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The Effect of Speed on Hindlimb Muscle Recruitment during Swimming in the Toad, *Bufo marinus*

by

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This paper was prepared

under the direction of

Professor Gary B. Gillis

for eight credits.

For my family:

My parents, Jagath and Miriam Gunaratne, for teaching me to believe in magic.

My grandparents, Ranjit and Rita De Livera, for the foundation they built me.

My grandfather, Adrian Toussaint, for lessons on unconditional love.

My sister, Anjuli Gunaratne, for guiding me through some of the toughest years of my life, and for always protecting me.

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ABSTRACT

Animals must vary their locomotor behavior to successfully maneuver in their environments, and one parameter that varies widely within the locomotor repertoire of many animals is speed. Changing speed depends on the anatomical and functional properties of muscles that are involved in force production during locomotion. Analysis of the recruitment intensity of such muscles gives insight into how an animal deals with the mechanical demands of increasing speed. In this study simultaneous data on limb movement and muscle activity were collected to address how the toad *Bufo marinus* changes speed. The muscles analyzed were major extensors and flexors of the knee and hip joints, and I predicted that changes in speed are brought about more by altering the recruitment intensity of hindlimb extensors than flexors. Results show a significant positive relationship between recruitment intensity and speed in both flexors and extensors. These results indicate that toads alter speed by pushing against the water with greater force and retracting their limbs at a greater speed, a pattern unlike what's used during terrestrial locomotion in most tetrapods, where only the propulsive phase appears functionally linked to changes in speed.

INTRODUCTION

From powering a sailfish as it propels itself through the water at speeds close to 110km/h to providing the mechanical energy required for a hovering hummingbird to beat its wings 90 times per second, muscles allow a large spectrum of movements in different kinds of environments in almost all animals. Providing the forces necessary to propel bodies through an environment is one of the most important functions of skeletal muscles. Muscles generate these forces through complex interactions between neuronal networks, contractile proteins, bones and the environment.

The basic microscopic machinery by which muscles produce force is common in most animals. Since the famous Vesalius' drawings in 1538 of the human muscular system (Rifkin and Ackerman, 2006), we have come to understand how the macroscopic arrangement and properties of muscles vary in animals ranging from cockroaches to fish to wallabies (Biewener et al., 1998; Watson and Ritzmann 1998; Altringham and Ellerby 1999). These variations allow such different animals to perform locomotor functions appropriate to the environments they inhabit and movements they utilize.

Skeletal muscle structure and recruitment

Skeletal muscles have a highly complex, hierarchical structure consisting of organizational units called fascicles. All the fascicles that form a muscle are collectively enclosed within a connective tissue sheath called the epimysium.

The epimysium provides pathways for blood vessels and nerves. Each individual fascicle is itself enveloped by a connective tissue membrane called the perimysium and consists of many muscle fibers (Fig. 1A). A single muscle fiber is the cellular unit of muscle (McKinley and O'Loughlin, 2008).

Within a muscle fiber forces are generated via the interactions between the heads of myosin thick filaments and actin thin filaments (Eisenberg and Greene, 1980). Actin and myosin proteins are organized into bundles called myofibrils, which are segmented into sections called sarcomeres (Fig 1B). When the muscle is relaxed myosin cannot bind to actin (McKinley and O'Loughlin, 2008).

Muscle fibers are innervated by motor neurons. A single motor neuron and all the muscle fibers it innervates is called a motor unit (Fig. 1C). A single muscle is comprised of many motor units, and gradations in force during a muscle's contraction are achieved by varying the number of motor units activated by the nervous system (i.e., the muscle's recruitment intensity). An action potential that originates from the central nervous system and travels through a motor neuron to muscle fibers results in a muscle action potential, an electrical signal that travels along the innervated fibers. This electrical signal is actually a travelling influx of sodium ions into the muscle fibers, which in turn leads to calcium ions being released from the sarcoplasmic reticulum, a membrane system in which calcium ions are sequestered in a muscle fiber. The release of calcium ions enables myosin heads of the thick filaments to bind to actin thin filaments forming cross-bridges (McKinley and O'Loughlin, 2008).

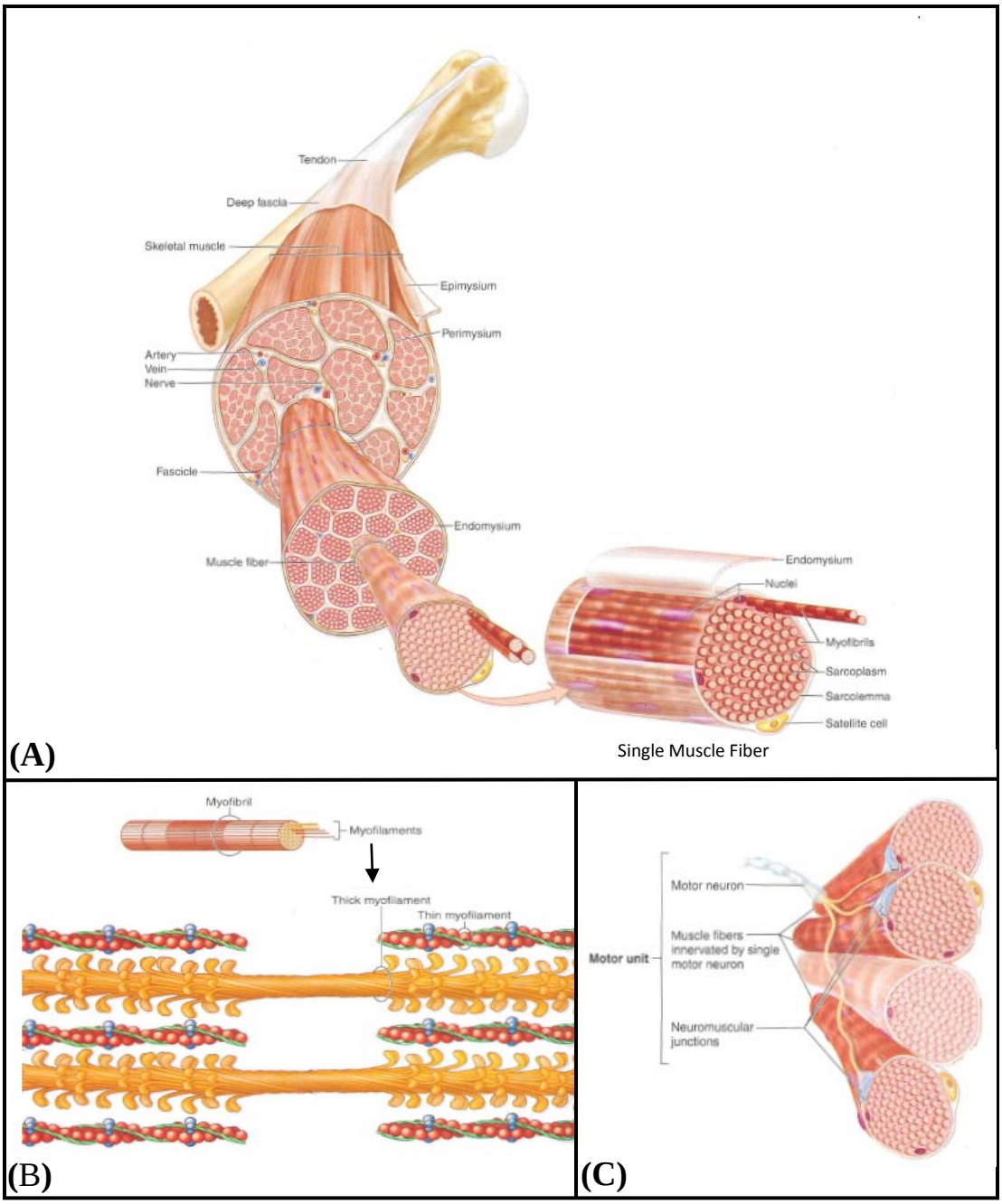

Figure 1: Structure and organization of skeletal muscle and motor units. (A) This image shows the different layers and organizational units that form a skeletal muscle. (B) This image shows the myofibirils that form a muscle fiber. Each skeletal muscle cell contains multiple myofibrils. The myosin thick filaments and actin thin filaments that form a single myofibril can be seen in this image. (C) This image shows the elements that create a motor unit. Groups of motor units function together to coordinate the contraction of a single skeletal muscle. Figure was compiled from figure 10.1, 10.5, and 10.10 in McKinley and O'Loughlin's Human Anatomy 2nd ed. 2008)

Through ATP hydrolysis, the myosin thick filaments pull against and slide past the actin thin filaments resulting in contraction, or shortening of the muscle fiber (Eisenberg and Greene, 1980). When many fibers simultaneously contract, a skeletal muscle develops the force necessary to move a joint. The coordinated contractions of multiple muscles and corresponding movements of many joints results in locomotion.

Electromyography

An experimental technique that enables scientists to quantify the timing, degree, and frequency of muscle activity *in vivo* is electromyography (EMG). In this experiment bipolar electromyography was used. This technique uses an electrode with two electrical contacts.

As stated above, when an action potential arrives at a muscle fiber it causes a chain of events that result in an influx of sodium ions into the muscle cell. The change in ion concentrations causes changing conductivities across the muscle cell membrane and extracellular fluid (Loeb and Gans, 1986). This changing cell membrane conductivity travels as a wave along the muscle cell. A bipolar electrode placed within a muscle detects the potential difference as this wave travels past its two recording contacts. The potential difference gives rise to an electrical current in the electrode. These electrical currents are relatively weak; therefore, an amplifier is used to convert them into larger voltages (Loeb and Gans, 1986). These voltages are represented in digital wave forms using

specialized computer software. The amplitude of these recorded waves represents the intensity of muscle recruitment. A raw EMG signal from a thigh muscle of a toad is shown in figure 2. Such an EMG signal is a random sample of all the active motor units within a muscle (Loeb and Gans, 1986).

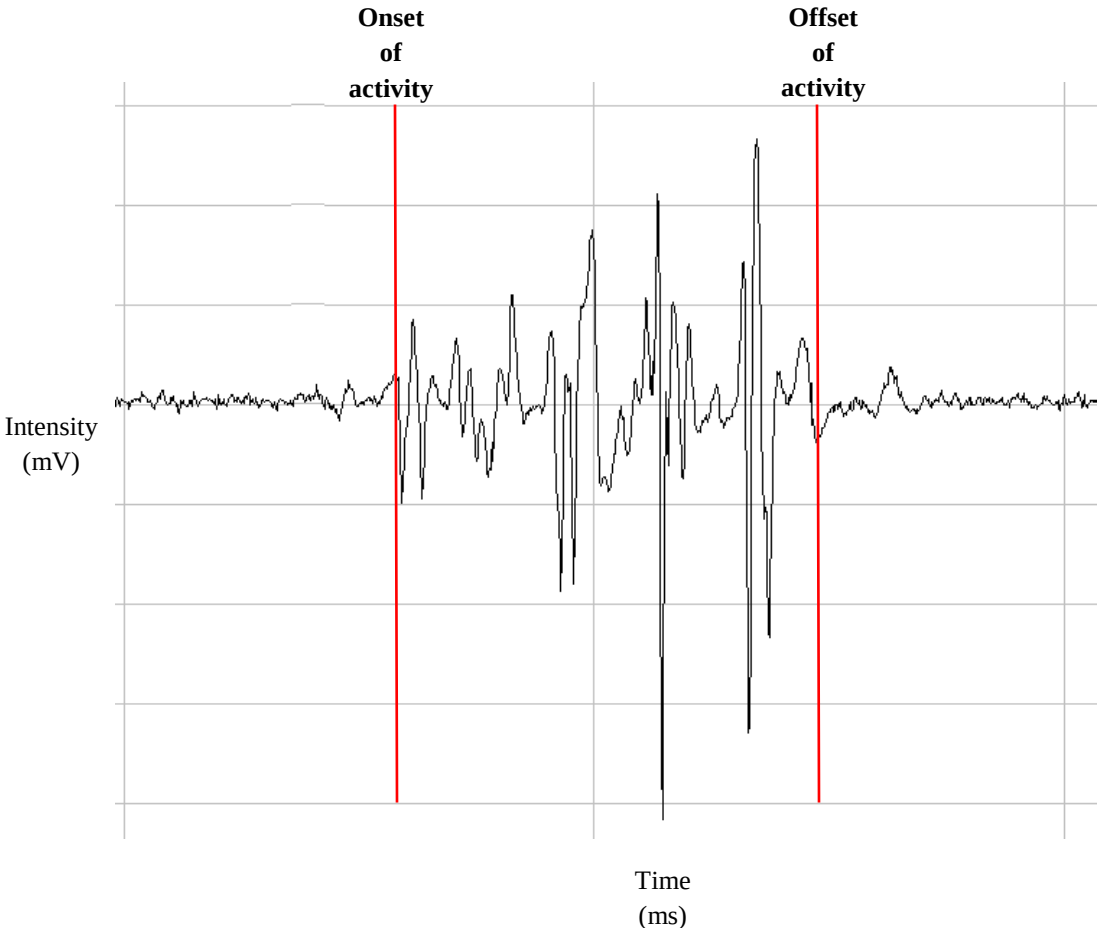

Figure 2: Raw EMG signal from the cruralis, a knee extending muscle of a toad. The vertical axis gives the potential difference between the two recording points of the electrode (intensity of activity) in millivolts. The horizontal axis shows time in milliseconds. Each such burst has an onset, offset, duration, and average intensity (i.e., spike amplitude).

Muscle recruitment patterns in relation to locomotor speed in vertebrates

Animals have to constantly be able to adjust their locomotor behavior in order to successfully maneuver in their environments. The presence of threats such as predators require many animals to be able to vary their speed of locomotion quickly and over a broad range. The ability to do this depends greatly on the anatomical and functional properties of the muscles involved.

Aquatic environments present demands that differ from those encountered during terrestrial locomotion. Gravity is counteracted by hydrostatic lift (buoyancy); however drag forces, which resist objects moving through fluids, are significant because of water's high density. Most fish swim using undulatory locomotor movements. Waves of curvature pass posteriorly along the length of an animal's body, pushing against the surrounding fluid, and propelling the animal forward. The body musculature of a fish differs from most other vertebrates in that its muscle fiber types are separated within the segmental structures called myotmes (Altringham and Ellerby, 1999). Slow-twitch or oxidative red muscle is generally organized into a small mass of laterally positioned muscle fibers, while fast-twitch or glycolytic muscle forms the bulk of a fish's cross-section and is more medially positioned. Slow, steady swimming is powered exclusively by slow-twitch muscles. As swimming speed increases fast glycolytic muscle fibers are additionally recruited (Altringham and Ellerby, 1999). Therefore, fish increase the number and types of muscle fibers recruited in order to provide the additional force required for increasing speed.

The effect of speed on muscle recruitment during terrestrial locomotion in mammals is quite well understood. Unlike aquatic locomotion in fish, terrestrial locomotion in mammals involves the use of limbs and their interaction with the substrate. During limbed locomotion, the stance phase is the phase in which the foot is in contact with the ground. This is the phase in which forces are generated to propel the body forward. The swing phase is the phase in which the foot is in the air and the limb is returned to its original position to begin exerting force against the ground again (Alexander, 2002). As speed increases, the duration of the stance phase decreases to a greater extent than the swing phase (Pierotti et al., 1988; Weyand et al., 2000; Gillis and Biewener, 2001). This enforces an increasing mechanical demand on the extensor muscles that power the stance phase. A study by Pierotti et al. (1988) showed that as locomotor speed is increased in cats, hindlimb muscle recruitment intensity increased more in extensors than flexors. Increased extensor recruitment provides the additional force and thereby work required to increase locomotor speed. The muscles involved in swinging the limbs forward seemed less important for changing speed.

