

## INTRODUCTION

Behaviors are produced via motor circuits in the form of movement (Tytell *et al.*, 2011). It is movement that is the common thread between the observable behavior and the underlying phenomena that are producing that behavior. Studying the interaction between observable movements and the activation of muscles during those movements is one way to investigate the connection between the body and the brain. Investigating these interactions within and across species can provide insight into the development and evolution of behavior and their motor control. In this study, I examine the locomotor behavior of anurans (frogs and toads) and focus on the relationship between forelimb movement and the recruitment of the underlying musculature in tree frogs. Additionally, I examine how these animals prepare for landing during jumps to a vertical surface, a novel locomotor behavior among anurans present in only tree frogs.

### *Muscle Structure and Function*

Muscle is a contractile tissue. Muscle tissue originates as the mesodermal layer in embryonic germ cells. Three types of muscle develop: skeletal, smooth and cardiac. Skeletal muscle, which is responsible for locomotion, is affixed by tendons to bone (Alberts *et al.*, 2010). Making up 40% of the adult human, this “voluntary muscle” is striated and has the ability to contract and relax moving the elements to which it is attached (Loeb and Gans, 1986). Skeletal muscle is

arranged in a hierarchical structure (Figure 1). The muscle is encased by epimysium, a dense layer of connective tissue that provides a pathway for the blood and nerve supply and protects the muscle from friction with other structures (Loeb and Gans, 1986). Beneath the epimysium, individual muscle fibers are bound into bundles or fascicles by another layer of irregular connective tissue, the perimysium (Alberts *et al*, 2010). Muscle fibers or myocytes are composed of tubular, rod-like units called myofibrils, which are highly organized into regular bundles (Figure 3). These basic muscle units are made up of proteins such as actin and myosin (Figure 2) (Alberts *et al*, 2010). Sarcomeres are the functional units of a muscle, which are made up of these proteins organized into thin and thick filaments, which stretch the length of the myofibril (Figure 2). The interactions between these proteins or filaments are responsible for the generation of muscle force. Actin filaments and myosin filaments, or thin and thick filaments respectively, slide past one another shortening the muscle fiber (Loeb and Gans, 1986).

The nervous system's involvement in the control of muscular function begins at the level of these proteins. The interaction of actin and myosin filaments is triggered by signals from the nervous system, which travel along a motor neuron to the muscle. A motor neuron and all of the muscle fibers it innervates compose a motor unit. The connection between a motor neuron and a muscle fiber becomes a specialized synapse called the neuromuscular junction. The motor unit is activated when electrical impulses from the central nervous

system reach the muscle fibers (Figure 3). These electrical impulses are generated from the exchange of ions across the membrane of the axons that travels down the length of the axon (Loeb and Gans, 1986). The impulse reaches the neuromuscular junction, a specialized synapse connecting the motor neuron and the muscle fiber. Here, acetylcholine is released from the motor neuron and received in the postsynaptic receptors on the muscle fibers (Figure 4). This triggers a cascade of events in the muscle cell. First, action potentials propagate along the muscle cell membrane, which causes the release of calcium from an organelle within the cell called the sarcoplasmic reticulum (Loeb and Gans, 1986). The newly free calcium then binds to troponin, a cellular protein, which changes shape in response to the calcium binding. This causes the exposure of the myosin-binding site on actin filaments by removing the attached protein (Alberts *et al*, 2010). The newly available myosin-binding site allows these proteins to slide along one another causing contraction of the sarcomere. The shortening of the functional unit of the muscle fiber results in the contraction or shortening of the muscle. A muscle's ability to exert force is dependent on the quantity of motor units stimulated by motor neurons (Loeb and Gans, 2010). This project is focused on the neuromuscular control of locomotor behaviors in anurans (frogs and toads), specifically forelimb action that occurs before landing during a jump.

