, we considered the shortening prior to EMG activity to be passive and refer to the phase of more rapid shortening after EMG onset as active shortening (as was the case with the gluteus). Fascicle strain magnitude and rate during active shortening (mean strain 0.20, strain rate 1.98 $L\,s^{-1}$) were both much greater than during passive shortening. Comparisons between jumping and swimming reveal that total strain (active+passive) during swimming tends to be lower than that observed during jumping, although this difference is not significant after Bonferroni correction (Table 2). Strain rates during active shortening were not different between jumping and swimming (Table 2).

Plantaris

The plantaris is a bi-articular, pinnate muscle that extends across the knee and ankle joints inserting *via* the plantar aponeurosis at the base of the foot. Its main action is to extend the ankle. The plantaris is the second largest muscle of the four examined here, averaging 0.80 g (8.7 % of limb mass or 1.9 % body mass when muscles from both limbs are considered) in the subset of toads measured (N=7).

During jumping, EMG activity began shortly prior to the onset of animal movement and continued for an average of 130 ms (Figs 4, 5). The plantaris began to shorten an average of 29 ms (range 8–73 ms) after the onset of EMG activity and continued shortening for a long time (mean 170 ms; range 124–248 ms). The magnitude and rate of strain during shortening varied within and among individuals, averaging 0.18 and 1.07 $L\,s^{-1}$ respectively. As was observed in the gluteus, the rate of strain in the plantaris during hopping was often much lower during the first half of the shortening phase than during the second half.

As with the other muscles, a small degree of pre-lengthening occurred occasionally in the plantaris prior to muscle shortening (mean strain –0.01). As in the