Thus, in both swimming fish that use their bodies for propulsion and in terrestrial animals that use their limbs, increases in muscle recruitment intensity underlie the capacity to increase speed. In limbs, however, it appears that mainly extensor muscles are important in this respect.

Anuran locomotion and performance

Anurans (frogs and toads) use a variety of locomotor modes of which jumping and swimming are the most common. Swimming in anurans is distinct when compared to fish because anurans use their hindlimbs to propel themselves through the water. Limbed locomotion, even in water, introduces phases into the locomotor cycle: the propulsive phase, in which the limbs extend and the feet push against the water (similar to the stance phase in terrestrial locomotion) and the recovery phase, in which the limbs are retracted back into their flexed position common to the start of the stroke (comparable to the swing phase in terrestrial locomotion).

The primary and more common form of locomotion in most anurans is jumping (Zug, 1978; Wake, 1997, Gomes et al., 2009). This is exemplified by the morphology of the anuran hindlimbs. The hindlimb muscles are large while the forelimb muscles are reduced. These large hindlimb muscles generate the forces required for jumping. A fused tibia-fibula enables increased force generation during jumping, and a fused radio-ulna allows increased force absorption when landing (Handrigan and Wassersug, 2007). Elongated metatarsals allow additional attachment sites on which muscles can act (Handrigan and Wassersug, 2007).

As jumping is terrestrial, it differs from swimming in the type of forces encountered, the magnitude of the propulsive impulse produced, and inter-limb coordination (Nauwelaerts and Aerts, 2003). For example the external forces during jumping work in the vertical plane while the movement of the body is at an

angle to the horizontal plane. Alternatively, in swimming the resultant external forces are oriented parallel to the direction of motion (Nauwelaerts and Aerts 2003). Furthermore, muscle recruitment patterns also differ between jumping and swimming (Kamel et al., 1996; Gillis and Biewener, 2000; Gillis and Blob, 2001). In particular, jumping tends to elicit larger degrees of hindlimb muscle recruitment than swimming. However, performance modulation in both forms of locomotion is attained in the same phase (Nauwelaerts et al., 2001). In jumping, variation in performance is brought about by changes in the take-off phase as the hindlimbs extend. Shorter duration take-offs lead to longer distance jumps (Gillis and Biewener, 2000). Similarly, changes in speed during swimming are most likely brought about by changes in the propulsive phase of a swim cycle when the hindlimbs are extending. Therefore, swimming can simplistically be thought of as "jumping under water" (Nauwelaerts etal., 2001) (Fig. 3).

During swimming in frogs, alterations in speed lead to little change in kinematic variables such as the duration of locomotor phases (propulsive and recovery) and maximum and minimum joint angles (Nauwelaerts et al. 2001). However, a strong relationship is observed in frogs between instantaneous velocity at the end of the propulsive phase and average swimming speed, indicating that frogs are able to control and modify the impulse generated during the propulsive phase.

The goal of this study is to answer one question: how does the toad *Bufo marinus* alter hindlimb muscle recruitment to deal with changes in mechanical

demands brought about by increasing swimming speed? Animals of this species of toad were selected as they move relatively steadily, are good cyclic swimmers, and are easy to train. Based on the results of Nauwelaerts et al. (2001) I hypothesized that the recruitment intensity of hindlimb extensor muscles would change with increasing speed as these power the propulsive phase during swimming. In contrast, based on the work by Pierotti et al., (1988) and Weyand et al., (2000) on terrestrial, limbed locomotion, I predicted that hindlimb flexors would show little or no change in recruitment intensity with changing swimming speed. In short, I predicted that increasing swimming speed is brought about in anurans mainly by pushing their feet against the water harder during propulsion than by retracting their limbs more quickly during recovery.

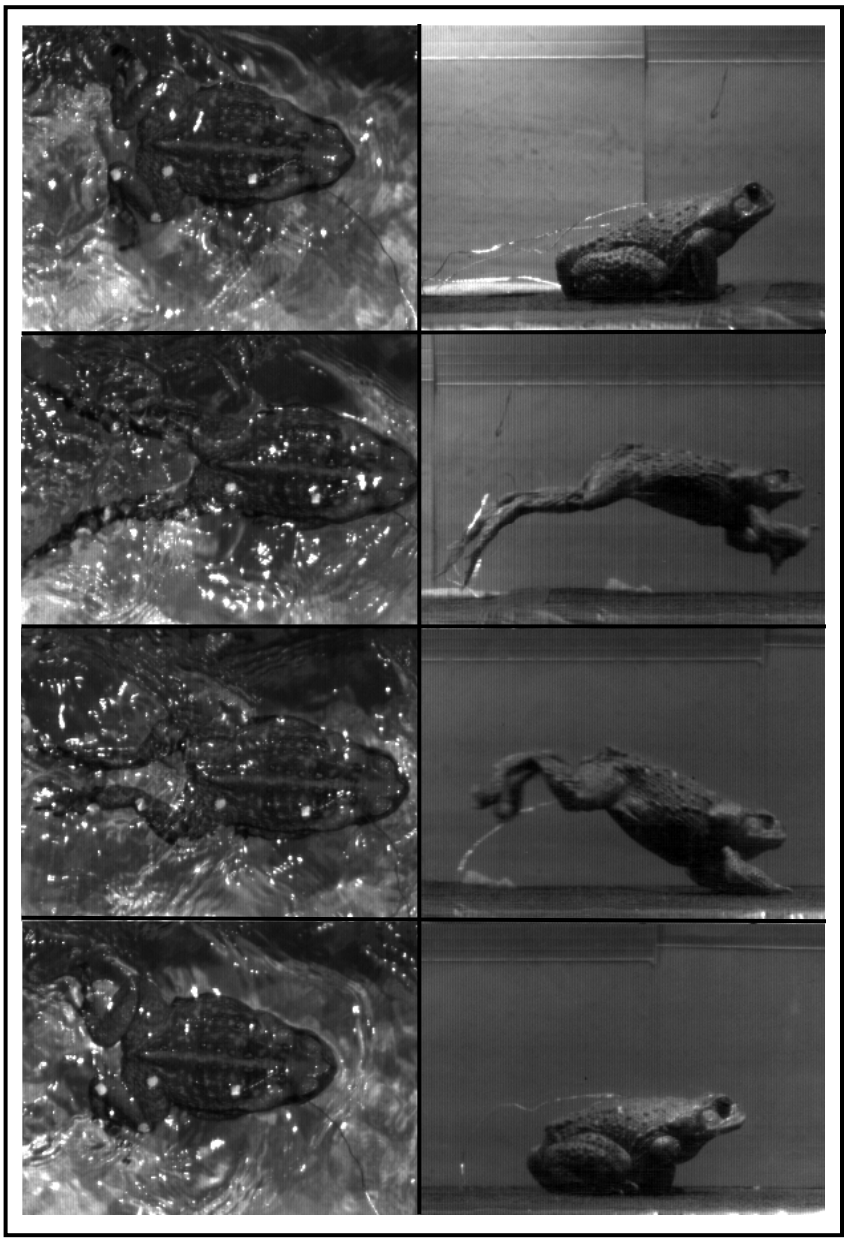

Figure 3: This image highlights the similarities between jumping and swimming in hindlimb movement. Both forms of locomotion begin with the hindlimbs fully retracted. The hindlimbs then push against the substrate propelling the body forward until they are maximally extended. The hindlimbs are then retracted again into their starting position in preparation for the next jump or swim.

METHODS AND MATERIALS

Animals

Ten cane toads (*Bufo marinus*) were used in this study. The toads were obtained from a commercial supplier and housed in groups of two to three in plastic containers at 24°C and were exposed to a 12h:12h light:dark cycle. They were provided with fresh water and a diet of mealworms. All experiments were approved by Mount Holyoke College's Institutional Animal Care and Use Committee.

Flow tank calibration

A 90l swim flume (Loligo Systems, Tjele, Denmark) was used in this experiment (Fig. 4). The swim flume consisted of a working section or swimming area measuring 65cm x 19.6cm x 19.5cm. A constant flow rate was generated by a propeller driven by a variable-speed motor. Turbulent water flow was minimized by two segmented sections, a metal grid and a honeycomb section, on either side of the swimming area.

The flow tank motor was calibrated with respect to water speeds using video recordings from a Photron 1280 PCI high-speed video camera (Photron, San Diego, CA, USA). The movement of a circular piece of cork across the first half of the swimming area was recorded at 250frames/s at different motor settings. For each motor setting, the distance moved by the cork was measured using the customized digitizing software Didge (Alistsair Cullum, Creighton University) during four separate trials. Each distance was divided by the time it took the cork

to move that distance in order to obtain the speed in cms^{-1}. Average speed from the four trials was plotted for each motor setting to obtain a standard curve relating any motor setting to the speed of water flow in the tank (Fig. 5). The equation obtained relating these two variables was:

$$Speed\ (cm/s) = 3.39(Motor\ Setting) - 4.69$$

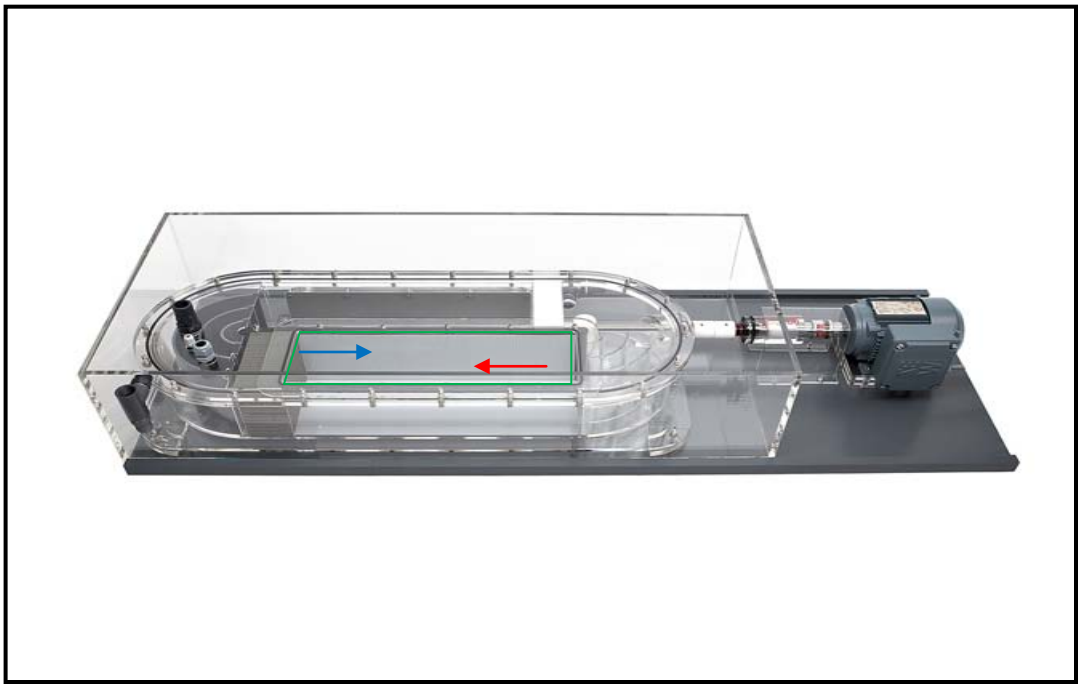

Figure 4: 90l swim flume. The area outlined in green highlights the swimming region measuring 65cmx19.6cmx19.5cm. The blue arrow shows the direction in which the toads swam while the red arrow shows the direction of water flow. The image was obtained from Loligo Systems http://www.loligosystems.com/index.php?menu=92

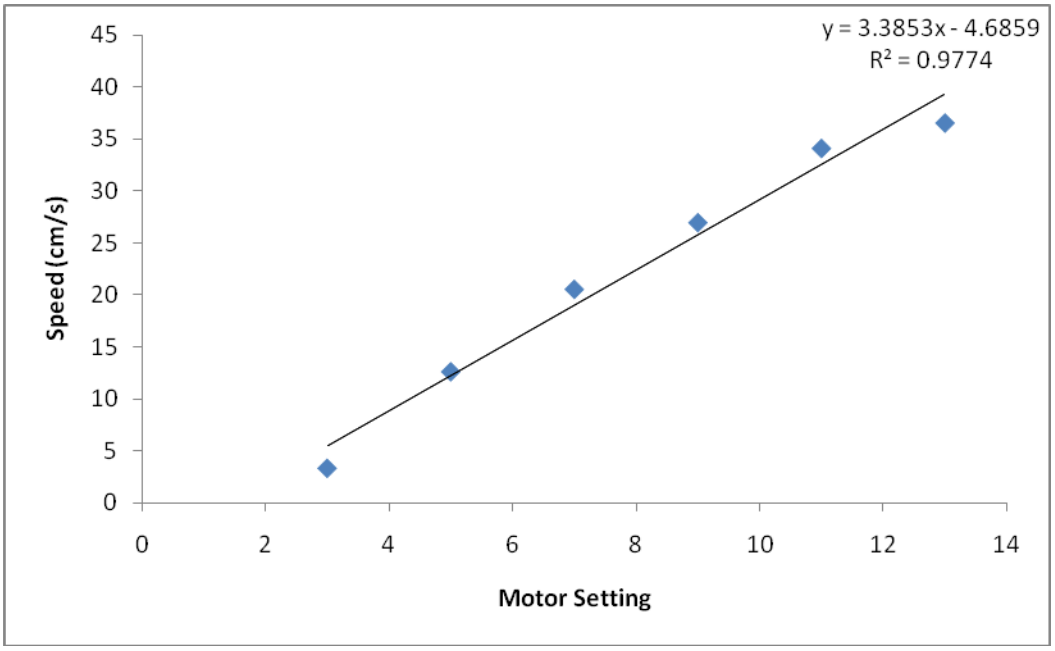

Figure 5: Standard curve relating motor setting of the swim flume to speed of water in the swim flume.

Determination of swimming speeds to use

In a series of preliminary swimming trials, toads were swum across a range of speeds in the swim flume in order to determine their performance limits. The slowest speeds at which steady synchronous swimming could be obtained consistently were between 15-16 cms^{-1} and the fastest speeds were between 27-28 cms^{-1}. Experiments in this study exploited this entire range and involved swimming animals at three speeds: 15.6 cms^{-1}, 21.4 cms^{-1}, and 27.5 cms^{-1}.

Experimental setup

To visualize animals during swimming, a mirror was placed at a 45° angle above the flow tank's working section, allowing a dorsal view of the animal (Fig. 6). A high-speed video camera was aimed at the mirror and focused on the water surface, where the animals swam. Recordings of swimming animals were taken at $250fps^{-1}$. The swimming arena was illuminated using two 500W Lowel Omni lights (Lowel Lighting, Brooklyn, NY, USA) and the water temperature was maintained at approximately 23°C.