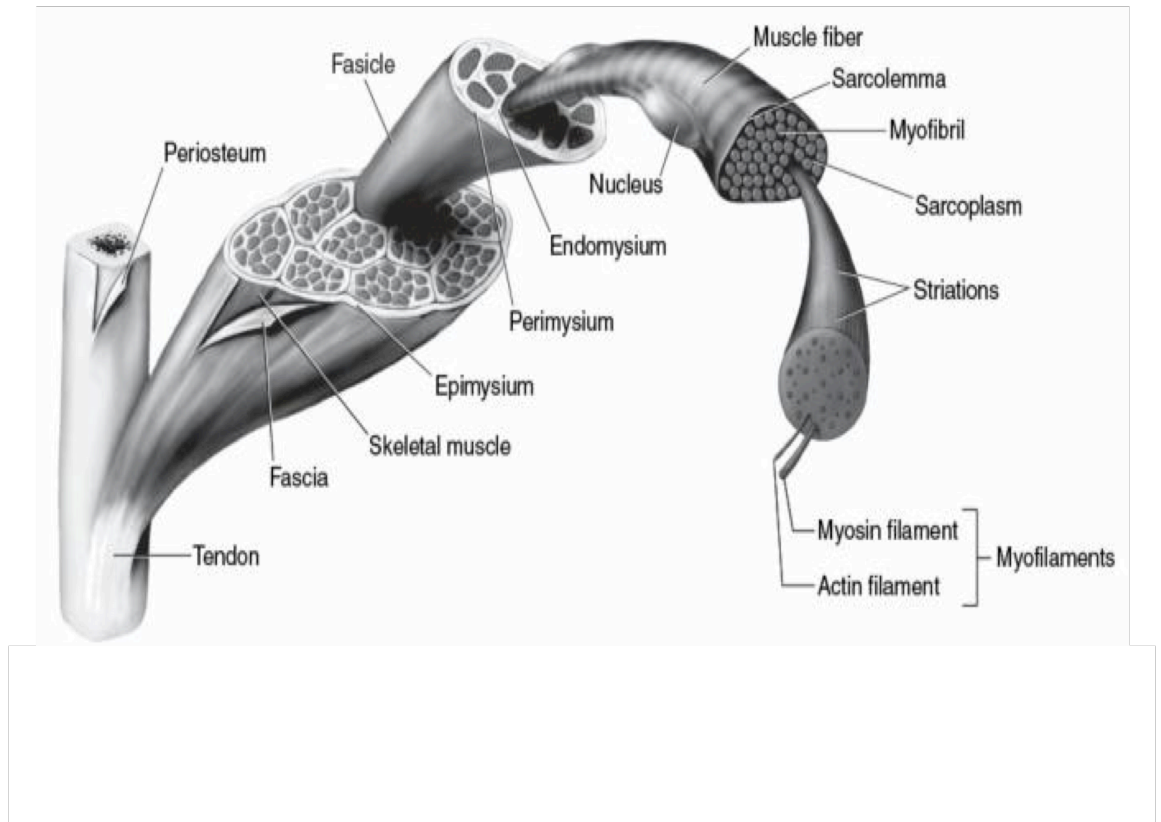


Figure 1. Structure and hierarchical organization of skeletal muscle. (Whiting and Rugg, 2005)

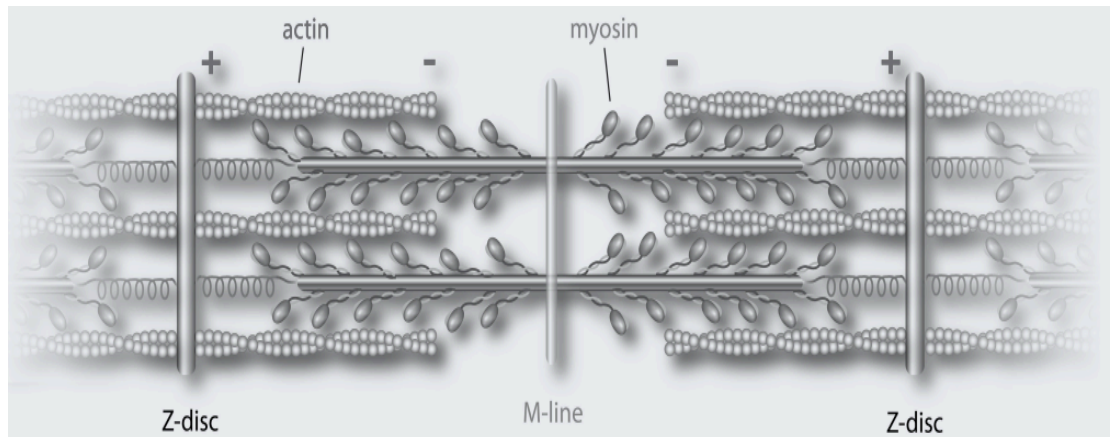


Figure 2. Myofilaments, actin and myosin, that form the sarcomeres. (uni-saarland.de)

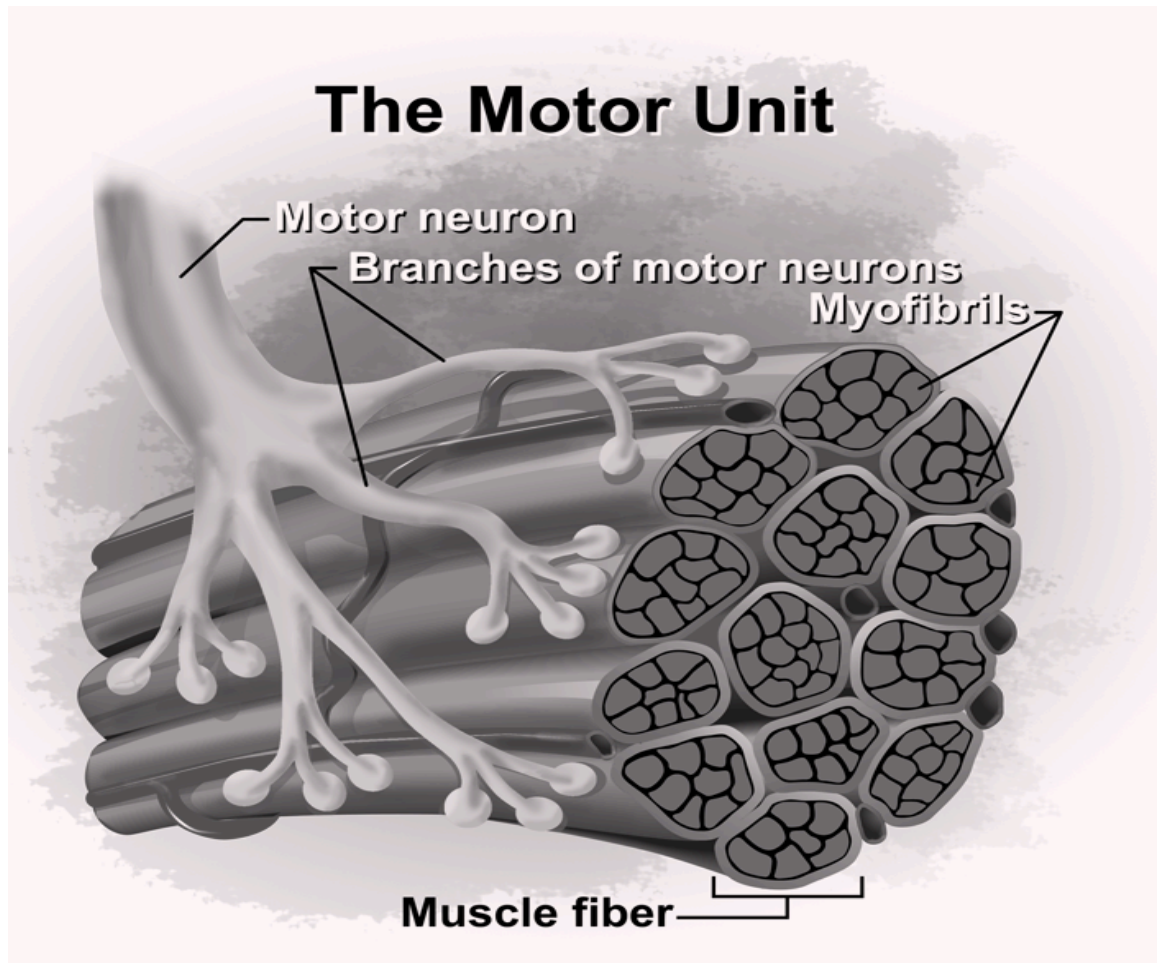


Figure 3. The composition of a motor unit, which coordinates muscle contraction.