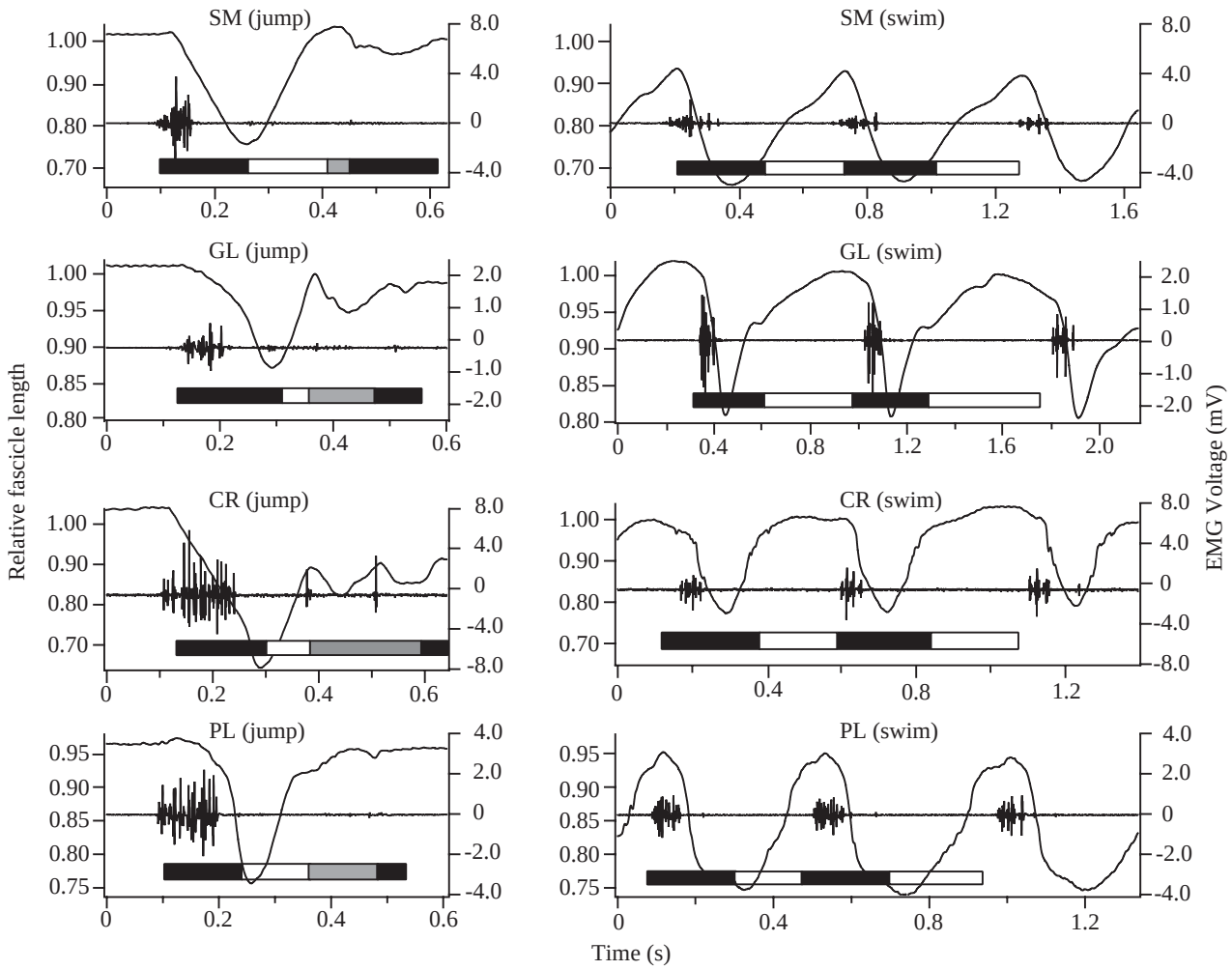

Fig. 4. Two columns of panels showing representative *in vivo* patterns of fascicle strain (left-hand vertical axes) and electromyographic (EMG) activity (right-hand vertical axes) *versus* time (horizontal axis). The data are from the semimembranosus (SM), gluteus (GL), cruralis (CR) and plantaris (PL) muscles during a single jump (left-hand column) and several swimming cycles (right-hand column). For each muscle, the jumping and swimming data are from the same individual. Bars in the jumping panels represent the temporal sequence of kinematic events. The first dark bar represents the time from the onset of toad movement to the time the animal leaves the ground. The white bar represents the aerial phase of the jump, and the gray bar represents the initial phase of landing, during which the toad is decelerating and supporting its body exclusively *via* its forelimbs. The final dark bar represents the second landing phase, from when the hindlimb first touches the ground until the animal's limbs are completely tucked back under its body and it is ready to jump again. Bars in the swimming panels also represent the temporal sequence of kinematic events. The dark bar represents the hindlimb extension phase (from when the knee begins to extend until the ankle finishes extending), and the white bar represents the limb's flexion (retraction) phase.

semimembranosus, when pre-lengthening did occur, the onset of fascicle shortening was significantly delayed and the overall duration of shortening was reduced. Pre-lengthening had no other significant effects on the activity or strain of the muscle.

During swimming, electrical activity in the plantaris typically began shortly before the limbs began to extend (Figs 4, 5) and continued for an average of 145 ms. While this duration was not significantly different from that observed during jumping, the average EMG burst intensity during swimming was only approximately half that observed during jumping (Table 2). Plantaris fascicles began to shorten 10–40 ms after the onset of EMG activity (mean 24 ms) and shortened over a relatively long period (mean 220 ms; Fig. 5). Total fascicle strain during shortening averaged 0.19, which was similar to that observed during jumping. In contrast to jumping, during many swimming sequences, the plantaris shortened at a relatively high strain rate over the first half of its shortening phase (mean $1.19\,L\,s^{-1}$) and then shortened more slowly during the second half (mean $0.39\,L\,s^{-1}$; Fig. 4). This latter, slower phase of shortening typically occurred after the offset of EMG activity (Fig. 4) and may reflect a shift from active to passive shortening. The higher (active) strain rates observed during the first half of the shortening phase during swimming were quite similar, on average, to the strain rates observed during jumping.

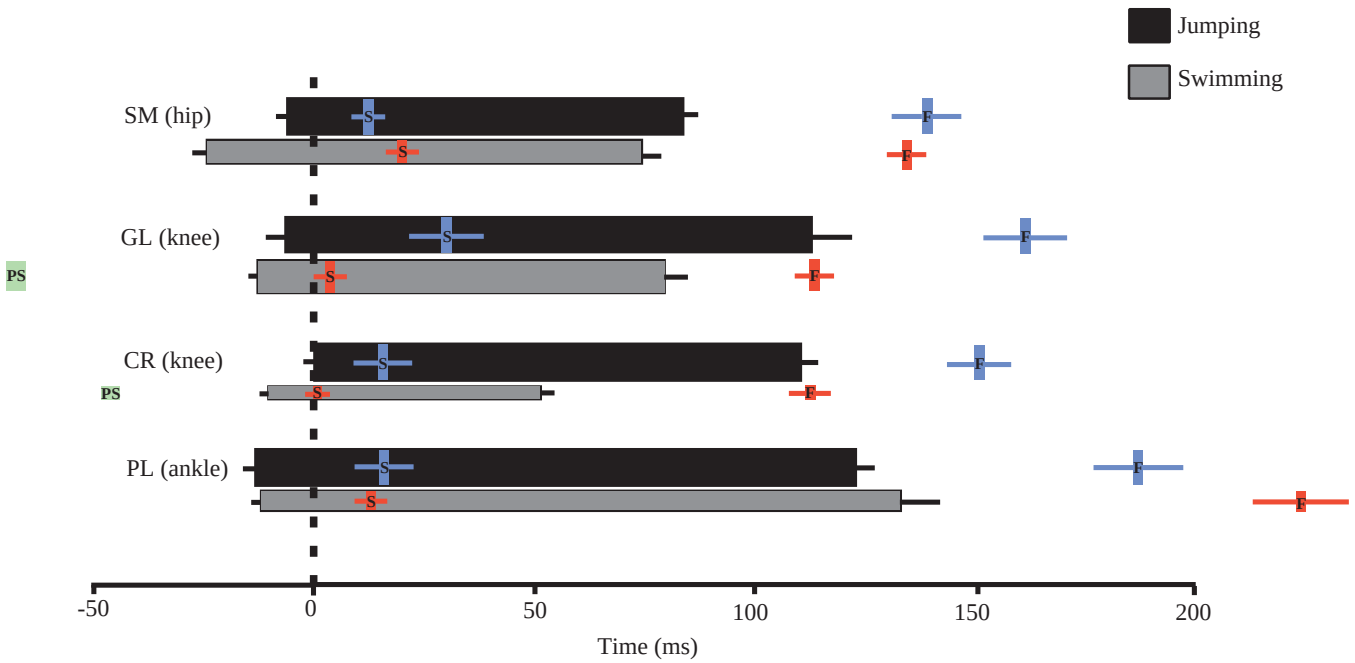

Fig. 5. Schematic diagram representing the average patterns of muscle activity and muscle strain during jumping and swimming. Horizontal bars represent mean periods of electromyographic (EMG) activity (dark bars, jumping; light bars, swimming). Differences in the height of these bars represent differences in EMG burst intensity between locomotor modes. Colored vertical rectangles (blue, jumping; red, swimming) represent the times at which fascicle shortening starts (S) and finishes (F), respectively. The onset of passive shortening in the gluteus and cruralis during swimming is marked by PS. Error bars represent ±1 S.E.M. The vertical dashed line represents either the onset of toad movement (during jumping) or the onset of limb extension (during swimming). During jumping, limb extension begins shortly after the first perceptible movement of the toad. SM, semimembranosus; GL, gluteus; CR, cruralis; PL, plantaris.