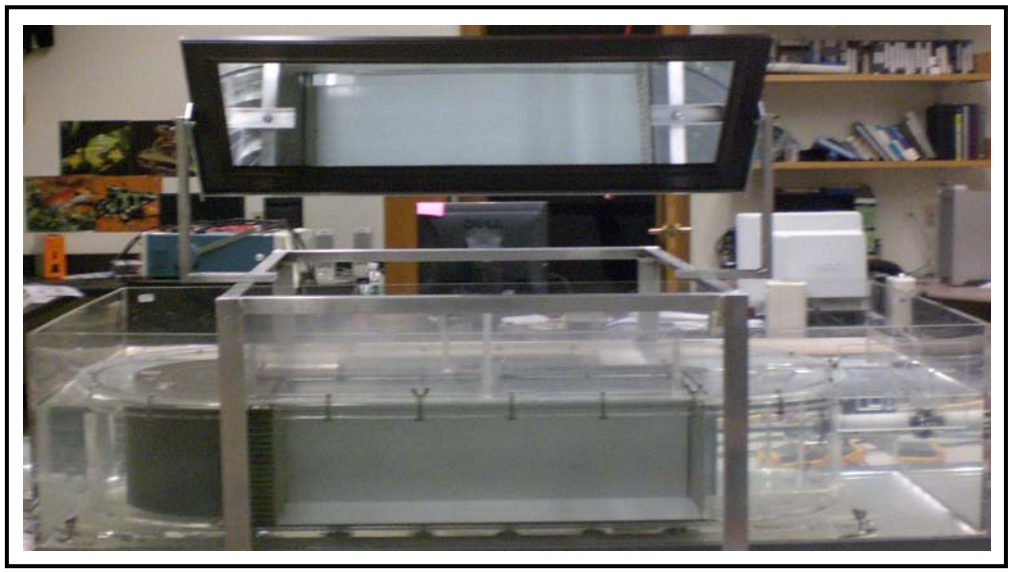

Figure 6: Experimental setup. The image shows the mirror placed at a 45° angle to give a dorsal perspective of the working section of the flow tank.

Myology

Four major hindlimb muscles were analyzed: the hindlimb extensors the *m. cruralis*, the *m. adductor magnus* and the *m. gracilis major,* and the hindlimb flexor the *m. semitendinosus* (Figs. 7 and 8). The *m. cruralis* forms the dorsal head of the *m. triceps femoris* muscle. It is a biarticular muscle that originates from the acetabulum of the pelvis, extends across the anterior border of the knee and femur and inserts onto the tibiafibula. The *m. cruralis* is in position to extend the knee (Duellman and Trueb, 1986). The *m. adductor magnus* is a large muscle that originates via two heads from the pelvic rim. Its fibers run along the ventromedial surface of the thigh and insert onto the medial surface of the femur. This muscle is relatively deep and is covered superficially by the *m. sartorius* and *m. gracilis major*. When the *m. adductor magnus* contracts it adducts the hip joint and brings the femur closer to midline of the body (Duellman and Trueb, 1986). The *m. gracilis major* originates from the posterior border of the ischium of the pelvis and inserts via two tendons onto the aponeurosis of the knee and medial region of the tibiofibula head. It is a broad muscle that has fibers that run along the ventrolateral surface of the femur (Duellman and Trueb, 1986). The *m. gracilis major* is in position to extend the hip joint. The *m. semitendinosus* is the only major hindlimb flexor studied. It is a deep muscle that consists of two heads that originate via a tendon from the pelvic rim. The muscle extends along the ventromedial surface of the femur and inserts onto the ventral surface of the

tibiofibula via a tendon and is in a position to flex the knee (Duellman and Trueb, 1986).

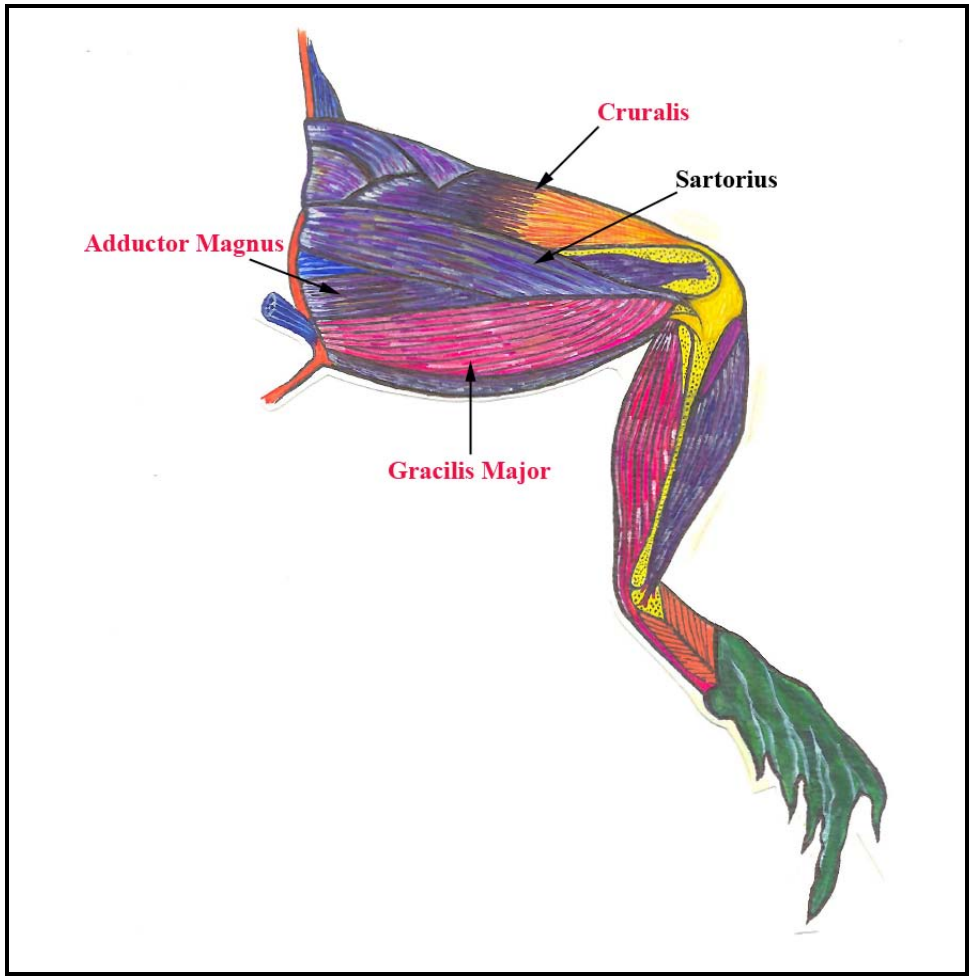

Figure 7: This image shows a ventral view of a left toad leg. The skin has been removed to reveal the underlying limb musculature. The muscle names in red are the three extensor muscles implanted in this experiment.

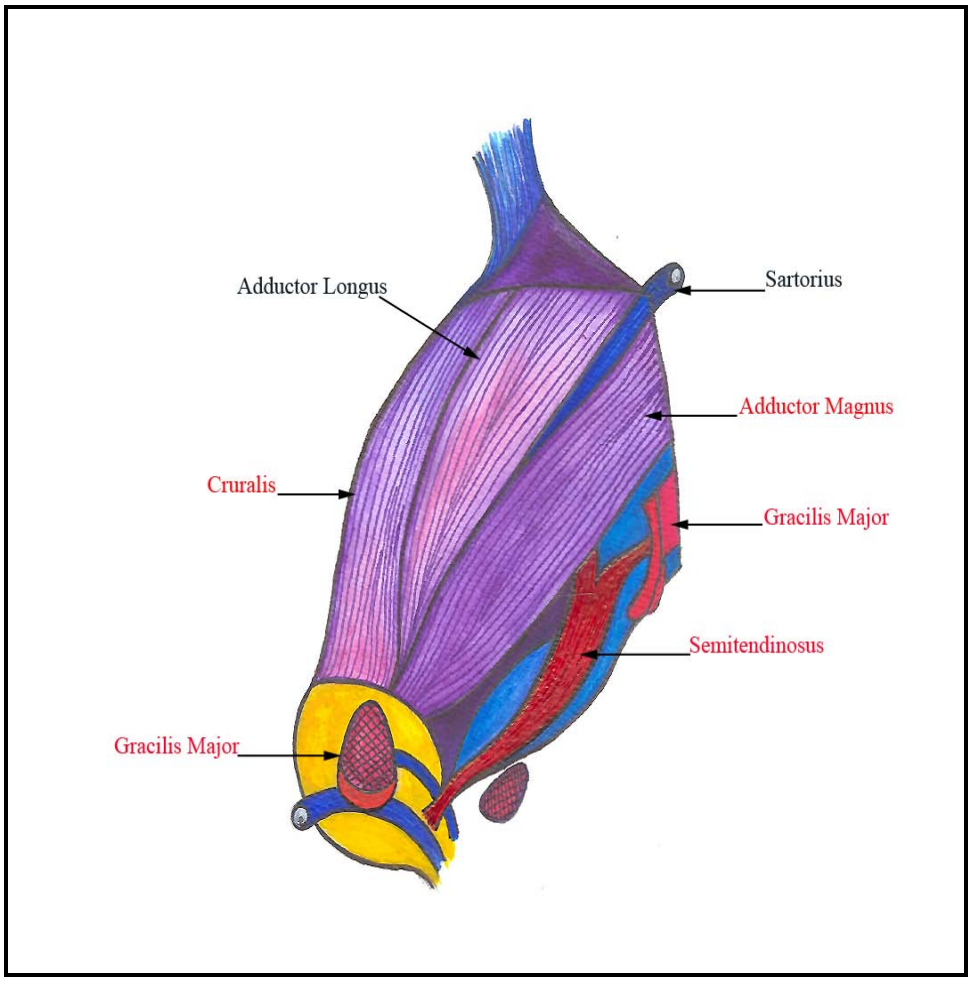

Figure 8: In this ventral view of the right thigh of *Bufo marinus* the superficially positioned sartorius and gracilis major have been transected to show the deeply positioned and double-headed knee flexor, the semitendinosus. The muscle names highlighted in red are those that were implanted in this study.

Surgical procedure and electromyography

To implant electrodes for recording electromyograms, toads were anesthetized by immersion in 1l of tricane methanosulfate solution (MS-222, 1.2g/l) for approximately 45min. An incision about 2cm in length was made on the ventral surface of the thigh. Silver finewire bipolar electrodes (twisted, offset hook) were implanted into the four muscles of interest. An electrode was constructed by twisting together two silver, insulated wires 1m in length with a diameter of 0.1mm (California Fine Wire Company. CA, USA). One millimeter of the insulation was removed from both ends of the two wires. One end from which the insulation was removed was implanted into a muscle of interest using a 23G hypodermic needle. Silk thread (6.0) was used to suture the electrodes onto the muscle in order to prevent them from slipping out of their insertion sites. The incision was closed using 4.0 silk thread. The electrodes were further secured by suturing them at multiple points onto the back of the animal.

The other bared ends of the electrodes were soldered into a 15pin female connector. The female connecter was connected to its complementary male connector whose wires carried signals to Grass P511 pre-amplifiers. Signals were amplified 1000X and filtered to eliminate 60 Hz noise and reduce frequency components lower than 100 Hz and higher than 3000 Hz. Signals were digitized at 5000 Hz using Axon Instruments' Digidata 1322A 16-bit A/D converter, and saved onto a computer. An animal was allowed to recover for approximately 1.5 hours before the swimming trials as described below were conducted. Muscle

electrical activity was recorded from the electrodes as the animal swam across a range of speeds.

Kinematics

To obtain kinematic data, small cloth squares (VetWrap) were glued onto the skin covering the hip, knee, ankle and metatarsalphalangeal joints, as well as at the midpoint of the vertebral column using Duro™ super glue (Henkel Loctite Corporation, USA) (Fig. 9). The markers on the limb were used to calculate joint angles at the hip, knee and ankle, while the marker on the animal's back was used for tracking its position and calculating velocity. The animal was placed in the swimming arena, and stimulated to swim by touch. Trials in which the animal swam with minimum acceleration, using synchronous kicks, in the middle of the swim arena were recorded using the high speed video and saved onto a personal computer. For each swim trial, three consecutive swim cycles were saved. The number of swim trials recorded at each of the three speeds for each toad is shown in table 1.

After EMG and high-speed video recordings were completed the animal was euthanized by extended immersion (~24 hours) in 1.2g/l tricane methanesulfate solution. The toad was dissected the next day in order to confirm the placement of the electrodes. Table 2 shows the mass of each toad used, and the muscles successfully implanted in each animal.

Table 1: Number of trials recorded at each speed for each toad.

Toad	Number of Trials Recorded		
	Speed (cms^{-1})		
	15.6	**21.4**	**27.5**
1	4	4	4
2	4	5	4
3	4	5	5
4	4	4	5
5	4	5	5
6	4	4	2
7	5	4	5
8	5	6	6
9	4	4	4
10	5	6	4
Total	43	47	44

Table 2: Mass and snout-vent length of each toad used, and muscles successfully implanted in each toad. 'X' indicates a successfully implanted muscle from which usable EMG data was obtained in each toad.

Toad	Mass/g	Snout- Vent Length/cm	Muscles			
			Cruralis	Adductor Magnus	Gracilis Major	Semitendinosus
1	122.8	11.7	X			
2	156.7	11.7		X	X	
3	114.8	11.1	X	X		
4	117.2	10.6	X	X		
5	115.3	10.6	X			
6	120.6	11.2	X	X	X	
7	85.5	10.4	X		X	X
8	107.9	10.3	X			X
9	172.3	11.9	X		X	X
10	119.8	11.9	X		X	X
Total			9	4	5	4

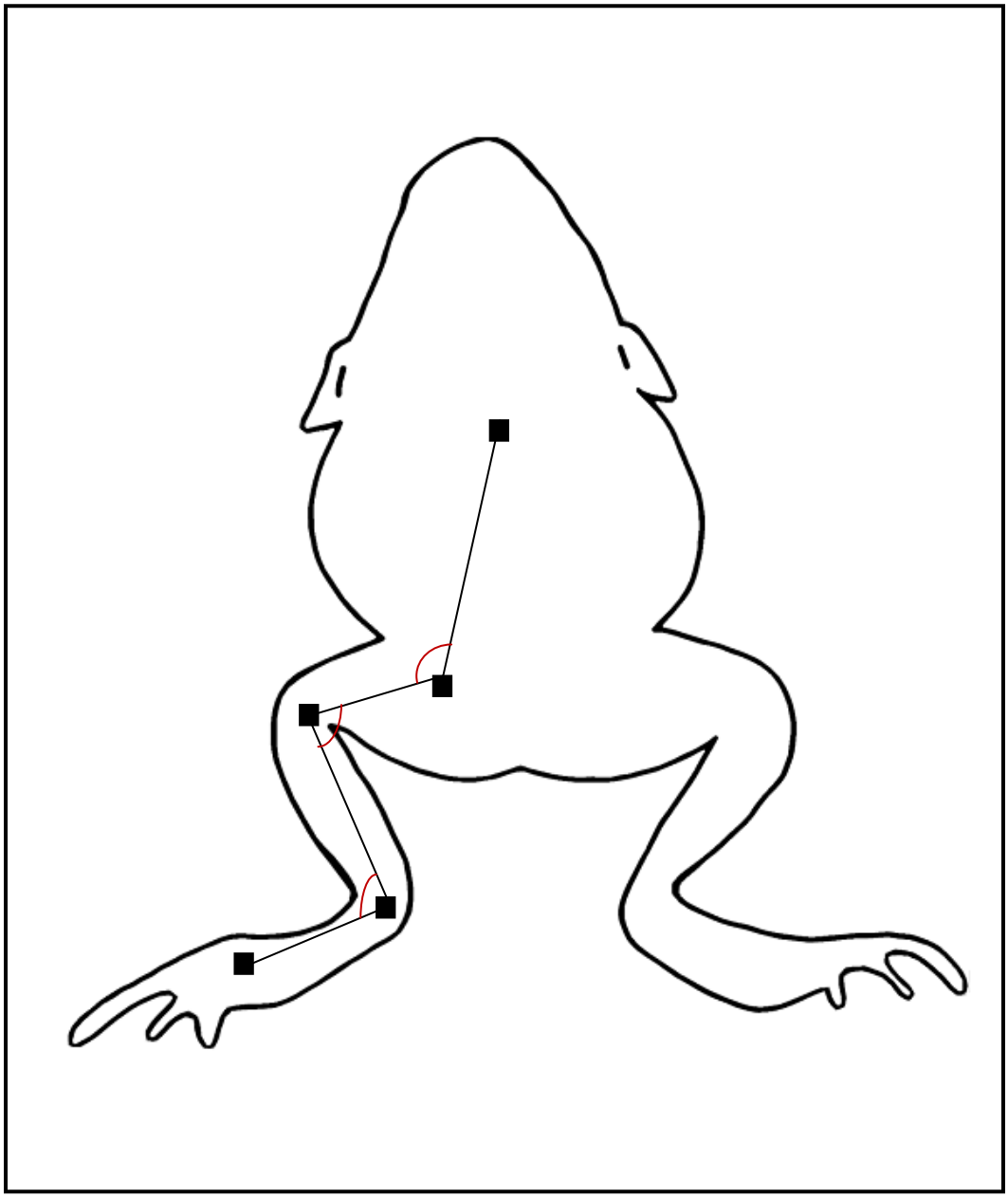

Figure 9: Dorsal view of a toad showing markers used to digitize animal movements, and hip, knee, and ankle joint angles.