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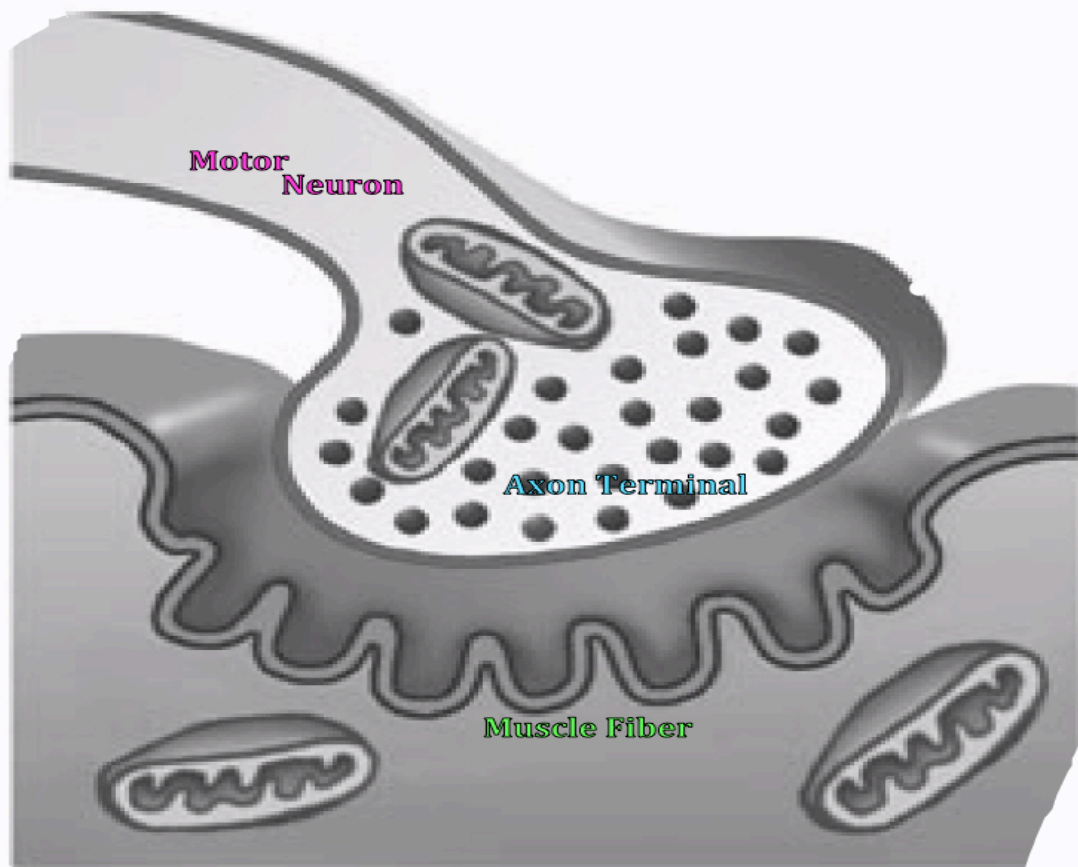


Figure 4. The neuromuscular junction where the motor neuron meets the muscle fiber and the release of acetylcholine triggers muscle activity.

([www.rci.rutgers.edu](http://www.rci.rutgers.edu))

### *Anuran Locomotion*

Many anurans' main locomotive behavior is jumping (or hopping, depending on distance covered). These organisms' ability for this saltatorial mode of locomotion is in part due to the morphology of their hindlimbs. When beginning a hop (the propulsion phase) the hind limbs exert force against the substrate pushing the animal into the air (the flight phase). Anatomically, the elongation of the hindlimbs and the allocation of substantially more muscle mass to the hindlimbs compared to the forelimbs are examples of adaptations that improve jumping ability. The forelimbs are responsible for absorbing the impact of the landing phase by dissipating the kinetic energy generated during the propulsive phase of the hop (Nauwelaerts and Aerts, 2005). In addition to muscular adaptations, skeletons of anurans also display forms indicative of the jumping locomotor mode. For example, tibia-fibula fusion in the hindlimb and radius-ulna fusion of the forelimb help to support the force of propulsion and resistance to impact respectively (Zug, 1972).

Studies performed on landing in humans and other mammals have shown that a common strategy involves limb muscle activation prior to impact. Early work in humans, for example, demonstrated that pre-landing activation of the muscles is important in the stabilization of the limb and that the muscle activation was anticipatory rather than a reflexive response to impact (Melvill and Watt, 1971). Other studies furthered these observations and identified tuning of the timing and intensity of pre-landing muscle activity. For example, greater step



heights led to greater recruitment of the muscle prior to a presumably greater impact. Further, the timing of the pre-landing activation was adjusted based on the perceived moment of impact; the greater the step height, the later the activation to account for a longer time to impact. (Melvill and Watt, 1971). Monkeys also adjusted the onset of preparatory muscle activity in the biceps and triceps with precision based on visual estimate of distance (Dyhre-Poulsen and Laursen, 1984). This phenomenon was also observed in cats, which activated extensor muscles in the limbs to contribute to the deceleration of the body upon impact (Prochazka *et al.*, 1971).