Discussion

General patterns of movement during jumping and swimming

A number of studies have characterized the fundamental kinematic patterns involved in anuran jumping, subdividing the movements of the animal into specific phases on the basis of the actions and positions of the body, forelimbs and hindlimbs (Emerson and DeJongh, 1980; Olson and Marsh, 1998; Peters et al., 1996). We follow the terminology of Olson and Marsh (1998), which divides the jump into three phases: (i) the take-off phase, lasting from the time the animal begins to move until the last hindlimb digits leave the ground; (ii) the aerial phase, lasting from when the hindlimb leaves the ground until the forelimb digits first re-establish contact with the ground, and (iii) the landing phase, lasting from when the forelimbs first touch the ground until the hindlimbs come back into contact with the ground (shortly after this, the animal's movements stop and the toad is ready to jump again).

The start of a toad jump can readily be defined by the onset of the animal's movement (assuming that it begins the jump from rest, as is the case in this study). Often, this initial phase of movement involves a slight rotation of the body in the dorso-ventral plane (which can be counter-clockwise or clockwise and can be independent of hindlimb movements). This rotation is probably generated by slight movements of the forelimbs, or by the activity of muscles acting about the ilio-sacral joint (for more details regarding the activity of these muscles, see Emerson and DeJongh, 1980). Simultaneously or slightly after this initial body motion, the hindlimbs begin to extend. During this time, the animal's center of mass moves anteriorly and vertically, and the forelimbs lose contact with the ground. There is no simultaneous extension of the forelimbs against the ground, and they are unlikely to contribute much, if any, power to the jump. Nevertheless, the forelimbs probably do serve a useful role during anuran jumping in terms of body support and positioning prior to take-off (Peters et al., 1996). After the forelimbs leave the ground, the hindlimbs continue to extend until they too leave the ground. At this point, the hindlimbs are near full extension (Fig. 1).

The duration of the take-off phase varies with jump distance (Fig. 3A). Longer jumps are produced with shorter take-off phases, while shorter jumps have longer take-off phases. Because of its ballistic nature, jump performance, as measured by the linear horizontal distance traveled by the animal, is largely a function of take-off velocity and angle (Marsh, 1994). Given similar amounts of limb extension, jumps with shorter take-off phases should have higher accelerations of the center of mass and, given similar take-off angles, should cover greater distances. Take-off angle can vary substantially among jumps (range 14–51 °, mean 31 °), and such variation is probably responsible for some of the scatter among data relating take-

off duration to jump distance (Fig. 3A). However, this variation does not obscure the negative relationship between take-off duration and jump distance in toads and other anuran species (e.g. *Rana catesbeiana*; Olson and Marsh, 1998).

The duration of the aerial phase of the jump increased significantly with jump distance (Fig. 3B). The average duration of the aerial phase in the present study (100 ms; N=69 jumps) was substantially lower than that reported by Peters et al. (1996) for leopard frogs (*Rana pipiens*) of similar body mass (166 ms; 60 jumps). The longer average aerial phases recorded by Peters et al. (1996) suggest that the leopard frogs were capable of generating more work and power during take-off, as would be predicted from their musculoskeletal anatomy and physiology (e.g. Marsh, 1994). However, the average jump distance reported by Peters et al. (1996; 29.5 cm; 60 jumps) is nearly identical to that found for toads in this study (30 cm; 69 jumps) but is substantially lower than that reported for *R. pipiens* of similar size in other studies (e.g. Emerson, 1978; Rand, 1952). Olson and Marsh (1998) showed that take-off angles greater than 60° in *Rana catesbeiana* led to substantially longer aerial phase durations for a given jump distance. Taken as a whole, these data suggest that the frogs studied by Peters et al. (1996) probably used highly inclined take-off angles, on average, and perhaps also performed submaximally.

The landing phase duration decreased significantly with jump distance (Fig. 3C). Despite substantial scatter among the data underlying this relationship, it is likely that the forces required to decelerate and stabilize the body during landing become large enough in longer jumps to begin to overwhelm the forces produced by the forelimb muscles during the initial phase of landing. Remarkably, some anurans (such as the toads studied here and the leopard frogs studied by Peters et al., 1996) are able to use their forelimbs to support their entire body above ground during landing for over 200 ms, while slowly rotating their flexed hindlimbs beneath them to prepare for the next jump once the hindlimbs come back into contact with the ground.

A number of researchers have also characterized the kinematic patterns of hindlimbs during swimming by subdividing the movements into a variety of different phases on the basis of the relative positions and actions of the hindlimbs and forelimbs (Emerson and DeJongh, 1980; Gal and Blake, 1988a,b; Peters et al., 1996). The simplest means of categorization is to divide the hindlimb stroke cycle into only two phases, extension and flexion. It should be noted that, in toads, the limb joints do not all begin to extend or flex simultaneously. The knee joint is typically the first joint that begins extending during limb extension and flexing during limb retraction, while the more distal ankle joint begins to extend and flex last (see Gal and Blake, 1988b, for similar results in the frog *Hymenochirus boettgeri*). In fact, the ankle joint is typically still actively flexing as the knee begins to extend. Similarly, the more proximal knee and hip joints begin to flex and retract while the more distal ankle joint is finishing its extension. To avoid confusion related to the differential timing of angular excursions among joints, but retain the simplicity of only two phases, we define the extension phase as lasting from when the first joint (the knee) begins to extend until the last joint (the ankle) finishes its extension. The flexion or retraction phase is defined as lasting from when the ankle finishes extension until the knee begins to extend again.