Data analysis

Timing and duration of phases

For each toad one swimming cycle was analyzed per trial; a swimming cycle was defined as lasting from the start of one stroke to the start of the next stroke. Simultaneous EMG and video recordings were used to determine the frame number and thereby the time point for the onset of knee extension, maximum ankle extension, and the onset of knee extension in the next swim cycle. These time points were then used to calculate the duration (in ms) of the propulsive phase (onset of knee extension to maximum ankle extension), and the recovery phase (maximum ankle extension to onset of knee extension in the next cycle) for each swim cycle analyzed (Fig. 10).

EMG recordings from the cruralis were used to define each cycle, as this muscle begins its activity early, at the start of each stroke (Gillis, 2007). The onset of the cruralis was visually determined and defined as 0% of a swim cycle. A swim cycle lasted until the onset of activity in the cruralis in the next swim cycle. The timings of the kinematic events described above were converted into percentages of the swim cycle using the formula below:

$$\left(\frac{Time\ point\ at\ which\ kinematic\ event\ occurs(ms) - Onset\ of\ cruralis\ in\ cycle\ of\ interest\ (ms)}{Onset\ of\ cruralis\ activity\ in\ next\ cycle\ (ms) - Onset\ of\ cruralis\ activity\ in\ cycle\ of\ interest(ms)}\right)*100$$

These percentage points were then superimposed onto the muscle activity profiles obtained by the binning analysis described below in order to see how muscle activity patterns corresponded to the toad's limb movements.

The total cycle duration was plotted against speed, as was the propulsive phase duration (as a percentage of total cycle duration). This was done in order to see if toads shortened their swim cycles, or allocated a different fraction of the swim cycle to the propulsive phase to increase swimming speed.

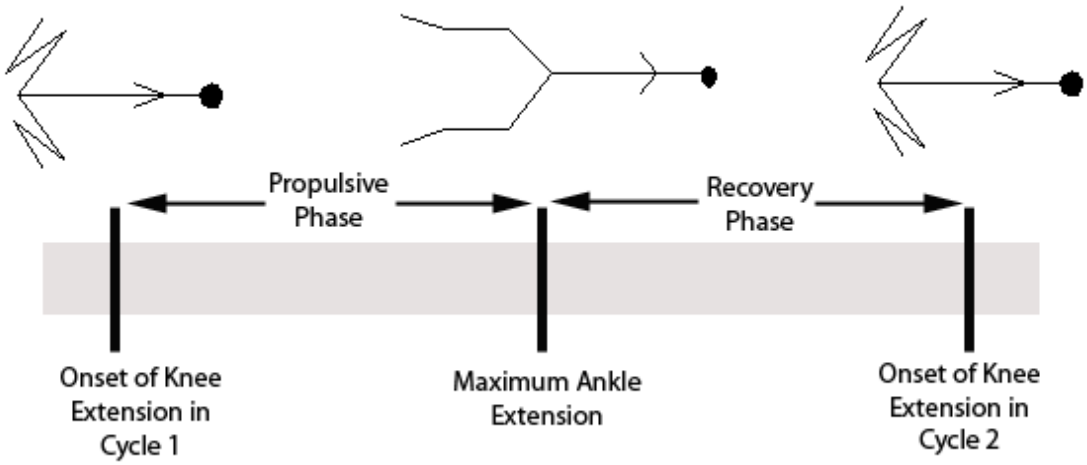

Figure 10: Phases in a single swim cycle. Each swim cycle consisted of two phases defined by three kinematic events. The propulsive phase began when the animal extended its knee until the ankle reached maximum extension. The recovery phase began when the ankle had reached maximum extension and lasted until the animal began to extend its knee in the next swim cycle.

Binning analysis

A binning analysis was conducted in order to ensure that the muscle recruitment patterns were similar to those obtained in previous experiments. The binning analysis was carried out by obtaining the raw muscle activity signals in mV for a swim cycle from the recording program Axoscope. The values for each muscle for every swim cycle analyzed were rectified and divided into one-hundred equally spaced intervals call bins. Within each muscle in every animal, the values in each of the one-hundred bins were averaged in order to obtain a single value for each bin. Figure 11 shows how a binning analysis would be conducted on a swim cycle using twenty-five bins for a single muscle. Twenty-five bins were used in this image as it was not possible to illustrate one-hundred bins on the graph.

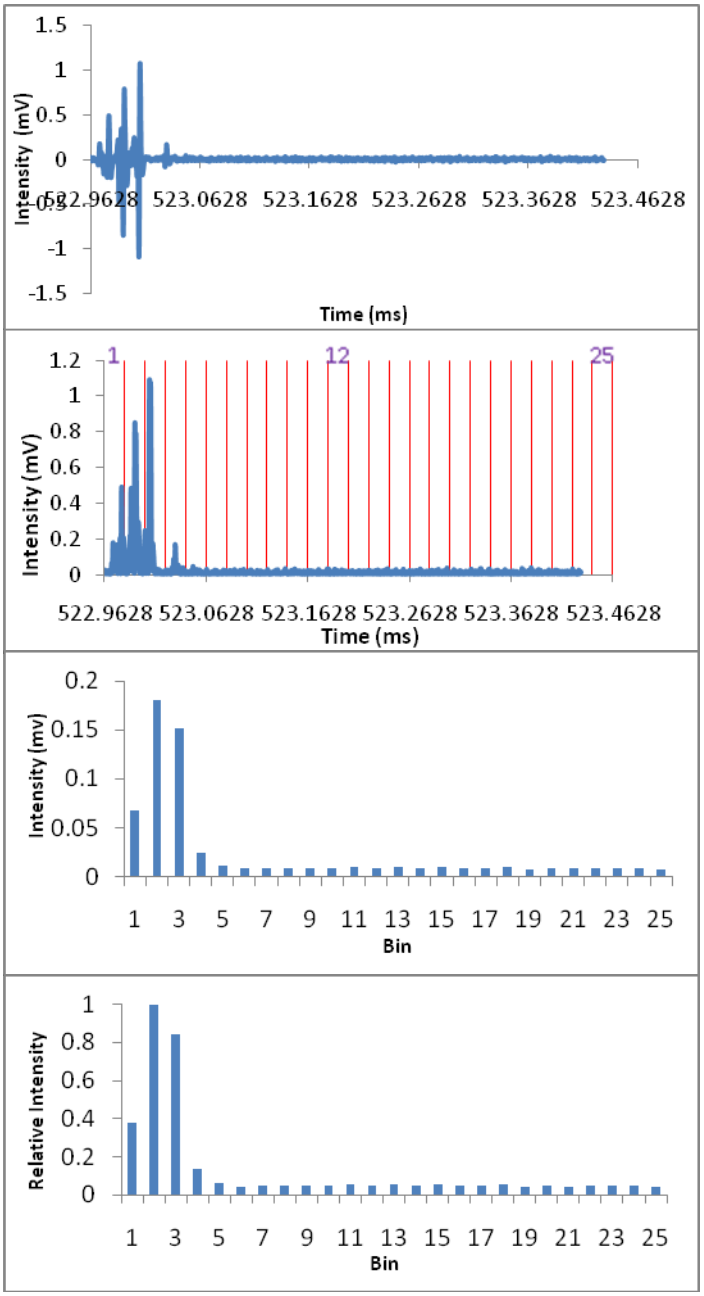

Figure 11: Binning analysis. (A) Step 1 in the binning analysis. A raw EMG signal for a swim cycle is obtained from Axoscope. This signal is from the cruralis muscle of toad 7. (B) Step 2 in the binning analysis. The signal is rectified and divided into 25 equally spaced time intervals called bins. In this experiment 100 bins were used, however, all 100 bins could not be clearly shown on this graph. The values in each bin are averaged to obtain one value for each bin. Therefore, instead of thousands of data points, which make data analysis difficult, binning “scrunches” the data making it easier to analyze. (C) The 25 values obtained after binning plotted using a bar graph. (D) Scaled bin values. The values in each bin are scaled to the maximum value observed for the muscle in all swim cycles analyzed in a toad. This allows muscle recruitment patterns to be averaged for all cycles and compared across toads.

Determination of average muscle recruitment intensity and burst duration

The onset and offset times of muscle activity bursts for each of the muscles implanted were determined by eye. These time points were then used to calculate muscle burst durations for each trial. The burst duration values were scaled for a particular muscle within a toad by dividing the values obtained by the maximum burst duration value observed for that muscle. These scaled values were named relative burst duration.

The average muscle intensity between the onset and offset of muscle activity for each trial was determined by subtracting the baseline from the muscle voltage values obtained from Axoscope, rectifying the signal, and averaging all the values obtained. The average muscle activity values for all the trials for a particular muscle within a toad were scaled by dividing the values obtained by the maximum average value observed for that muscle. These scaled values were named relative muscle recruitment intensity.

Determination of actual swimming speed

As toads tended to accelerate and decelerate during a trial the average speed of the animal in each trial was calculated. The distance moved by the animal from onset of knee extension in the cycle of interest to onset of knee extension in the following cycle was calculated by digitizing the x,y coordinates of the cloth square superglued to the animal's back in the images representing the start of the two consecutive swim cycles using the custom digitizing program

Didge (Alistsair Cullum, Creighton University). This distance was then divided by the time taken to travel it in order to obtain the speed of the animal in cms^{-1}. This speed was then added to the speed of the flow tank current (against which the animal swam) in order obtain the actual swimming speed of the animal.

Graphical representations of EMG data

Regressions were plotted with speed on the x-axis and relative muscle burst duration, relative muscle recruitment intensity on the y-axis for all toads in order to identify the presence or absence of relationships between these variables and speed.

The relative recruitment intensities of a muscle at different speeds were plotted against all other muscles in order to analyze the relationships of recruitment intensities between muscles.

Joint angles

In three toads the joint angles at the hip, knee and ankle for a swim cycle were determined using the vet wrap squares covering each joint and the software program Image J (Fig. 9). Joint angle profiles were smoothed with respect to time using a five point running average and plotted against the percentage of the swim cycle.

The maximum and minimum joint angles at the four highest speeds and four lowest speeds were averaged and analyzed for each of three toads in order to

see if there was variability present. The joint angles were also analyzed without averaging highest and lowest speeds for each of the three toads.

Statistical analysis

Linear regression (SPSS) was used to examine the relationships between speed and relative muscle recruitment intensity, relative muscle burst duration, cycle duration, and propulsive phase duration (as a percentage of the total cycle duration). P-values less than 0.05 were defined as significant. This method was also used to analyze the recruitment intensity relationships between different muscles.

RESULTS

A total of 129 swim cycles were analyzed. Table 3 gives a summary of the number of cycles analyzed per toad, the range of swimming speeds of each toad, and their average speeds. The overall range of swimming speeds was between 12.18cms^{-1}- 45.84cms^{-1}. The average swimming speed for all toads was 22.88cms^{-1}.

Table 3: Data showing the number of swim cycles analyzed per toad, the speed range for each toad, and the average speed of each toad.

Toad	Number of Cycles Analyzed	Speed Range (cm/s)	Average Speed (cm/s)
1	11	16.98-30.18	22.71
2	12	14.60-33.22	24.59
3	14	17.56-29.12	22.16
4	13	18.71-32.26	25.8
5	13	14.42-32.24	22.29
6	10	12.40-22.11	17.05
7	13	12.18-25.40	19.10
8	16	17.15-45.84	24.92
9	12	17.09-35.27	25.30
10	15	15.24-36.58	24.89

Kinematics

Figure 12 shows the average timings of the onset of knee extension and maximum ankle extension in a typical swim cycle. Onset of knee extension occurred 2.30 ± 2.4% into the cycle while maximum ankle extension occurred 27.8 ± 4.7% into the cycle. Therefore, the propulsive phase was the shorter phase and lasted on average for 25.5% of the swim cycle. The recovery phase was longer and composed, on average, 74.5% of a swim cycle.

Muscle recruitment patterns

Figure 13 shows representative raw EMG data from the cruralis and semitendinosus from a single toad with the propulsive and recovery phases superimposed for context. The cruralis consistently showed a single burst of activity early in the propulsive phase while the semitendinosus showed two bursts of activity, one during propulsion and one during recovery. The adductor magnus and gracilis major showed activation patterns similar to the cruralis (i.e., single bursts early in propulsion).

Figure 14 shows the overall average timing and intensity profiles obtained from the binning analysis for all four muscles. The cruralis began its activity earliest among the muscles studied and was used to define the beginning of a swim cycle. Between the four muscles implanted, the cruralis was active for the shortest period of time in a swim cycle during the propulsive phase (Fig. 14). On average cruralis activity lasted for 64.6 ±26.6ms and occupied on average 11.6 ±

3.7% of a swim cycle (N_{cycles}= 117) from onset to offset of activity. The adductor magnus and gracilis major began their activity shortly after the cruralis and were active for longer durations. The adductor magnus was active on average for 117.8 ± 32.7ms in the propulsive phase and occupied 18.5± 4.8% (N_{cycles}=37) of a swim cycle. The gracilis major was active on average for 110 ± 29.3ms in the propulsive phase, which composed 18.1± 8.0% of a swim cycle (N_{cycles} = 49).

The semitendinosus showed a biphasic pattern of activity. The first burst of activity (semitendinosus extensor burst) was typically of a higher intensity than the second burst of activity (semitendinosus flexor burst) (Figs. 13 and 14). The first burst of activity began shortly after cruralis activity. It lasted on average for 102.3 ± 50.7ms and occupied 17.0± 8.0% (N_{cycles} = 54) of a swim cycle in the propulsive phase. The second burst of activity took place in the recovery phase and lasted on average for 155.3 ± 49.3ms and composed 26.5± 8.6% (N_{cycles}=51) of a swim cycle.