Frogs land using their forelimbs (Aerts and Nauwelaer, 2009). Recent published work from the Gillis lab (Gillis *et al.*, 2010; Akella and Gillis, 2011) emphasized the role of the forelimbs and their underlying muscles in the landing of cane toads (*Bufo marinus*) to slow and stabilize the body after impact and in transitioning between hops. Their electromyographic (EMG) data revealed that the muscles of the forelimbs are most active in the aerial phase and upon landing allowing them to infer that the forelimb muscles are activated for appropriate positioning of the arms in preparation for resisting impact. In addition, they also showed that in a number of muscles, pre-landing recruitment intensity was tuned to distance, such that muscles exhibited higher levels of pre-landing activity during a long hop than during a short hop (Gillis *et al.*, 2010; Akella and Gillis, 2011). This demonstrated that much like humans, toads prepare differently for landing depending on the magnitude of impact. This presumably allows the

animals to stabilize their limbs during landing across a range of jump distances to prevent limb collapse or injury (Gillis et al., 2010; Akella and Gillis, 2011). There is some evidence that bullfrogs also exhibit the ability to stabilize their forelimbs, specifically the elbow joint, in preparation for landing, similarly to the toads (Hicks *et al.*, 2012). The extent to which other anurans outside of the true toads, or bufonids, “prepare” for landing is unknown and will be tested in this thesis by exploring jumping and landing behavior in Cuban tree frogs.

### *Cuban Tree Frogs*

The Cuban tree frogs (*Osteopilus septentrionalis*) are the largest tree frogs found in North America ranging from 1.5 to 5 inches in length (Duellman and Treub, 1994). This non-native species was introduced to Southern Florida from the Caribbean and is now considered an invasive exotic species threatening Florida’s wildlife biodiversity ("Cuban Treefrog *Osteopilus septentrionalis* ", Florida Wildlife Extension). These extremely resilient arboreal animals are indiscriminate predators, willing to consume any frog, snake, or lizard small enough to eat including individuals of their own species.

Cuban Tree Frogs are also exceptionally capable jumpers and climbers. Studies such as one by Peplowski and Marsh (1997) concluded that the peak power that these frogs are able to exert over the course of a jump exceeds the amount of power the leg muscles are even capable of producing by sevenfold. Elasticity of the tendons is what allows for the massive power amplification in the system, as energy that is used to stretch the tendon, as limb muscles contract, can

be returned much more quickly as tendons recoil, much like slingshots, propelling the animal into the air. The propulsion of the tree frog from the ground via the swift extension of the hind limbs is a take-off behavior shared by most jumping anurans. Like Peplowski and Marsh's work, most investigations of the muscular basis of anuran locomotion have focused on the power of the hind limbs in take-off. For example, a variety of studies out of the Roberts Lab at Brown University have shown the importance of this elastic recoil mechanism in a variety of jumping frogs and toads (e.g., Roberts, Abbott, and Azizi. 2011). In contrast to the considerable research that has been done on hindlimb muscle function during take-off in anurans, very little attention has been paid to landing. Once these animals have used their robust hind limbs to propel themselves, how and with what mechanisms do they land?

Research has been done on the morphology and function of the limbs during climbing in tree frogs. Morphologically, arboreal anurans or tree frogs are characterized by their large head and eyes, slim pelvic region, relatively low body mass, and long limbs (Zug, 1972). There is speculation that their toe pads, smaller mass, and longer limb length are the adaptive traits associated with the development of arboreality (Reilly and Jorgensen, 2010). Reilly and Jorgensen (2010) suggested that pelvic morphologies may not be responsible for arboreal frogs' transition to climbing and hopping behavior; instead other morphological traits, like toe pads, may be more influential. These species of frogs use toe pads for suction on surfaces (Duellman and Trueb, 1994) and show a specific grasping

ability dubbed a “power grip”, in which they activate flexor muscles of the hand to grip the substrate to produce a stabilizing torque (Manzano *et al.*, 2008). However, the literature is sparse regarding the involvement of the forelimbs in landing in these arboreal species. The limited research in regard to the forelimbs of tree frogs has focused on manipulation of prey and gripping of narrow substrates in climbing behaviors. Toe pads are essential to the landing and climbing capabilities of tree frogs. The toe pad mechanism relies on the presence of fluid or a watery mucous between the pad and the substrate formed by glands within the pad (Smith *et al.*, 2006). A study by Smith *et al.* (2006) assessed the relationship between body size and adhesion over the life of arboreal frogs. Their results showed increased toe-pad surface area was not responsible for the scaling of adhesion through maturation, but rather suggested it is increased adhesion efficiency, which supports increased body mass (Smith *et al.*, 2006). These researchers also determined that adults had a lower adhesive ability than juveniles (showing an inverse relationship between adhesion and mass) implying a selective preference for hopping speed and distance rather than adhesion (Smith *et al.*, 2006). This might insinuate the greater importance of muscle development and control in the hind end of the frog responsible for take off than the front end involved with landing (Hanna and Barnes, 1991).

### *Experiments*

I chose to implant the deltoideus scapularis and the coracoradialis to analyze the muscle activation patterns during jumping and landing in the Cuban

tree frog. The deltoideus contracts to abduct the forelimb. The coracoradialis is responsible for flexing the elbow and moving the forearm forward. (Wingerd, 1988). These muscles appeared to be involved in many of the motions of both the mid-air and pre-landing behaviors of the tree frog.

In particular, I asked what, if any, differences exist between the roles of the forelimbs during landing on horizontal versus vertical surfaces? I hypothesized that when hopping horizontally, these frogs prepare for landing much like toads, perhaps even tuning pre-landing muscle activity to distance. Given that the positioning of the arms is completely different in preparation for a vertical landing, I predict the forelimb muscle activity patterns associated with vertical landings will be distinct from those of horizontal landings.