Muscle activity during jumping and swimming

It is well established that anurans synchronously activate hindlimb extensor muscles and several muscles acting about the articulation of the pelvic girdle and vertebral column to initiate a jump (Emerson and DeJongh, 1980; Kamel et al., 1996; Olson and Marsh, 1998). Similar results for hindlimb extensor muscles have been found here for jumping in *B. marinus*, in which all the muscles are activated within 10 ms of when movement is first observed at the start of a jump (Fig. 5). Synchronous activation has also been demonstrated in extensor muscles of various anurans during limb extension in swimming (Emerson and DeJongh, 1980; Kamel et al., 1996). In the present study, toad hindlimb extensors become activated approximately 20 ms prior to the onset of limb extension, and the average difference in the onset of activity among all muscles was only 10 ms (<5 % of the extension phase). Thus, nearly simultaneous onset of EMG activity also characterizes limb extensors during swimming in toads.

In contrast to the near synchrony in activation of hindlimb extensor muscles during toad locomotion, the offset times for EMG activity in these muscles differ more substantially because of differing EMG burst durations among the hindlimb extensors (Fig. 5). During jumping, these differences in offset times are not particularly pronounced, although comparisons between the semimembranosus (the muscle with the shortest average EMG burst duration) and the plantaris (the muscle with the longest average EMG burst duration) reveal an average difference of 35 ms between offset times (22 % of the take-off phase). During swimming, differences in EMG offset times are much more apparent. The cruralis exhibited short average burst durations of only 52 ms, while the plantaris EMG activity lasted nearly three times as long (average 145 ms). As in toads, Kamel et al. (1996) found that, in *R. pipiens*, the semimembranosus and plantaris had the shortest and longest average EMG burst durations, respectively, during jumping and that the cruralis and plantaris exhibited the shortest and longest average EMG burst durations, respectively, during swimming. Thus, it appears that, across a fairly broad phylogenetic spectrum of anurans, near-simultaneous activation but staggered deactivation characterizes the general pattern of activity of hindlimb extensor muscles during both jumping and swimming. Moreover, regardless of environment, it is the most distal extensor muscle, the plantaris, that remains active the longest.

The average relative intensity of EMG bursts was substantially greater during jumping than during swimming in the cruralis and plantaris, and slightly (although not significantly) greater, on average, in the semimembranosus. Kamel et al. (1996) also found, on average, larger EMG bursts

during jumping than during swimming in these same three hindlimb extensors of *R. pipiens*. A number of other workers examining muscle activity during locomotion in water and on land in a variety of taxa have found both larger EMG bursts during terrestrial than during aquatic locomotion (Clarac et al., 1987; Gillis, 2000) and higher degrees of muscle stress and strain on land than in the water (Biewener and Gillis, 1999; Corning and Biewener, 1997). These data are derived from studies involving invertebrates and vertebrates (and, within vertebrates, axial- and appendicular-based locomotion). Such results imply that animals using the same structures to propel themselves in water and on land often utilize higher levels of muscle recruitment and probably generate more force during terrestrial locomotion than during aquatic locomotion, although this is not necessarily the case for all muscles (de Leon et al., 1994; Roy et al., 1991). In cases where higher recruitment levels are observed, much of the additional increment in muscle force generation on land is probably used to counteract gravitational forces that are largely negated by buoyancy in the water.

Within a single environment, the intensity of EMG bursts is also often related to locomotor performance. In vertebrates, increased locomotor speed has been shown to be linked to higher-intensity EMG bursts during swimming (Rome et al., 1984), flying (Tobalske, 1995) and running (Jayne et al., 1990), although such a linkage is not always found in all muscles (Roy et al., 1991). For anurans, jump distance, as opposed to locomotor speed, is an appropriate measure of performance on land. Frogs probably recruit their hindlimb extensor muscles maximally during their longest jumps (e.g. Hirano and Rome, 1984), and it would seem that the degree of muscle recruitment during jumping directly influences jump performance. Interestingly, Kamel et al. (1996) found no significant relationship between the intensity of EMG bursts in hindlimb extensor muscles in *R. pipiens* and jump performance (jump distance or height). However, their regressions were based on pooled data from all individuals, which might have masked some intra-individual correlations between EMG intensity and jump distance.

In the present study, some individuals did show a significant positive relationship between EMG burst intensity and jump distance for certain muscles. Individuals that did exhibit such a relationship typically also exhibited a greater range of jump distances and/or EMG burst intensities than did individuals that showed no relationship between these variables. It appears that a minor increase in EMG burst magnitude in a single limb muscle will not necessarily lead to a proportional increase in jump distance. But, given a sufficient degree of variation in jump distance, longer jumps tend to be associated with larger EMG bursts, and presumably higher degrees of muscle recruitment, than shorter jumps.

Muscle strain during jumping and swimming

Despite the nearly simultaneous activation of limb extensors during jumping and swimming, the onset of muscle shortening during these locomotor behaviors is temporally staggered. Furthermore, the order in which muscles begin to shorten differs between jumping and swimming. During jumping, the semimembranosus, cruralis and plantaris all start to shorten at approximately the same time, while the gluteus, on average, starts to shorten slightly later (Fig. 5). A delay in the onset of gluteus shortening was also reported by Olson and Marsh (1998) for the bullfrog *Rana catesbeiana*. Using three-dimensional kinematics, Lutz and Rome (1996a) recorded the temporal pattern of angular excursions at the hip and knee joints during jumping in *Rana pipiens* and found that the hip began to extend before the knee in the initial phase of limb extension. Olson and Marsh (1998) suggested that this temporal sequence of joint extension (hip before knee) was probably responsible for the delay in shortening observed in the gluteus in *R. catesbeiana*. Given that a similar delay in shortening is observed in the gluteus of members of a phylogenetically distant genus (*Bufo*), this pattern may be common among many jumping anurans. The cruralis, which converges with the gluteus onto a broad aponeurosis spanning the knee joint, does not show such a delay, reflecting its different origin and position within the limb, relative to the gluteus.