Joint angles

Figures 15 and 16 illustrate how the angles at the hip, knee and ankle joints change over a swim cycle. Each figure is from a single swim cycle from two different animals to show some of the variation present in limb movements during swimming. The joint angles at all three hindlimb joints increased in the propulsive phase indicating that the hindlimbs were extending during this period. The joint angles at all three joints decreased in the recovery phase indicating that the limbs are flexed in this interval. The duration of activity of muscles were

plotted above this graph to help show the roles of the different muscles in limb extension versus flexion. The cruralis, adductor magnus, and gracilis major were all active when the hindlimbs were extending (Fig. 15), highlighting their role in knee and hip extension. The first, extensor burst of the semtendinosus was also active when the joint angles were increasing (Fig 16), indicating an active role during limb extension. The second, flexor burst of the semitendinosus was active for most of the recovery phase when joint angles were decreasing.

Maximum and minimum joint angles were analyzed for three toads in order to determine if they changed when speed was increased. Angles at the hip ranged from 126.2°-162.6°. Angles at the knee ranged from 46.9°-131.4°. Angles at the ankle ranged from 54.6°-153.4°. Figure 17 shows a bar graph with averaged maximum and minimum joint angles at the four fastest and four slowest speeds for each of the three animals. A significant relationship was seen for minimum joint angles in one toad at the hip, one toad at the knee, and one toad at the ankle such that the joint angle is lower at faster speeds (i.e., the limbs were more flexed during recovery. When toads were analyzed for all speeds at an individual level this relationship was seen as well (Tables 4 and 5).

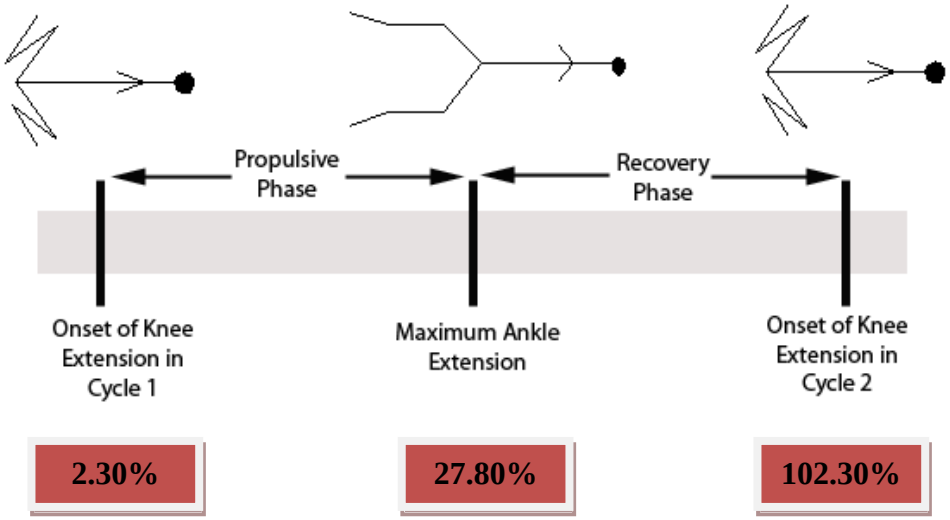

Figure 12: Average percentage time points of the onset of knee extension, maximum ankle extension and onset of knee extension in the following cycle. The values were obtained by averaging the time points at which these kinematic events occurred in every cycle analyzed for every toad except toad two. The propulsive phase was shorter than the recovery phase, and lasted on average 25.5% of a swim cycle. The recovery phase lasted on average 74.5% of a swim cycle.

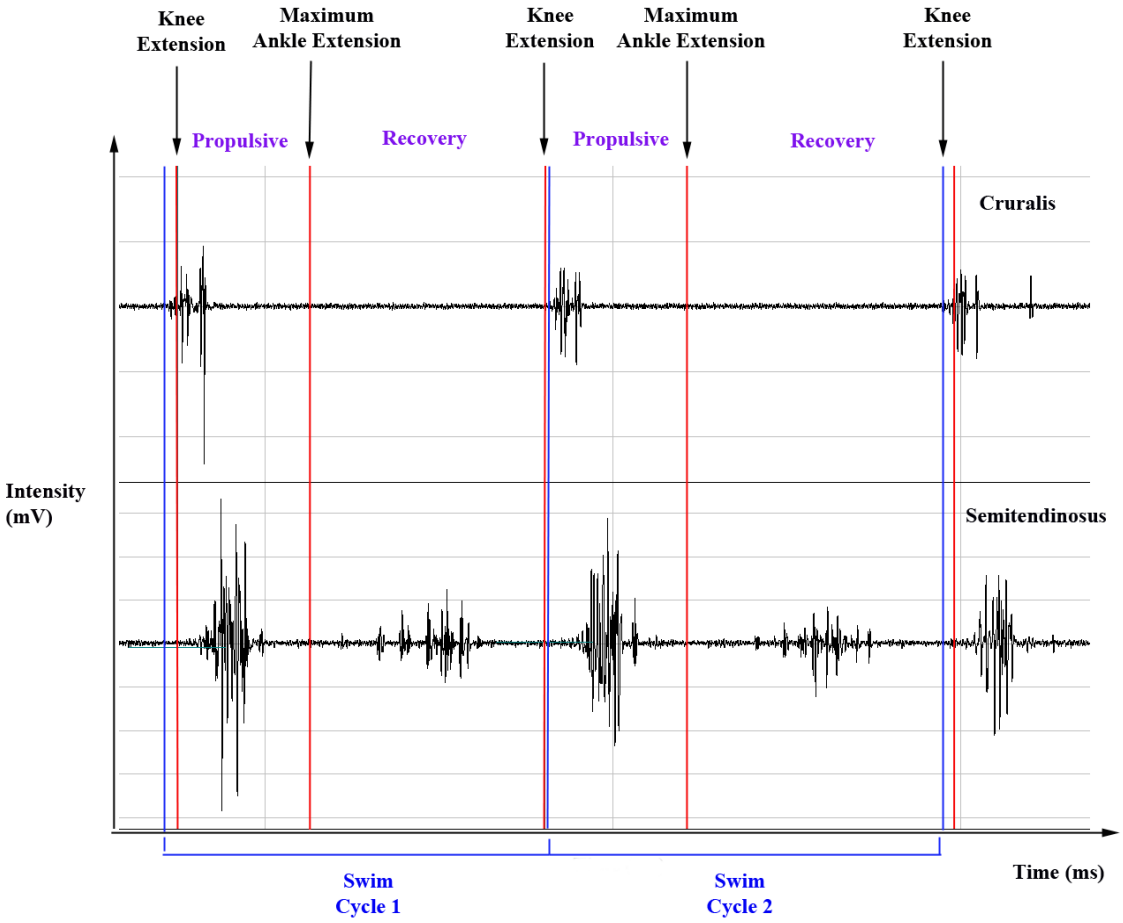

Figure 13: Raw EMG data from the cruralis and semitendinosus of a single toad with kinematic events superimposed. The cruralis showed one burst of activity in the propulsive phase while the semitendinosus showed a double burst pattern, with one burst during propulsion and another during recovery. The adductor magnus and gracilis major showed similar activity patterns to the cruralis.

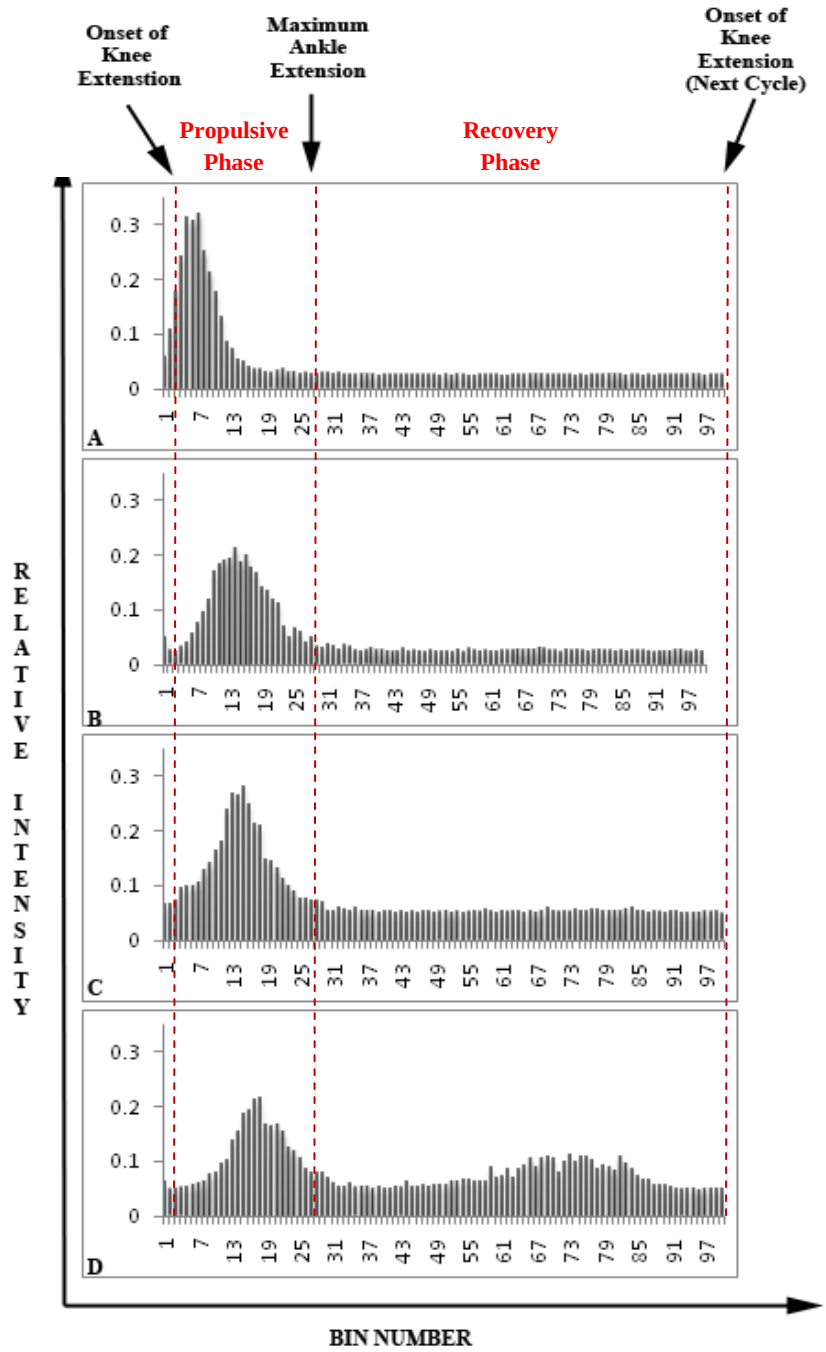

Figure 14: Intensity profiles for (A) cruralis, (B) adductor magnus, (C) gracilis major and (D) semitendinosus. The graphs were obtained by averaging the values in each of the 100 bins from all toads for a particular muscle. The cruralis, adductor magnus, and gracilis major showed one burst of activity in the propulsive phase while the semitendinosus showed a biphasic activity pattern.

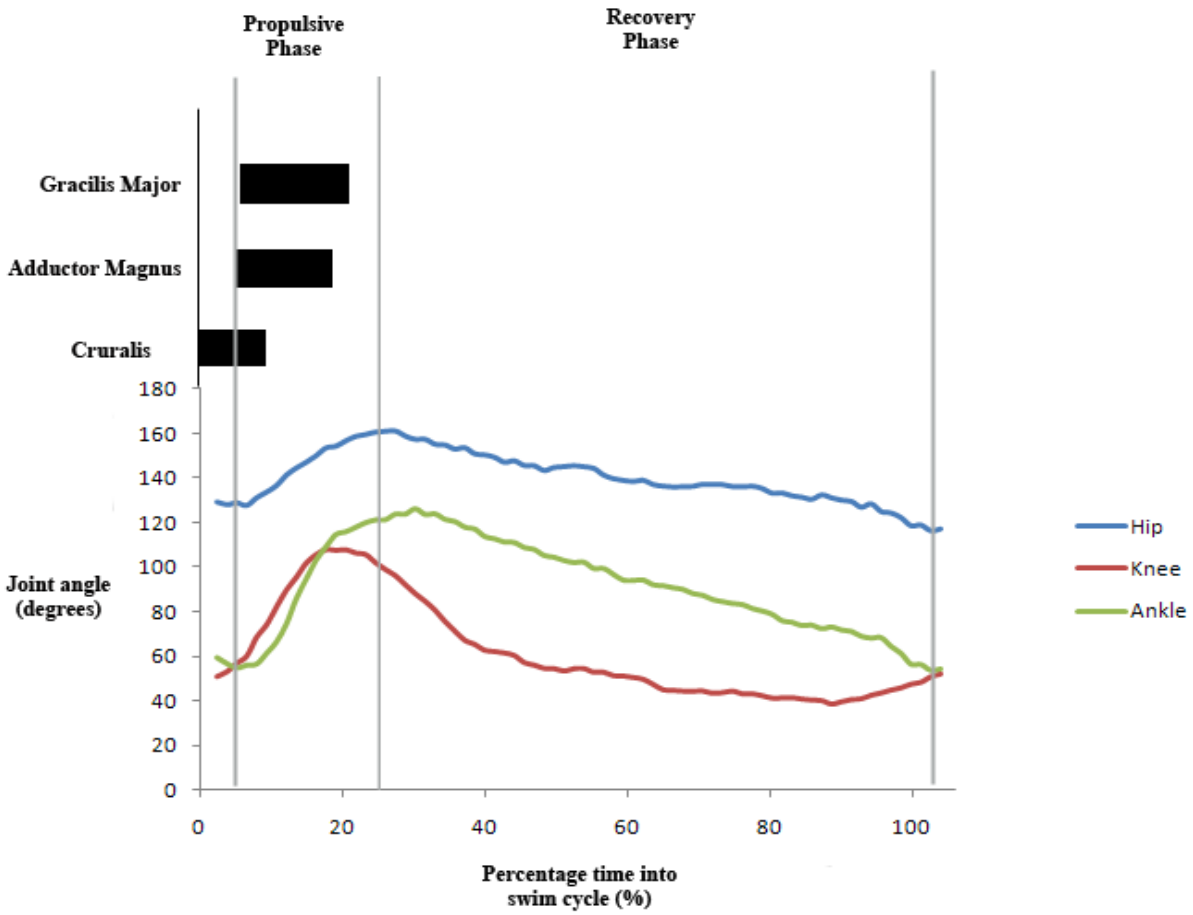

Figure 15: Diagram showing how joint angles at the hip, knee and ankle change over a swim cycle. The timing of activity of the cruralis, adductor magnus, and gracilis major has been plotted above. The data were compiled using a single, representative swim cycle from toad 6.