## MATERIALS AND METHODS

### *Animals*

Four Cuban tree frogs with a mean mass of 28.5 grams, ranging from 21.6 to 37.0 grams, were used in this study. After being obtained from a commercial supplier, the animals were housed in plastic containers in groups of two or three in a temperature-controlled room (24° C) with a 12 hour:12 hour light:dark schedule. All experiments were approved by the Mount Holyoke College Institutional Animal Care and Use Committee.

### *Myology*

The structure of the anuran forelimb is composed of the skeletal elements the humerus and the radioulna (Duellman, 1994; Wingerd, 1988). The humerus joins the thorax of the organism at the glenohumeral joint, a synovial or free-moving joint more commonly known as the shoulder (Duellman, 1994). The radioulna, a fusion of the radius and ulna, articulates with the humerus at a hinge joint also known as the elbow. These structural components are actuated by contracting muscles of the chest and forelimb via their tendon attachments (Wingerd, 1988). The joints of the elbow and shoulder are essential in the stabilization of the forelimb upon impact. The deltoideus scapularis muscle, which is integral in the movement of the shoulder joint and the coracoradialis muscle, which acts at the elbow are the two muscles considered in this study (Figure 5).

The coracoradialis is an elbow flexor and also acts as a humoral adductor, drawing the forelimb toward the ventral surface along the sagittal plane of the body (Duellman, 1994). Originating at the anterior aspect of the epicoracoid cartilage of the pectoral girdle, the m. coracoradialis inserts on to the proximal end of the radioulna at the elbow via a long tendon that runs the length of the humerus (Figure 5) (Wingerd, 1988). Previous work has shown that this muscle is essential in mid-hop arm positioning and stabilization of the forelimb during impact in cane toads (Akella & Gillis, 2011; Gillis et al, 2010).

The m. deltoideus scapularis is one of three heads of the deltoideus muscle in anurans. This specific head acts to rotate and extend the humerus laterally. It originates at the proximal scapulo-clavicular joint via an aponeurosis, which are layers of broad, flat tendons. It inserts on the distal tuberosity of the proximal lateral humerus (Figure 5) (Wingerd, 1988). Although little research has focused on the function of this muscle in hopping anurans, based on its use in grasping in tree frogs, it is likely important in arm positioning in preparation for landing (Manzano et al., 2008).

#### *Electrode Construction*

I constructed electrodes by twisting together two insulated silver wires approximately 1.5 meters in length and 0.1mm in diameter (California Fine Wire Company, CA, USA). Approximately one millimeter of insulation was removed at both ends of each of the two twisted wires. At one end, one wire was approximately one millimeter shorter than the other in order to prevent contact

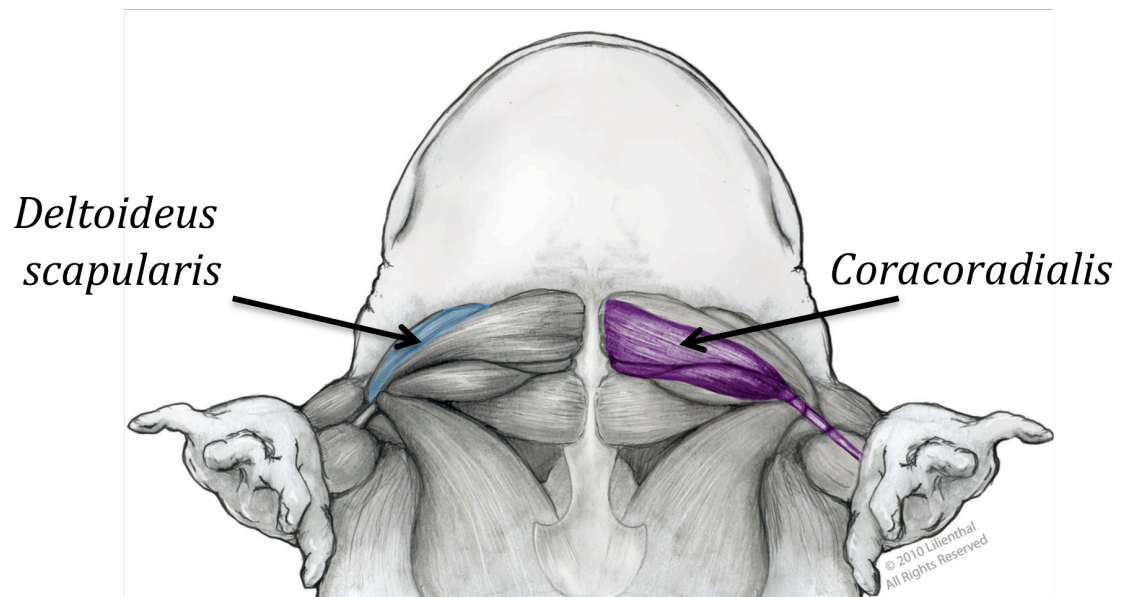


Figure 5. A ventral view of *Bufo marinus*, the cane toad, highlighting the coracoradialis muscle in purple and the deltoideus scapularis in blue. Image drawn by Anneliese Lilienthal.



between the bared tips; this end was implanted into a muscle of interest using a 26-gauge hypodermic needle (described in more detail in the *Surgical Procedure*). The bared tips at the opposing end were soldered into a 15-pin female connector. After being tested to ensure that the current flow through the two wires was separate and a proper connection was made between the wires and the connector, the connector was sealed with epoxy resin.

### *Electromyography*

When studying muscles and their activation and recruitment patterns, electromyography is a useful tool for experimentalists to quantify the timing and intensity of muscle activity *in vivo* (Loeb and Gans, 1986). In each selected muscle, I implanted a single electrode with two electrical contacts. When the experiment was about to begin, the female connector mentioned above was attached to a male connector, which would then transfer the signals to Grass P511 pre-amplifiers. The resultant recording represented the amplified muscle action potentials detected by the two contacts (Loeb and Gans, 1986). These signals were also filtered to eradicate noise above and below prescribed cutoff frequencies and at 60 Hz. Signals were digitized at 5000 Hz using an Axon Instruments Digidata 1322A 16-bit A/D converter and each trace was saved on a personal computer.