The actual strain patterns observed during take-off revealed that, within several muscles, the fascicle shortening velocities were often not constant. For example, in most jumps, both the gluteus and plantaris shortened at a much slower rate during the first half to two-thirds of the take-off phase (i.e. during the period of EMG activity) than during the latter portions of take-off (Fig. 4). A less prominent increase in shortening velocity during take-off was also observed on occasion in the cruralis and semimembranosus during jumping. Olson and Marsh (1998) reported changes in fascicle shortening velocity in the gluteus muscle of *R. catesbeiana* similar to those reported here for the gluteus and plantaris of *B. marinus* (much faster shortening late in take-off). They also suggested that the shortening velocity of the semimembranosus in *R. pipiens* during take-off was not constant during many jumps and, instead, tended to increase until take-off. It appears that the inherent musculoskeletal dynamics involved in limb extension during take-off might lead to unsteady (often increasing) shortening velocities in at least some of the hindlimb extensors of jumping anurans.

While the functional basis for this increase in shortening velocity late in take-off is currently unknown, it is tempting to postulate that it may be related to enhancing muscle power output during the jump. Others working on anuran jumping have shown that some degree of power amplification is probably necessary to explain jump performance in various anuran species given measurements of hindlimb muscle mass and *in vitro* measures of the mass-specific power available from such muscles (e.g. Marsh, 1994). The extended period of relatively slow shortening that occurs consistently over much of the take-off phase in the gluteus and plantaris, and on occasion in the cruralis and semimembranosus, would probably lead to higher levels of force development than would occur if the muscles shortened more rapidly over this same

period. If these forces reach peak levels as the muscles begin to shorten more rapidly, this would then also enhance the power output of the muscles.

Power amplification during anuran jumping is also thought to be derived from the rapid release of energy stored elastically in compliant collagenous structures in the limb (Marsh, 1994; Marsh and John-Alder, 1994; Olson and Marsh, 1998; Peplowski and Marsh, 1997). Such an amplification mechanism has been reported for various taxa known for their remarkable jumping capacity, including animals as divergent as insects (e.g. the locust *Schistocerca gregaria*; Bennet-Clark, 1975) and mammals (e.g. the bushbaby *Galago senegalensis*; Aerts, 1998). Recent work by Roberts and Marsh (1997) on *R. catesbeiana* provided preliminary evidence that the long plantaris tendon is a likely candidate for a site of elastic energy storage. This energy can be released rapidly late in the take-off phase to amplify power during frog jumping. Predicted and observed length trajectories of the plantaris, acting as a power amplifier during jumping in *R. catesbeiana*, suggest that the muscle should initially shorten rapidly over a short distance, stretching the in-series compliance of the muscle–tendon unit, then slow down, as the joint begins to be extended, and finally accelerate again as the animal nears the point of take-off (Marsh, 1999). Although an initial phase of rapid shortening was not observed in any of the muscles examined in the present study, the latter two phases were regularly observed in the plantaris and gluteus. Whether this precludes the possibility that elastic energy is being stored and rapidly released in any of the elastic structures of the toad hindlimb to amplify power during jumping cannot be determined at this time, but deserves further study.

During swimming, the knee extensors often undergo a small degree of slow passive shortening before the onset of EMG activity, after which they begin actively to contract and extend the knee. This passive shortening occurs as the hip and ankle continue to be flexed and the tibiofibula rotates laterally, leading to a small degree of extension at the knee. After this period of passive shortening and the onset of EMG activity, the knee extensors begin to shorten rapidly. The onset of this rapid, active shortening also occurs before the hip and ankle extensors begin to shorten. It is currently not clear why, despite nearly simultaneous activation of muscles acting to extend the hip, knee and ankle, the knee extensors begin to shorten actively first. One possibility is that differential timing in the deactivation of flexors acting at the hip, knee and ankle leads to different degrees of resistance that need to be overcome at each joint by active extensors at the start of limb extension. In any case, temporally advanced extension at the knee joint (relative to the hip and ankle) has been reported for other anurans during swimming (Gal and Blake, 1988b), and it has been suggested that this is important in allowing for the positioning of the feet, which will provide the majority of thrust during the swimming stroke.

During both jumping and swimming, the offset of muscle shortening is also temporally staggered. During jumping, it might be expected that all the muscles would finish shortening when the limb leaves the ground, since any work generated after this point cannot be used to power the jump. However, for all muscles, there is substantial variation about when the muscle stops shortening relative to the time of take-off (Fig. 6). In addition, while the hip and knee extensors finish shortening near the time when the foot leaves the ground, the plantaris almost always continues to shorten and extend the ankle for a variable period after this (Fig. 6). It seems probable that this additional shortening occurs when levels of force in the muscle have diminished substantially (thus wasting little useful power), but *in vivo* recordings of plantaris forces during jumping are required to verify this.