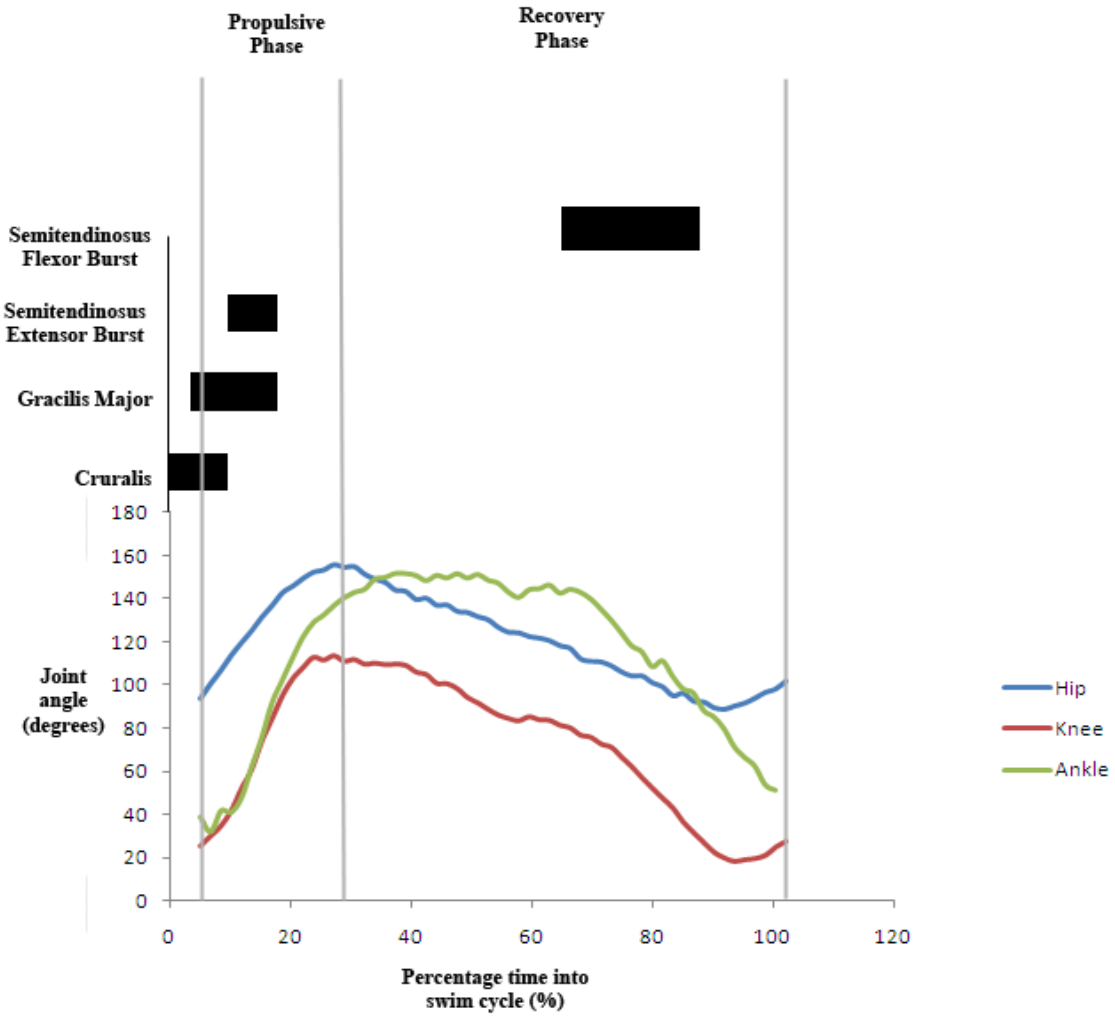

Figure 16: Diagram showing how joint angles at the hip, knee and ankle changed over a swim cycle. The timing of activity of the muscles studied are also plotted above. The data were compiled using a single, representative swim cycle from toad 10.

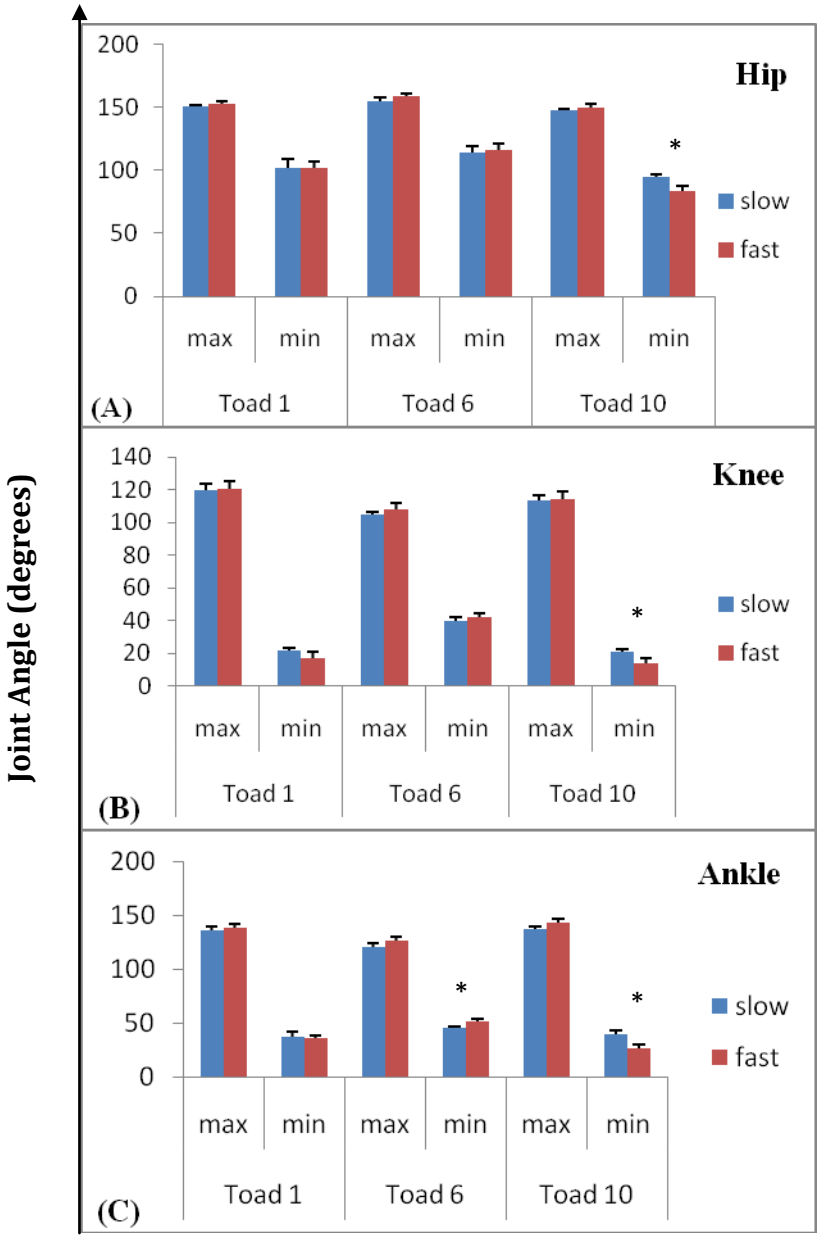

Figure 17: Maximum and minimum joint angles at the (A) Hip, (B) Knee, and (C) Ankle for toads 1, 6 and 10. The joint angles for the four slowest and four fastest speeds were averaged for each toad. Minimum joint angle showed a significant decrease in some toads as speed increased. '*' indicates significant relationships.

Table 4: R^2 and p values for relationships between maximum joint angle and speed. '*' indicate statistically significant relationships

Toad	Number of swim Trials Analysed	Maximum Hip		Maximum Knee		Maximum Ankle	
		R^2	p	R^2	p	R^2	p
1	8	0.077	0.505	0.027	0.695	0.007	0.849
6	9	0.015	0.719	0.004	0.852	0.134	0.268
10	11	0.438	0.052	0.38	0.077	0.51	0.031*

Table 5: R^2 and p values for relationships between minimum joint angle at hip, knee and ankle, and speed. '*' indicate statistically significant relationships

Toad	Number of swim Trials Analysed	Minimum Hip		Minimum Knee		Minimum Ankle	
		R^2	p	R^2	p	R^2	p
1	8	0.011	0.806	0.516	0.045*	0.107	0.428
6	9	0.715	0.001*	0.374	0.046*	0.715	0.001*
10	11	0.168	0.273	0.171	0.269	0.392	0.071

Relationships between speed, swim cycle duration, and propulsive phase duration

Swim cycle durations ranged between 418.7ms-1201.1ms (average = 647.5ms, N_{cycles}=117). A significant negative relationship was observed between speed and absolute swim cycle duration (R^2= 0.083, p = 0.002) (Fig. 18A). However, when toads were analyzed on an individual level only one out of nine toads showed a significant relationship.

The duration of the propulsive phase ranged between 88ms-272ms (average=161.1ms, N_{cycles}=117). The relationship between speed and propulsive phase duration (as a percentage of the total swim cycle duration) was analyzed. The percentage duration of the propulsive phase ranged from 10.3%-44.4% (average = 25.7%, N_{cycles}= 117). No significant relationship was observed when all toads were plotted on the same graph (R^2 = 0.002, p = 0.629) (Fig. 18B), and when toads were analyzed individually, only two out of nine animals showed significant negative relationships.

Relationship between speed and muscle recruitment intensity

Compiled graphs with all toads for relative muscle recruitment intensity versus speed are shown in figure 19. All muscles except the gracilis major showed significant positive relationships, suggesting a link between increased speed and increased muscle recruitment. However, such a positive relationship between speed and muscle recruitment intensity wasn't consistently observed for all animals (Table 6). For example, in the cruralis, only one out of nine animals

showed a significant relationship between speed and recruitment intensity. For the adductor magnus none of the individual toads showed a significant positive relationship. For the gracilis major two out of five toads showed significant positive relationships, and for the semitendinosus extensor burst and flexor burst only one out of four toads and two out of four toads showed significant positive relationships, respectively.

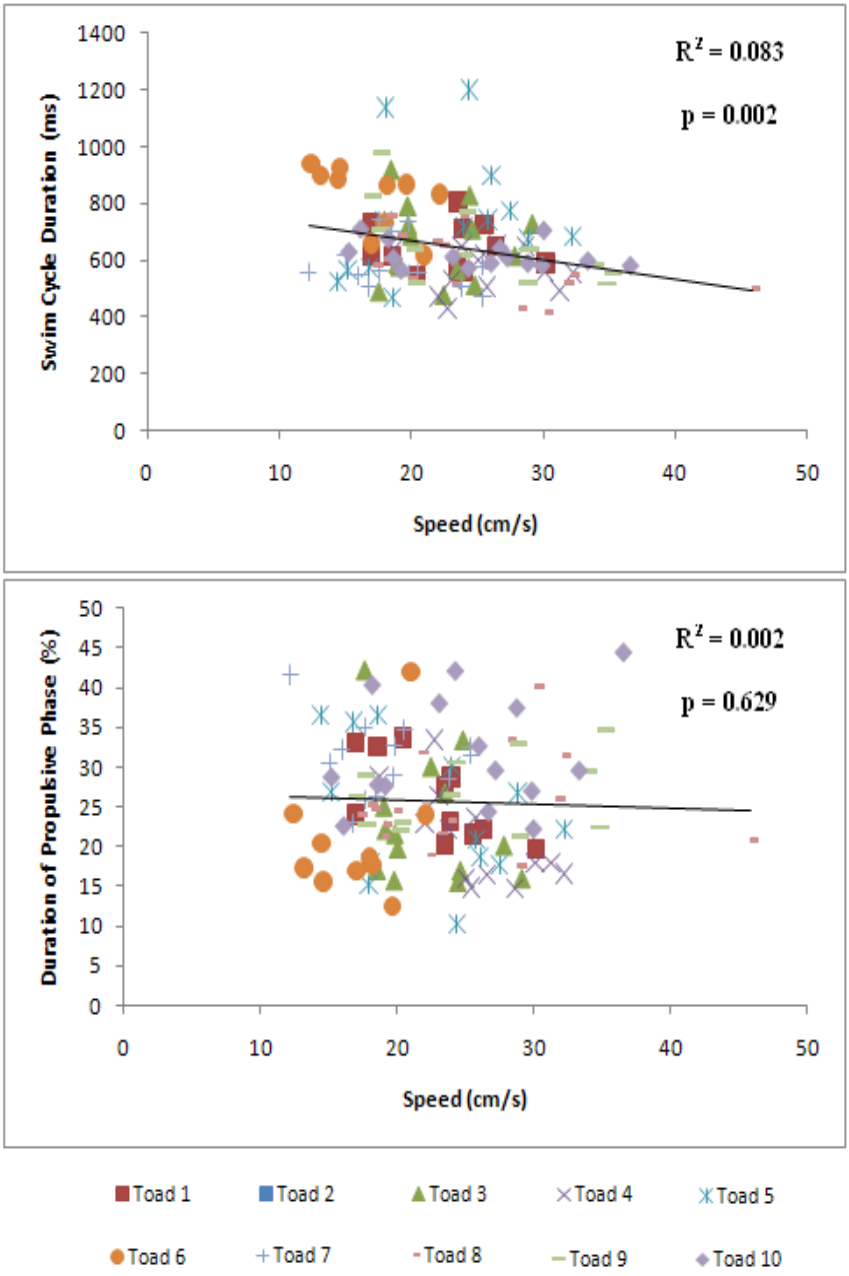

Figure 18: Relationship between speed and (A) swim cycle duration (ms), and (B) propulsive phase duration (as a percentage of the total cycle duration). A significant negative relationship was present between cycle duration and speed. The relationship between propulsive phase duration and speed was not significant.

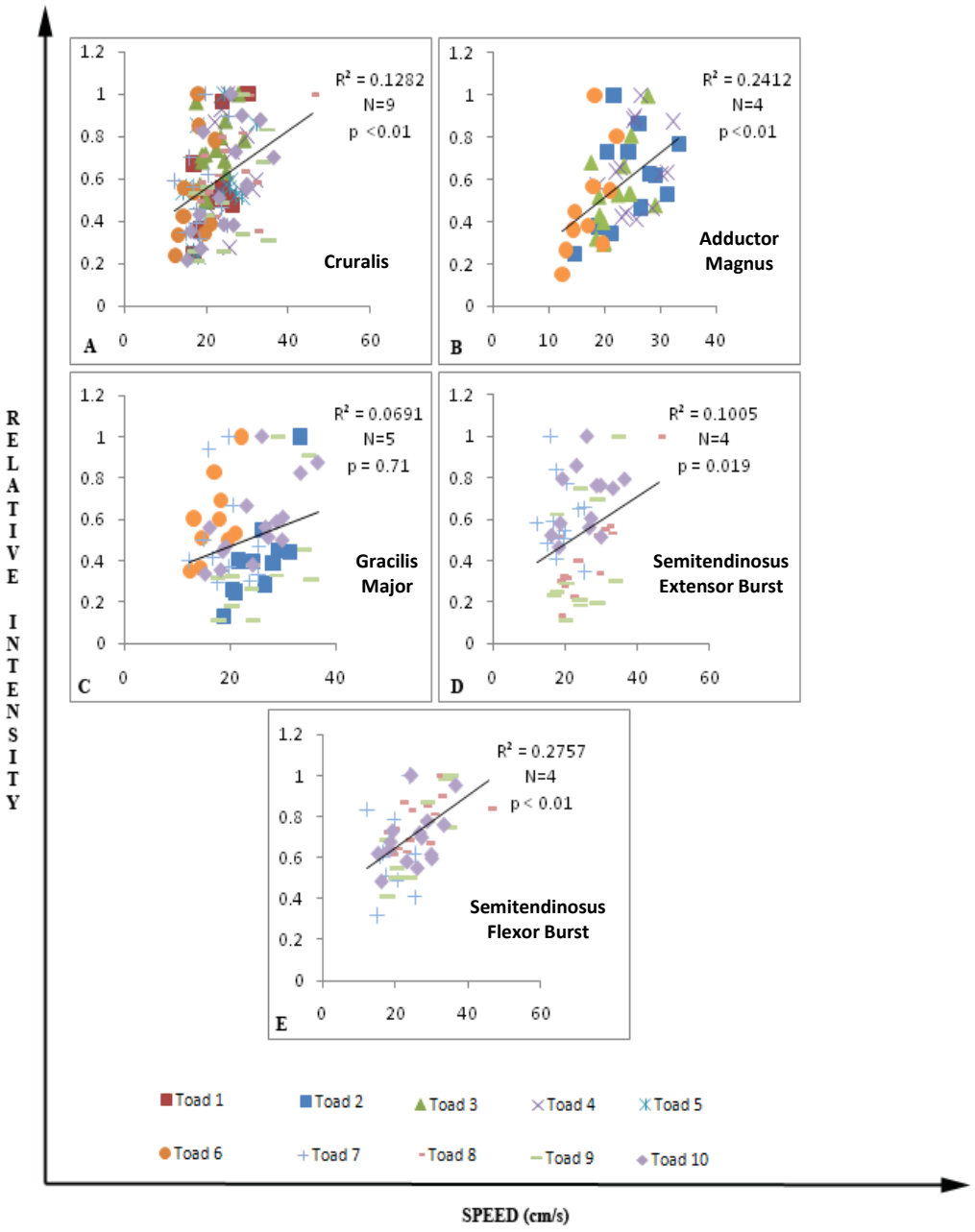

Figure 19: The effect of speed on relative muscle recruitment intensity. (A) cruralis, (B) adductor magnus, (C) gracilis major, (D) semitendinosus extensor burst, and (E) semitendinosus flexor burst. Excluding the gracilis, all muscles showed an overall significant positive relationship with increasing speed. However, a significant positive relationship wasn't observed when toads were analyzed on an individual basis, perhaps indicating that sample size within individuals was too small.