### *Surgical Procedure*

The frogs were anesthetized by immersion in tricane methanosulfate (MS-222, 0.7g/L) and baking soda (1g/L), for approximately 30 minutes. Once the

animals appeared unresponsive, a T-shaped incision was made across the ventral surface of the chest. The incision extended approximately 3 cm along the sternum to expose the coracoradialis muscles and 3cm perpendicularly along the clavicles to each side of the central incision to expose the deltoid muscles. One bipolar electrode was implanted into these muscles bilaterally in each animal using a 26-gauge hypodermic needle. The electrode was threaded into the needle to create a small hook at the end of electrode, which increased the likelihood of it staying in the muscle once it was implanted. The electrode was held in place with forceps as the needle was removed. The electrode was secured with 6.0 silk thread suture at the point of entry into the muscle. After all of the implantations were complete, the skin incision was sutured closed also using 6.0 silk-thread leaving a small gap at the ends of the incision near the shoulders for the electrodes to exit. The electrodes were also sutured to the skin on the dorsal surface of the animal to prevent superfluous movement. The electrodes were then combined into a single cable from the back of the frog to the connector using rubber cement. For additional stability, the electrodes were secured to the skin using cyanoacrylate gel. After the cyanoacrylate dried, the animals were rinsed with fresh water and allowed to recover for 1.5 to 2 hours before the jumping trials commenced.

#### *Jumping Trials and data collection*

After recovering from the anesthesia, the frogs were placed in a large 183 by 65 by 62 cm glass tank resting on its side with the open side laterally. A small piece of glass was secured to part of the open side to help prevent escape. The

tank was lit from above by one 600-watt light bulb. The animals were then stimulated using a variety of sounds or a gentle poke with a wooden rod to encourage jumping. The animals were allowed to hop to either a vertical or horizontal surface, but a slightly more subtle stimulus was used to elicit a horizontal hop. Two Hi-Spec high-speed video cameras were used to capture video of the animals jumping from lateral and superior perspectives at 500 frames per second and a resolution of 1280 X 1024 pixels. The electromyographical recordings were digitized and analyzed using a second computer and Axoscope software. After approximately 18 to 25 hops, the animals were euthanized via extended immersion in MS-222 (0.7g/L). Dissections of the animals were performed the next day to confirm accurate placement of the electrodes.

#### *Data Analysis*

High-speed video was used to determine seven key events of each hop: (1) the onset of animal movement, (2) forelimb liftoff, (3) hindlimb liftoff, (4) initiation of aerial forelimb movement, (5) movement of the forelimbs forward, beyond perpendicular to the ground, accompanied by the rotation of the wrist, (6) impact or forelimb touchdown, and (7) hind-limb recovery (Figure 6). The size of the hop was determined by aerial time, the time between hindlimb liftoff and impact or forelimb touchdown. After determining the timing of these kinematic events in the high-speed video, they were mapped onto EMG readings. Each EMG recording was then analyzed using a binning analysis to determine the quality of each individual recording and establish the trend of activity between