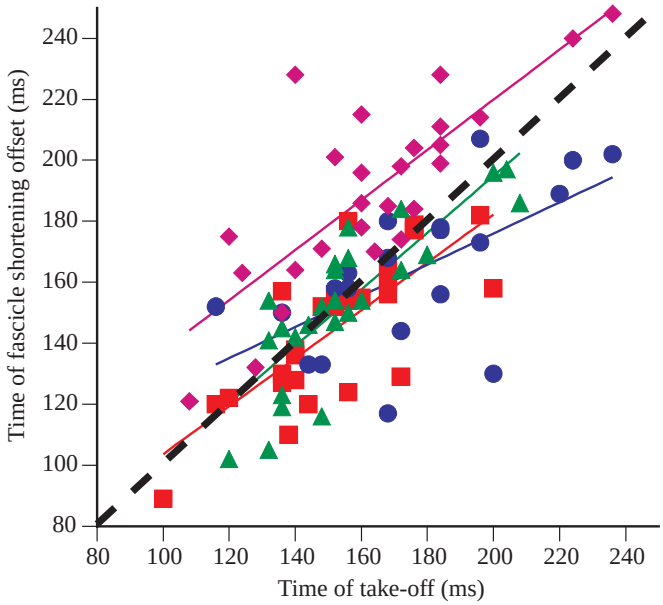

Fig. 6. Graph showing when muscles complete their shortening phase relative to take-off during a jump. The dashed line represents equality between the time at take-off and the time at which a muscle finishes shortening. Different symbols represent different muscles (squares, semimembranosus; circles, gluteus; triangles, cruralis; diamonds, plantaris). Individual symbols represent different jumps. Note that, for any muscle, substantial variation exists with respect to when muscles finish shortening and when the animal takes off. The semimembranosus, gluteus and cruralis generally finish shortening (on average) near the time of take-off, although variation about the line of equality is greatest in the gluteus. The plantaris nearly always finishes shortening after take-off.

During swimming, the plantaris is also the last muscle to finish shortening. In contrast to the other muscles, which appear to shorten at a nearly constant velocity after being activated, the plantaris often exhibits a decrease in shortening velocity late in the limb extension phase, after the cessation of EMG activity (Fig. 4). As suggested by Gal and Blake (1988a,b), unsteady forces are probably important during anuran swimming, and this final, slow phase of shortening in the plantaris may reflect passive extension at the ankle created by fluid forces associated with an 'added mass' of water (*sensu* Daniel, 1984) entrained by the foot as it was actively accelerated earlier in limb extension. This phase of reduced

shortening velocity occurs after the other limb segments have been fully extended as the feet rotate towards one another and make (or nearly make) contact, before subsequently being retracted parallel to the animal's axis of forward movement (Fig. 2). Gal and Blake (1988b) have suggested that interactive effects between the feet as they come together during this phase of the swimming stroke might be important for generating additional locomotor forces.

A summary of toad hindlimb muscle function during jumping and swimming

Toads use their hindlimbs to produce propulsive thrust during both jumping and swimming. We undertook this study to investigate the function of four major hindlimb extensor muscles during the propulsive stroke in each of these locomotor behaviors. It is possible that the limb muscles are activated and operate mechanically in a very similar fashion during both behaviors. If this were the case, any differences in hindlimb movements between jumping and swimming would be passive and would be the result of differences in the physical properties and nature of the resistance encountered by the limb in an aquatic *versus* a terrestrial environment. However, it is also possible that toads (and possibly other anurans) actively mediate hindlimb function depending upon the external environment *via* alterations in the motor output to key muscles. Below, we address this issue by summarizing the functional differences of each of the four extensor muscles examined in this study between jumping and swimming.

The semimembranosus has been the subject of numerous studies examining *in vivo* muscle function (activity and/or length change) during anuran locomotion (Kamel et al., 1996; Lutz and Rome, 1996a,b, 1994; Olson and Marsh, 1998; Peters et al., 1996). While conclusions regarding the constancy of shortening velocity during a contraction have varied among some of these studies, agreement exists on the importance of this muscle in powering hip extension during a jump. During swimming, this muscle also powers hip extension and, in doing so, only one variable appears to be consistently altered (relative to jumping): the delay between the onset of EMG activity and the onset of fascicle shortening is increased. This difference probably reflects the presence of a transient flexor moment at the hip early in limb extension. Such a moment could be due to extended hip flexor activity during limb retraction or to the shortening of a bi-articular muscle such as the gluteus which, when contracting to extend the knee (which is the first joint to extend during swimming), might also exert a flexor moment at the hip.

The *in vivo* function of the gluteus magnus has also been examined previously during locomotion in anurans (Olson and Marsh, 1998). Because of its small size, it is likely to contribute less power during jumping. Olson and Marsh (1998) provide observations suggesting that, in *R. catesbeiana*, the gluteus might be more important during swimming, where it appears to begin to shorten rapidly after activation and undergoes higher degrees of strain than during jumping. However, in *B. marinus*, there does not appear to be any significant alteration in the muscle's degree of strain between jumping and swimming. In addition, the intensity and duration of muscle activity do not differ significantly between jumping and swimming (Table 2). However, one factor did consistently differ with respect to this muscle's mechanical behavior during jumping and swimming. During swimming, the gluteus often begins to shorten passively prior to the onset of EMG activity, after which it shortens rapidly and at a fairly constant velocity. In contrast, during jumping, the muscle also often begins to shorten slowly early in limb extension, but this shortening is not passive and occurs when the muscle is electrically active. It seems likely that, during this slow shortening phase of jumping, muscle force generation can be enhanced (as a result of the inherent force–velocity properties of muscle). Thus, once the muscle begins to shorten rapidly prior to take-off, power output can be higher than that generated during swimming (under constant-velocity conditions) despite similar EMG activation patterns and levels of fascicle strain.