Table 6: R^2, p and slope values for relationship between relative intensity and speed for individual toads. Only 6 toads showed significant relationships between these two variables. '-' indicates that data for that muscle were not obtained in that toad. '*' indicates a significant relationship.

Toad	Cruralis			Adductor Magnus			Gracilis Major			Semitendinosus Extensor Burst			Semitendinosus Flexor Burst		
	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope
1	0.327	-0.934	0.032	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	0.146	0.214	0.016	0.596	0.005*	0.037	-	-	-	-	-	-
3	0.152	0.168	0.016	0.281	0.051	0.029	-	-	-	-	-	-	-	-	-
4	0.022	0.631	0.009	0.062	0.414	0.013	-	-	-	-	-	-	-	-	-
5	0.149	0.193	0.015	-	-		-	-	-	-	-	-	-	-	-
6	0.025	0.201	0.034	0.394	-0.948	0.048	0.354	0.070	0.036	-	-	-	-	-	-
7	0.025	0.603	0.009	-	-	-	0.018	0.666	0.008	0.032	-0.438	-0.008	0.001	-0.084	0.002
8	0.159	0.126	0.010	-	-	-	-	-	-	0.701	<0.0001*	0.023	0.375	<0.0001*	0.010
9	0.197	0.149	0.016	-	-	-	0.313	-0.941	0.025	0.177	0.173	0.020	0.691	0.003*	0.025
10	0.335	0.024*	0.022	-	-	-	0.406	0.011*	0.019	0.112	0.251	0.009	0.209	-0.914	0.010
All Toads Used	0.128	<0.0001*	0.014	0.241	<0.0001*	0.021	0.069	0.710	0.010	0.101	0.019*	0.012	0.276	<0.0001*	0.013

Relationships between speed and relative muscle activity burst duration

Compiled graphs with all toads for relative muscle recruitment burst duration versus speed are shown in figure 20. None of the muscles showed a significant relationship with speed. When toads were analyzed on an individual basis, a single muscle, the cruralis, within a single animal, showed a significant negative relationship between speed and muscle activity burst duration (Table 7).

Relationships between muscle recruitment intensities

The relative intensity of one extensor muscle plotted against another extensor muscle is shown in figure 21. All extensors except the adductor and the gracilis major, for which the sample size was small, showed significant positive relationships. When the toads were analyzed on an individual basis, nine out of fifteen showed significant positive extensor/extensor relationships (Table 8). The relative intensity of flexor muscles plotted against the relative intensity of extensor muscles is shown in figure 22. No significant flexor/extensor relationships were observed. Data for individual toads are shown in table 8.

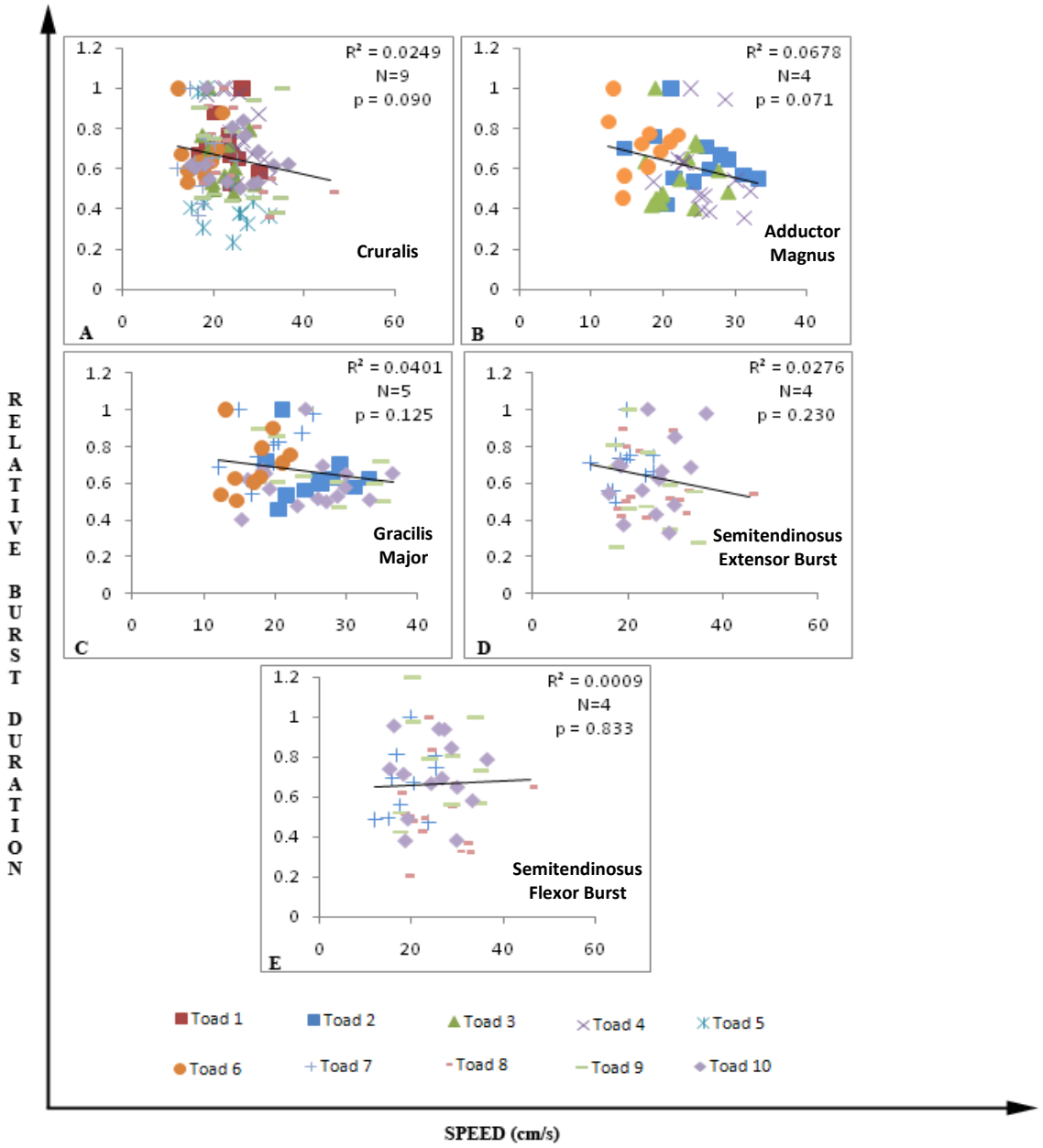

Figure 20: The effect of speed on relative muscle burst duration. (A) cruralis, (B) adductor magnus, (C) gracilis major, (D) semitendinosus extensor burst, and (E) semitendinosis flexor burst. No significant relationships were observed.

Table 7: R^2, p and slope values for relationship between relative burst duration and speed for individual toads. Only toad 4 showed a significant relationship between these two variables in the cruralis. '-' indicates that data for that muscle was not obtained in that toad. '*' indicates statistically significant relationships.

Toad	Cruralis			Adductor Magnus			Gracilis Major			Semitendinosus Extensor Burst			Semitendinosus Flexor Burst		
	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope
1	0.001	-0.074	0.001	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	0.082	0.368	-0.008	0.002	-0.334	-0.004	-	-	-	-	-	-
3	0.062	-0.610	-0.010	0.000	0.983	<0.001	-	-	-	-	-	-	-	-	-
4	0.347	0.034*	-0.025	0.046	0.480	-0.011	-	-	-	-	-	-	-	-	-
5	0.189	0.138	-0.018	-	-	-	-	-	-	-	-	-	-	-	-
6	0.001	0.953	-0.001	0.007	-0.184	-0.004	0.059	0.500	0.011	-	-	-	-	-	-
7	0.051	0.458	0.010	-	-	-	0.051	0.459	-0.008	0.016	0.677	-0.006	0.114	0.341	0.013
8	0.202	0.081	-0.011	-	-	-	-	-	-	0.014	0.658	-0.003	0.001	0.896	-0.001
9	0.006	0.811	-0.003	-	-	-	0.197	0.172	-0.011	0.152	0.235	-0.014	0.001	0.926	0.001
10	0.017	0.649	-0.003	-	-	-	0.003	0.854	0.001	0.088	0.305	0.010	0.001	0.927	<0.001
All Used Toads	0.025	0.090	-0.005	0.068	-0.929	-0.009	0.040	0.125	-0.005	0.028	0.230	-0.005	0.001	0.833	0.001

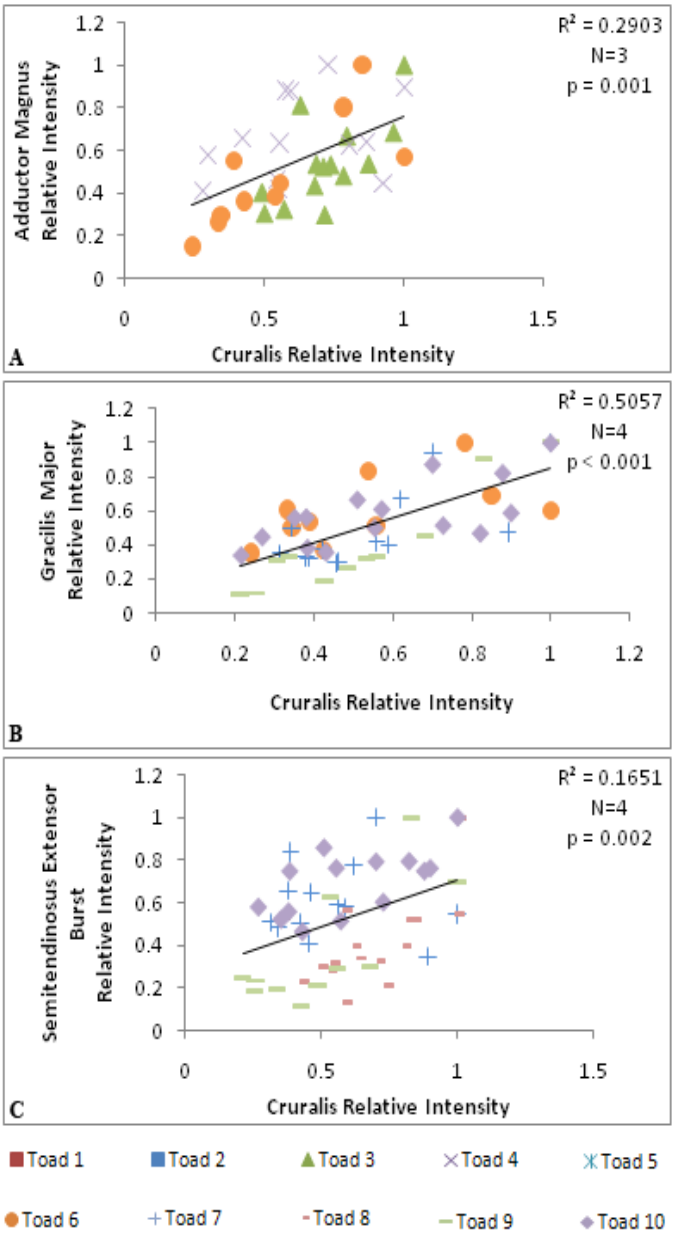

Figure 21A: Relative intensity of cruralis on the x-axis versus the relative intensity of the extensors (A) adductor magnus, (B) gracilis major, (C) semitendinosus extensor burst on the y-axis. All muscles showed significant positive relationships.

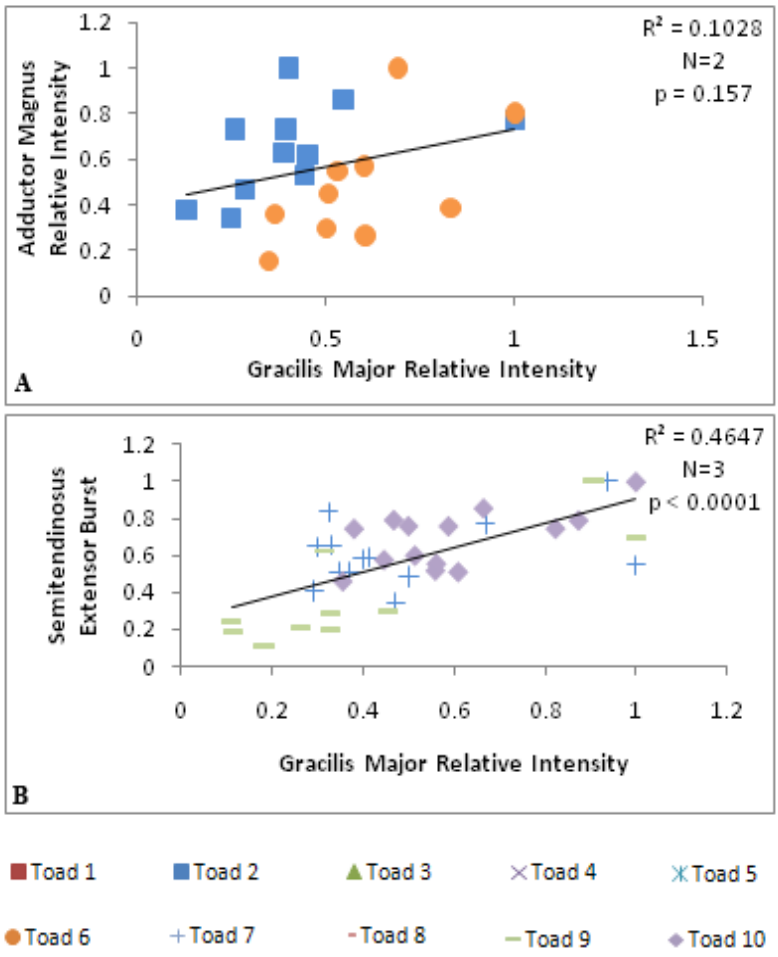

Figure 21B: Relative intensity of gracilis major on the x axis versus the relative intensity of the extensors (A) adductor magnus, (B) semitendinosus extensor burst on the y-axis. The gracilis major and semitendinosus showed a significant positive relationship while the adductor magnus and gracilis major did not, although the trend was in the same direction.