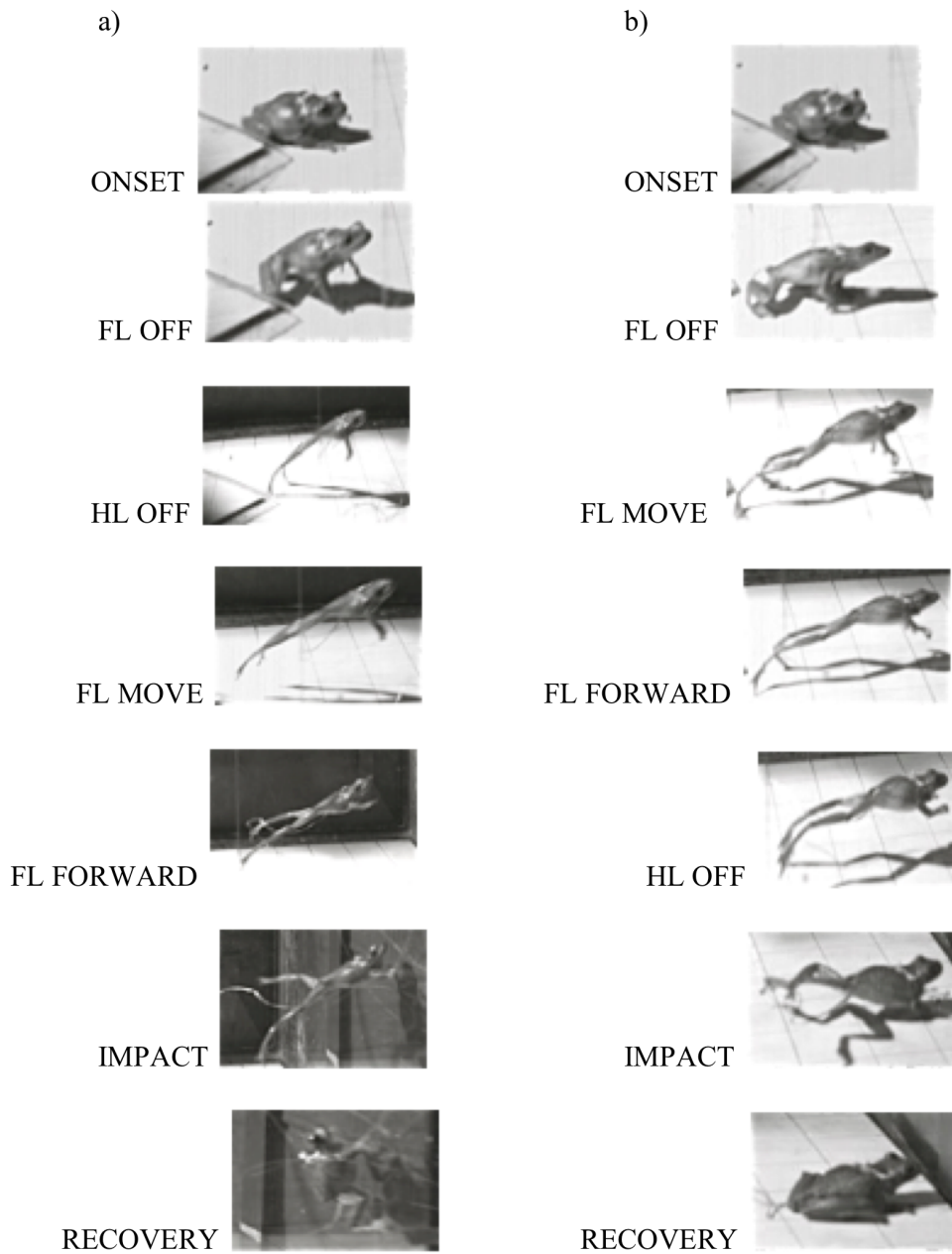


Figure 6. Images of the various kinematic phases of a) a vertical hop and b) a horizontal hop obtained from the high-speed video recording. ONSET = the onset of animal movement; FL OFF = forelimb liftoff; HL OFF = hindlimb liftoff; FL MOVE = initiation of aerial forelimb movement; FL FORWARD = movement of the forelimbs forward, beyond perpendicular to the ground, accompanied by the rotation of the wrist; IMPACT = forelimb touchdown; RECOVERY = hindlimb recovery.

onset and impact. Binning involved averaging the rectified signal amplitude into 100 equally spaced “bins” spanning the time between the onset of movement and forelimb impact. These bins were then plotted using a bar graph to create a generalized map of the muscle activity pattern from both muscles for each hop to identify the typical signal characteristics for a specific muscle. If any hops exhibited a highly irregular pattern compared to the other hops, that hop was eliminated.

The EMG signals were then analyzed with regard to the intensity of pre-landing muscle activation. To investigate pre-landing intensity, I determined the average amplitude of the EMG signal in the 50 milliseconds prior to impact (Figure 7). This was used to evaluate how intensely these animals recruited their muscles just prior to landing. The EMG signals were also used to assess the time at which these animals recruited their muscles in the course of the hop. To evaluate when the muscles were activated, I used the measurement of onset latency. Onset Latency is the time between a specific event in the hop and the beginning of a muscle burst. In my analysis, I defined onset latency as the time between forelimb liftoff and the onset of muscle recruitment (Figure 7). This allowed me to determine if the time at which these animals activated their muscles followed a specific pattern.

I also measured the duration of the aerial phase, the time in which the animal was suspended in the air. This was used as a measurement for hop

magnitude and can be used as the independent variable when comparing pre-landing intensity and onset latencies of the muscles.

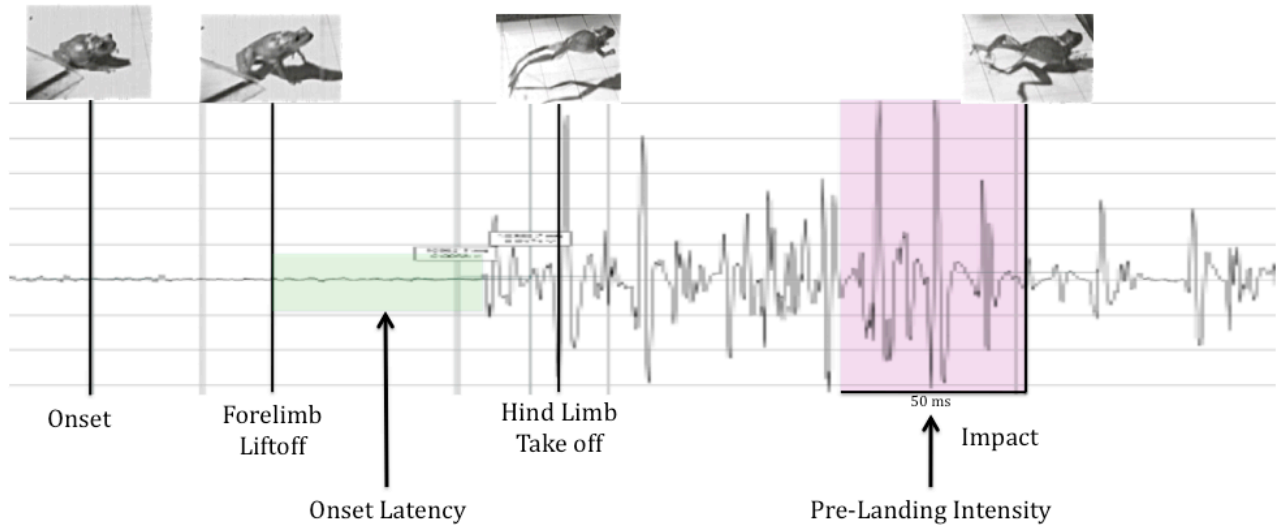


Figure 7. An EMG recording from the *coracoradialis* muscle detailing the variables obtained from each hop.

## RESULTS

Table 1 shows the animals, the number of hops, and hop durations that are described in this section.

Table 1. Overview of the data collected in this study.

<b>Frog</b>	<b>Mass (g)</b>	<b>Vertical Hops</b>	<b>Average Vertical Aerial Duration Mean <math>\pm</math> S.D. (ms)</b>	<b>Horizontal Hops</b>	<b>Average Horizontal Aerial Duration Mean <math>\pm</math> S.D. (ms)</b>
1	28.97	16	158 $\pm$ 32	3	73 $\pm$ 8
2	37.02	5	119 $\pm$ 29	12	92 $\pm$ 31
3	26.48	10	154 $\pm$ 43	10	99 $\pm$ 41
4	21.58	14	190 $\pm$ 45	4	81 $\pm$ 32

### *Kinematics*

When propelling themselves to vertical surfaces, tree frogs pushed themselves up and forward with their hind limbs. When their forelimbs first left the ground, they hung perpendicular to the launch surface (Figure 8). During the aerial phase they then flattened their forelimbs against the body before swinging them laterally and anteriorly to prepare for landing (Figure 8). At the point of contact with the glass surface, they did not appear to brace or stiffen their forelimbs for impact, so the ventral aspect of their bodies contacted the surface very shortly after the forelimbs. After landing on the vertical surface, frogs used sticky toe pads for adherence while they pulled their hindlimbs in behind them (Figure 8).