The cruralis is the largest muscle in the hindlimb. Electromyographic activity in this muscle has been recorded previously during anuran locomotion (Kamel et al., 1996; Olson and Marsh, 1998), but simultaneous direct measurements of relative length changes during locomotion are presented here for the first time. As was the case with the gluteus, the cruralis often exhibits a short period of slow passive shortening during swimming (prior to the onset of EMG activity) that is not seen during jumping. However, the cruralis is the only muscle examined that consistently exhibits a shift in the duration of EMG activity (reduced by approximately half during swimming) and a consistent change in the total degree of fascicle strain which, on average, is approximately 40 % greater during jumping (Fig. 5; Table 2). In addition, the intensity of EMG activity in the cruralis is much greater during jumping than during swimming (Fig. 5; Table 2). These features are all consistent with its central role in generating high levels of power during jumping.

The plantaris is also a large muscle and, while its EMG activity patterns have been recorded during anuran locomotion before (Kamel et al., 1996; Olson and Marsh, 1998), this is the first study to incorporate simultaneous direct measurements of relative length change. This muscle tends to have the longest burst durations and also shortens over a longer period than the other muscles studied here (especially during swimming). Interestingly, the muscle exhibits a pattern of strain during jumping quite similar to that of the gluteus, in which in the initial phase of limb extension fascicle shortening is active but slow. Prior to take-off, the fascicles then begin to shorten rapidly, generating power for the jump. In contrast, during swimming, the muscle's fascicles actually exhibit a decrease in shortening velocity late in the propulsive stroke, probably reflecting a period of passive extension at the ankle caused by fluid forces associated with unsteady (accelerative) movements of the limb earlier in limb extension. In addition to these differences in strain trajectory, the plantaris also exhibits significantly higher intensity EMG bursts during jumping than during swimming.

Our original goal was to address whether changes in hindlimb muscle function occur in concert with a shift in locomotor mode from jumping to swimming in the toad *B. marinus*. It appears that, among extensors, higher-intensity EMG activity (greater than twofold in the cruralis and plantaris) is important for generating high levels of force, work and power during jumping. In addition, the strain trajectories observed consistently in the plantaris and gluteus during jumping (initial, slow active shortening followed by rapid shortening until take-off) also probably act to enhance force and power output when compared with the strain trajectories observed in these muscles during swimming (where some degree of passive shortening characterizes the muscles' behavior). Indeed, consistent marked alterations in the duration of activation and degree of shortening of the largest hindlimb muscle, the cruralis (but none of the others), support the idea that toad hindlimb function is actively altered between behaviors and that this muscle is important for mediating such functional differences. Further work examining alterations in limb muscle function during a variety of locomotor behaviors in other animals will be required to explain the ways in which the musculoskeletal system acts to accommodate the range of limb functions required by organisms confronting the variable conditions they encounter in nature.

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References

Aerts, P. (1998). Vertical jumping in *Galago senegalensis*: the quest for an obligate mechanical power amplifier. *Phil. Trans. R. Soc. Lond. B* **V353 N1375**, 1607–1620.

Ashley-Ross, (1995). Patterns of hind limb motor output during walking in the salamander *Dicamptodon tenebrosus*, with comparisons to other tetrapods. *J. Comp. Physiol.* A **177**, 273–285.

Bennet-Clark, H. C. (1975). The energetics of the jump of the locust, *Schistocerca gregaria*. *J. Exp. Biol.* **63**, 53–83.

Bertram, J. E. and Marsh, R. L. (1998). Muscle properties and organismal function: shifting paradigms. *Am. Zool.* **38**, 697–702.

Biewener, A. A. (1998). Muscle function *in vivo*: a comparison of muscles used for elastic energy savings *versus* muscles used to generate mechanical power. *Am. Zool.* **38**, 703–717.

Biewener, A. A., Corning, W. R. and Tobalske, B. W. (1998a). *In vivo* pectoralis muscle force–length behavior during level flight in pigeons (*Columba livia*). *J. Exp. Biol.* **201**, 3293–3307.

Biewener, A. A. and Gillis, G. B. (1999). Dynamics of muscle function during locomotion: accommodating variable conditions. *J. Exp. Biol.* **202**, 3387–3396.

Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V. (1998b). *In vivo* muscle force–length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681–1694.

Calow, L. J. and Alexander, R. McN. (1973). A mechanical analysis of the hind leg of a frog (*Rana temporaria*). *J. Zool., Lond.* **171**, 293–321.

Clarac, F., Libersat, F., Pflüger, H. J. and Rathmayer, W. (1987). Motor pattern analysis in the shore crab (*Carcinus maenas*) walking freely in water and on land. *J. Exp. Biol.* **133**, 395–414.

Corning, W. R. and Biewener, A. A. (1997). Gastrocnemius muscle mechanics in swimming and walking ducks. *Am. Zool.* **37**, 178A.

Daniel, T. L. (1984). Unsteady aspects of aquatic locomotion. *Am. Zool.* **24**, 121–134.

de Leon, R., Hodgson, J. A., Roy, R. R. and Edgerton, V. R. (1994). Extensor- and flexor-like modulation within motor pools of the rat hindlimb during treadmill locomotion and swimming. *Brain Res.* **654**, 241–250.

Denny, M. W. (1993). *Air and Water*. Princeton: Princeton University Press.

Dunlap, D. G. (1960). The comparative myology of the pelvic appendage in the Salientia. *J. Morph.* **106**, 1–76.

Emerson, S. B. (1978). Allometry and jumping in frogs: helping the twain to meet. *Evolution* **32**, 551–564.

Emerson, S. E. and DeJongh, H. J. (1980). Muscle activity at the ilio-sacral articulation of frogs. *J. Morph.* **166**, 129–144.