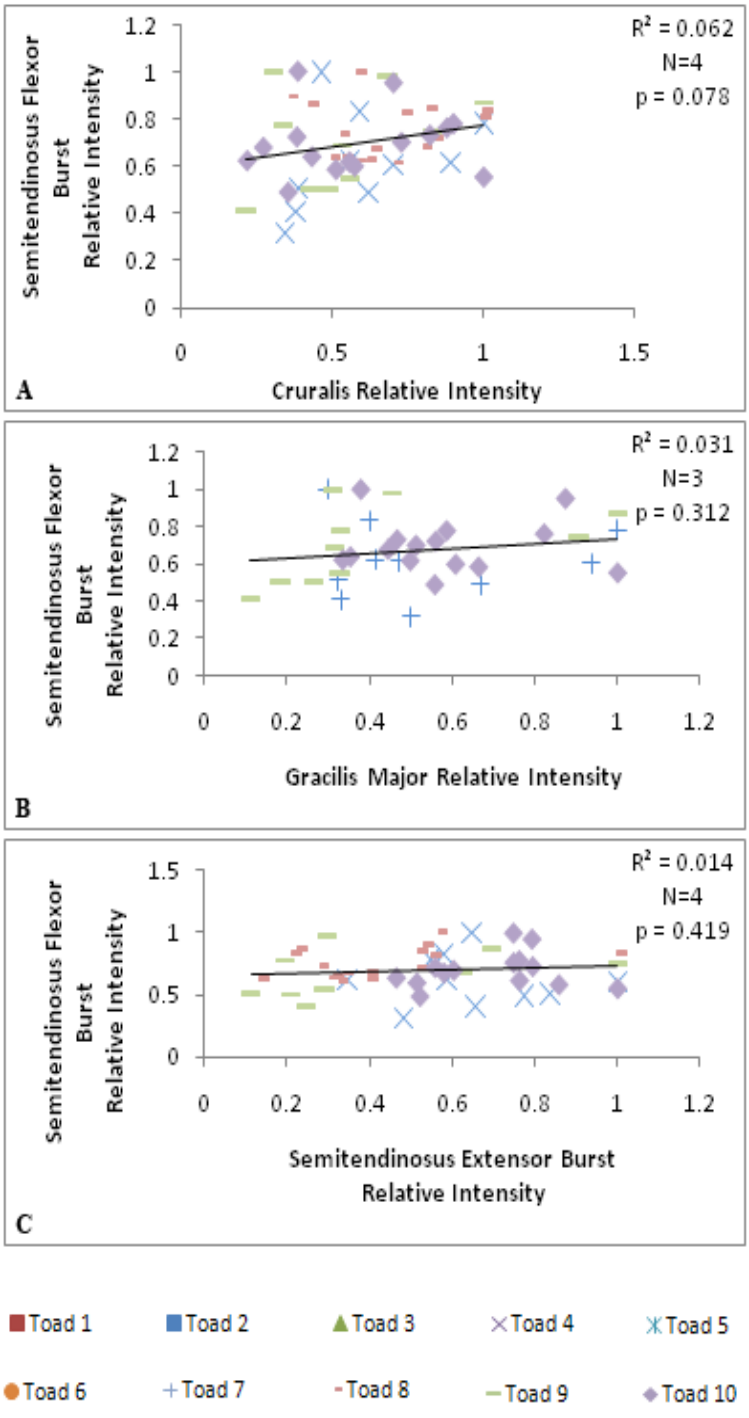

Figure 22: Relative intensity of semitendinosus flexor burst on the y axis versus the relative intensity of the extensors (A) cruralis, (B) gracilis major, and (C) semitendinosus extensor burst on the x-axis. No muscles showed significant relationships.

Table 8: R^2 and p values for muscle recruitment intensity relationships for each individual toad. Excluding the gracilis major versus adductor magnus relationships all extensor versus extensor relationships were significant positive relationships Nine out of fifteen individual toads showed significant positive extensor versus extensor relationships. None of the overall and individual toad extensor versus flexor relationships were significant. '*' indicates significant relationships.

	Extensor vs Extensor										Extensor vs Flexor					
Toad	Cruralis vs Adductor Magnus		Cruralis vs Gracilis Major		Cruralis vs Semitendinosus Extensor Burst		Gracilis vs Semitendinosus Extensor burst		Gracilis vs Adductor Magnus		Cruralis vs Semitendinosus Flexor Burst		Gracilis vs Semitendinosus Flexor Burst		Semitendinosus Extensor Burst vs Semitendinosus Flexor Burst	
	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p
2	-	-	-	-	-	-	-	-	0.244	0.123	-	-	-	-	-	-
3	0.446	0.009*	-	-	-	-	-	-	0.359	0.067	-	-	-	-	-	-
4	0.109	0.271	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0.620	0.007*	0.310	0.094	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	0.527	0.005*	0.001	0.913	0.146	0.020	-	-	0.142	0.283	0.001	0.948	0.005	0.845
8	-	-			0.320	0.020*	-	-	-	-	0.011	0.901	-	-	0.213	0.062
9	-	-	0.847	<0.0001*	0.569	0.007*	0.700	0.003*	-	-	0.139	0.290	0.242	0.149	0.176	0.261
10	-	-			0.420	0.012*	0.377	0.019*	-	-	0.015	0.660	0.000	0.928	0.032	0.542
All Toads Used	0.290	0.001*	0.506	<0.0001*	0.165	0.002*	0.465	<0.0001*	0.103	0.157	0.062	0.078	0.031	0.312	0.014	0.419

DISCUSSION

The main goal of this study was to analyze how swimming speed affects hindlimb movements and the underlying muscle recruitment patterns that drive those movements in the toad, *Bufo marinus*. Results indicate that the four major thigh muscles studied, which act in limb extension and flexion, alter recruitment intensity, rather than burst duration, to deal with the changing demands of increasing speed. This relationship between recruitment intensity and speed, however, isn't consistent, with some muscles in some animals showing clearer relationships than others. In addition, a strong positive relationship was observed in recruitment intensity between pairs of extensors, such that increasing levels of recruitment in a given limb extensor also meant concomitant increases in intensity in other extensors. No such relationship was seen between flexor bursts and extensor bursts, however, suggesting that changes in the effort of propulsion are not linked to similar changes in the effort of recovery.

Muscle recruitment patterns

All four muscles show similar activation patterns to those observed in previous studies of anuran swimming (Biewener and Gillis, 2000; Kargo and Rome, 2002; Gillis, 2007) (Fig. 11). The cruralis, adductor magnus, and gracilis major are active in the propulsive phase when the hindlimbs are extending, highlighting their role as hindlimb extensors. The semitendinosus shows a biphasic activation pattern. The first phase of its activity overlaps with

antagonistic activity of the hindlimb extensors. This first phase of activity is believed to be important in stabilization of the limb, which likely evolved for jumping, but is retained in swimming (Gillis, 2007). The co-activation strategy could also be important in controlling the degree of limb extension rather than limb stabilization during swimming (Gillis, 2007). The second burst of activity of the semitendinosus occurs when the limb is recoiling in the recovery phase. This burst does not begin at the start of the recovery phase indicating that the initiation of limb flexion is likely a passive process (Gillis, 2007).

Joint angles, duration of propulsive phase and total swim cycle duration

Maximum joint angles, and hence the degree to which the hindlimbs extend, do not change significantly with speed (Fig. 17, Table 4). However, minimum joint angles seem to decrease with speed at the hip, knee and ankle in several of the toads analyzed (Fig. 17, Table 5). This indicates that toads may control speed of locomotion by increasing their joint angle excursions, and they increase these excursions by flexing to a greater degree during recovery. As only three toads were analyzed, increasing the sample size may show if a consistent relationship is present or not. In a study by Nauwelaerts et al. (2001), however, no relationship was observed between joint angles and speed in the frog *Rana esculenta*.

Swim cycle durations across all toads showed a negative relationship with

speed (Fig. 18A). However, when toads were analyzed on an individual basis, only one out of nine animals showed a significant negative relationship between speed and swim cycle duration. Given the overall negative relationship, it is likely that one possible way for toads to increase speed is by swimming with shorter cycle durations, although this may not be true in all animals. Increasing the number of cycles studied within each animal might help to clarify the nature of this relationship.

No significant relationship was observed across all toads between propulsive phase duration (as a percentage of total cycle duration) and speed. When toads were analyzed on an individual level, two out of nine toads showed significant relationships. If toads keep the overall swim cycle duration constant, but decrease the duration of the propulsive phase, this would cause an increase in the power produced in the propulsive phase, enabling the toad to swim faster. However, as a relationship was not observed between propulsive phase duration and swimming speed, it is unlikely that this method is used by toads to alter speed. A similar observation was made by Nauwelaerts et al. (2001) in the frog *Rana esculenta*.

Relationship between speed, muscle recruitment intensity, and burst duration

Except for the gracilis major, all muscles (including the semitendinosus, a limb flexor) show a significant relationship between speed and muscle recruitment intensity (Fig. 19). However, when toads were analyzed on an

individual basis, only seven out of twenty-six relationships were significant (Table 6). Again, this might reflect low sample sizes (Table 3) within each animal, and by studying more swim cycles, a more consistent picture might emerge. During terrestrial locomotion, increases in limb extensor recruitment intensity tend to be more closely tied to increases in speed than increases in flexor intensity (Pierotti et al., 1988). Indeed, even in humans, increases in running speed are achieved largely by changes in the stance phase duration (when extensors are active) rather than the swing phase duration (when flexors are active) (Weyand et al., 2000). In contrast, in some swimming toads, even flexor muscle recruitment levels seemed to be linked to changes in speed, suggesting that animals not only push harder against the water during the propulsive phase, but also perhaps pull or flex their limbs harder during recovery at higher speeds.

There is no significant relationship between muscle burst duration and speed in any of the muscles (Fig. 20 and Table 7). The absence of such a relationship indicates that increased recruitment intensity is more strongly associated with increases in speed than changes in muscle burst duration. A similar observation was made in the plantaris muscle of the aquatic frog *Xenopus laevis* (Richards and Biewener, 2007). The plantaris muscle is an ankle extensor, and in this particular study the recruitment intensity of the plantaris muscle was strongly associated with force level of the muscle during contraction while duration of muscle activity was not. Plantaris force levels, in turn, were associated

with increased cycle power production, which is necessary for increased swimming speed (Richards and Biewener, 2007).

Relationship between muscles in recruitment intensity

Excluding the relationship between the relative intensity of adductor magnus and gracilis major, for which the sample size is small, all other extensor/extensor comparisons exhibit strong positive relationships with respect to their intensities (Fig. 21 and Table 8). Even when toads are analyzed on an individual basis, most relationships, despite their small sample sizes, are significant. This indicates that recruitment levels of one limb extensor are strongly related to those of other extensor muscles in the hindlimb. In short, when one extensor is recruited strongly, all the other extensors are also recruited strongly to help increase the force generated by the limb in the propulsive phase.

The recruitment intensity of the flexor burst of the only flexor muscle studied, however, is not related to the recruitment intensity of any of the extensors (Fig. 22 and Table 6). Thus, just because extensor muscles are recruited more strongly does not necessitate that flexor muscles at the same joint will also be more strongly recruited. This presence of positive relationships in recruitment intensity between extensors, but not between extensors and flexors has also been seen in the frog *Rana pipiens* during swimming (Noyes and Gillis, 2009).

The absence of a relationship between flexor and extensor recruitment intensities contradicts the significant positive relationship observed between speed

and flexor muscle recruitment. This inconsistency maybe a consequence of the cyclical locomotor pattern used by toads when swimming. A toad generates impulse by extending its hindlimbs in the propulsive phase, and this causes it to move forward. This forward movement is counteracted to a certain extent by drag forces. In the recovery phase, when the limbs are retracted, the impulse is negative and the animal decelerates. The variation of velocity in a single swim cycle in a toad is highlighted in figure 23. This varying velocity may be the reason why the relationship between speed and muscle recruitment is unclear. Namely, it's hard to characterize a swimming cycle with a single speed. Breaststroke is a swimming style in humans that uses cyclical hindlimb movement patterns similar to those used by toads during swimming. When compared to other styles of human swimming, the breaststroke is known to have the highest intra-cyclic velocity variation because the recovery phase of the limbs provokes strong resistance in the direction opposite to movement (Seifert et al., 2010).

Conclusion

The difficulty in characterizing speed in a single swim cycle together with small sample sizes for individual swim cycles makes the results of this study inconsistent. However, the results indicate multiple ways in which the toad *Bufo marinus* alters speed. Increasing joint angle excursions and decreasing cycle duration are two patterns that toads can use to increase speed. At the level of muscles, swimming toads seem to increase speed by altering the recruitment

intensity of both flexors and extensors. This causes toads to push against the water with a greater force, and retract their limbs at a greater speed. This technique of speed modulation is different from limbed terrestrial locomotion, where limb retraction speeds are not altered greatly with changes in speed.

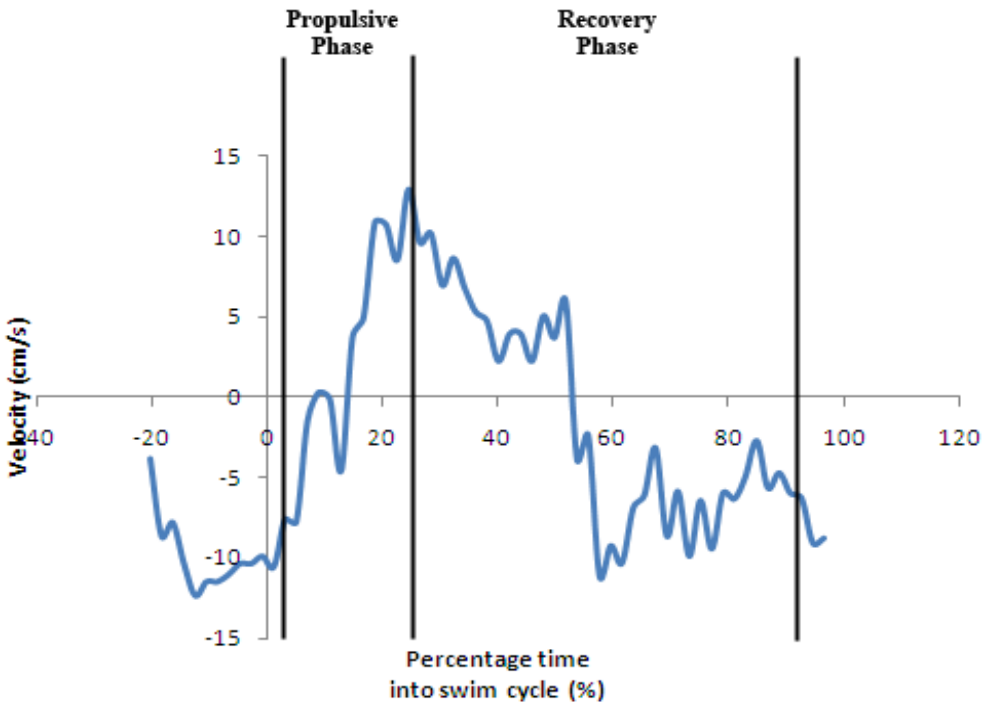

Figure 23: Velocity profile of a single swim cycle from toad 1. The graph shows that there is an overall increase in velocity during the propulsive phase and an overall decrease during the recovery phase. The changing velocity maybe the main reason why the relationship between speed and recruitment intensity is unclear.

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