Full, R. J., Autumn, K., Chung, J. I. and Ahn, A. (1998). Rapid negotiation of rough terrain by the death-head cockroach. *Am. Zool.* **38**, 81A.

Gal, J. M. and Blake, R. W. (1988a). Biomechanics of frog swimming. I. Estimation of the propulsive force generated by *Hymenochirus boettgeri*. *J. Exp. Biol.* **138**, 399–411.

Gal, J. M. and Blake, R. W. (1988b). Biomechanics of frog swimming. II. Mechanics of the limb-beat cycle in *Hymenochirus boettgeri*. *J. Exp. Biol.* **138**, 413–429.

Gatesy, S. M. (1999). Guineafowl hind limb function. II. Electromyographic analysis and motor pattern evolution. *J. Morph.* **240**, 127–142.

Gillis, G. B. (2000). Patterns of muscle activity during terrestrial locomotion in the American eel (*Anguilla rostrata*). *J. Exp. Biol.* **203**, 471–480.

Goldspink, G. (1977). Design of muscles in relation to locomotion. In *Mechanics and Energetics of Animal Locomotion* (ed. R. McN. Alexander), pp. 1–22. London: Chapman & Hall.

Goslow, J. E. Jr, Seeherman, H. J., Taylor, C. R., McCutchin, M. N. and Heglund, N. C. (1981). Electrical activity and relative length changes of dog limb muscles as a function of speed and gait. *J. Exp. Biol.* **94**, 15–42.

Hatta, I., Sugi, H. and Tamura, Y. (1988). Stiffness changes in frog skeletal muscle during contraction recorded using ultrasonic waves. *J. Physiol., Lond.* **403**, 193–209.

Hirano, M. and Rome, L. C. (1984). Jumping performance of frogs (*Rana pipiens*) as a function of muscle temperature. *J. Exp. Biol.* **108**, 429–439.

Jayne, B. C., Bennett, A. F. and Lauder, G. V. (1990). Muscle recruitment during terrestrial locomotion: how speed and temperature affect fibre type use in a lizard. *J. Exp. Biol.* **152**, 101–128.

Josephson, R. K. (1999). Dissecting muscle power output. *J. Exp. Biol.* **202**, 3369–3375.

Kamel, L. T., Peters, S. E. and Bashor, D. P. (1996). Hopping and swimming in the leopard frog, *Rana pipiens*. II. A comparison of muscle activities. *J. Morph.* **230**, 17–31.

Loeb, G. E. and Gans, C. (1985). *Electromyography for Experimentalists*. Chicago: University of Chicago Press.

Lutz, G. J. and Rome, L. C. (1996a). Muscle function during jumping in frogs. I. Sarcomere length change, EMG pattern and jumping performance. *Am. J. Physiol.* **271**, C563–C570.

Lutz, G. J. and Rome, L. C. (1996b). Muscle function during jumping in frogs. II. Mechanical properties of muscle: implications for system design. *Am. J. Physiol.* **271**, C571–C578.

Lutz, G. L. and Rome, L. C. (1994). Built for jumping: the design of the frog muscular system. *Science* **263**, 370–372.

Marsh, R. L. (1994). Jumping ability of anuran amphibians. *Adv. Vet. Sci. Comp. Med.* **38**B, 51–111.

Marsh, R. L. (1999). How muscles deal with real-world loads: the influence of length trajectory on muscle performance. *J. Exp. Biol.* **202**, 3377–3385.

Marsh, R. L. and John-Alder, H. A. (1994). Jumping performance of hylid frogs measured with high-speed cine film. *J. Exp. Biol.* **188**, 131–141.

Olson, J. M. and Marsh, R. L. (1998). Activation patterns and length changes in hindlimb muscles of the bullfrog *Rana catesbeiana* during jumping. *J. Exp. Biol.* **201**, 2763–2777.

Peplowski, M. M. and Marsh, R. L. (1997). Work and power output in the hind-limb muscles of cuban tree frogs *Osteopilus septentrionalis* during jumping. *J. Exp. Biol.* **200**, 2861–2870.

Peters, S. E., Kamel, L. T. and Bashor, D. P. (1996). Hopping and swimming in the leopard frog, *Rana pipiens*. I. Step cycles and kinematics. *J. Morph.* **230**, 1–16.

Rand, A. S. (1952). Jumping ability of certain anurans, with notes on endurance. *Copeia* **1952**, 15–20.

Reilly, S. M. (1995). Quantitative electromyography and muscle function of the hind limb during quadrupedal running in the lizard *Sceloporus clarki*. *Zoology* **98**, 263–277.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Roberts, T. J. and Marsh, R. L. (1997). Elastic energy storage in jumping frogs. *Am. Zool.* **37**, 174A.

Rome, L. C., Loughna, P. T. and Goldspink, G. (1984). Muscle fiber activity in carp as a function of swimming speed and muscle temperature. *Am J. Physiol.* **249**, R272–R279.

Roy, R. R., Hutchison, D. L., Pierotti, D. J., Hodgson, J. A. and Edgerton, V. R. (1991). EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming. *J. Appl. Physiol.* **70**, 2522–2529.

Tobalske, B. W. (1995). Neuromuscular control and kinematics of intermittent flight in the European starling (*Sturnus vulgaris*). *J. Exp. Biol.* **198**, 1259–1273.

Vogel, S. (1994). *Life in Moving Fluids*. Princeton: Princeton University Press.

Woledge, R. C., Curtin, N. A. and Homsher, E. (1985). *Energetic Aspects of Muscle Contraction.* London: Academic Press.