Although they propelled themselves into the air using their hindlimbs similarly in horizontal hops, their forelimb movement differed. When hopping horizontally, the frogs reached forward with their forelimbs almost immediately after forelimb liftoff (Figure 8). This positioned the frogs with their forelimbs extended forward and toward the ground over the entire course of the aerial phase of the hop (Figure 8). Upon impact, these animals appeared to use their forelimbs to press their bodies back, preventing the “belly flop” more typical of landing on a vertical surface. Animals also tended to pull their hindlimbs in under themselves prior to landing during horizontal hops, in contrast to the vertical hops, in which the hind limbs were left extended until well after impact (Figure 8).

Vertical and horizontal hops differed in a number of other characteristics as well. While propulsion phases were of similar duration,  $167.2 \pm 5.9$  ms (st. err.) versus  $173.0 \pm 7.9$  ms, respectively, aerial phase durations were much longer for vertical hops than horizontal hops:  $162.7 \pm 6.5$  ms versus  $90.8 \pm 6.0$  ms, respectively. The landing phases were extremely short in both types of jump, typically less than 25 milliseconds.

#### *Binning Analysis*

Consistently, the *m. deltoideus scapularis* exhibited a short burst coinciding with liftoff of the forelimbs during vertical hopping (Figures 9a and 10a). This initial burst had an average duration of 60.3 milliseconds across all four animals. Horizontal hops did not consistently exhibit this preliminary burst associated with take off (Figures 9b and 10b). Both the vertical and horizontal

hops exhibited a gradually growing burst of activity that began during the aerial phase and continued until impact (Figures 9 and 10). The initiation of this second burst coincided with the movement of the forelimbs forward, although in vertical hops, it tended to occur during the aerial phase, while in the horizontal hops, this gradual burst tended to begin shortly after the onset of movement (Figures 9 and 10).

For the coracoradialis, a ramping burst of activity beginning in the middle of the aerial phase, coincided with the forelimbs reaching forward, and continued to increase in intensity until impact in vertical hops (Figures 11a and 12a). A similar burst occurred during horizontal hops, but occurred earlier, shortly after the arms were lifted off of the ground (Figures 11b and 12b). Again, the initiation of the muscle activity occurred near the start of forward arm movement in both vertical and horizontal hops (Figures 11 and 12).

#### *Amplitude Analysis*

Relationships between pre-landing intensity and aerial duration were varied between vertical and horizontal hops in both muscles across all 4 animals. For vertical hops, significant positive relationships were seen in deltoideus scapularis in one animal and in the coracoradialis in one animal. For horizontal hops, relationships between pre-landing intensity and aerial duration produced consistent positive relationships across most animals. Significant positive relationships were seen in most animals for the deltoideus scapularis and

coracoradialis, although some of these individuals had very small numbers of horizontal hops (Figures 13 and 14).

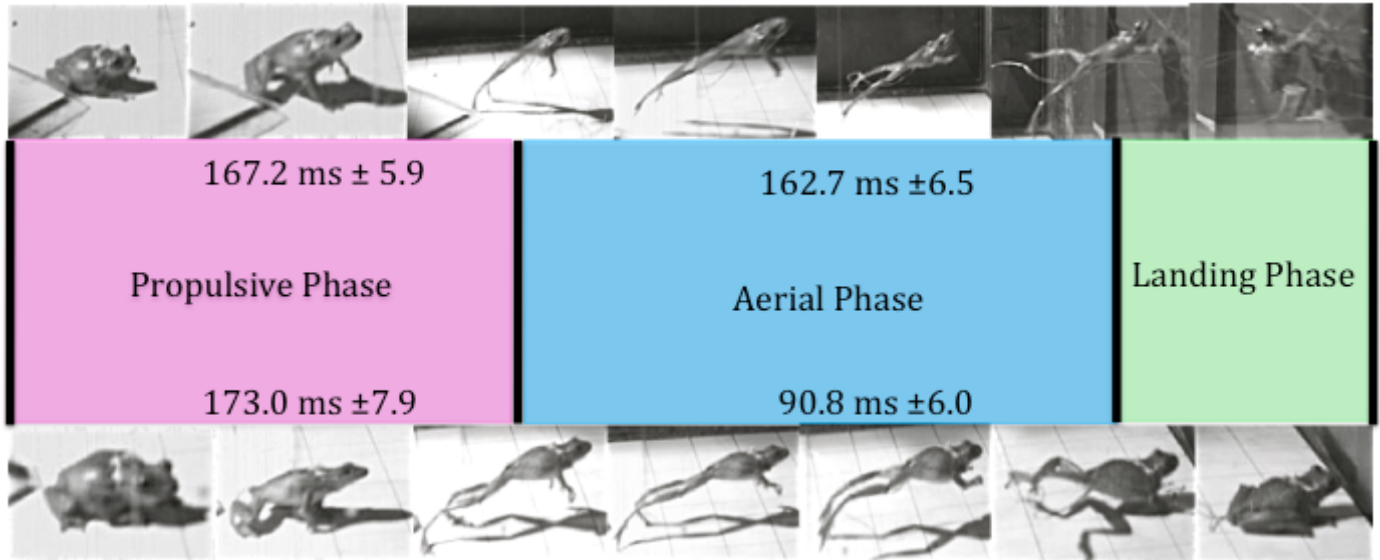
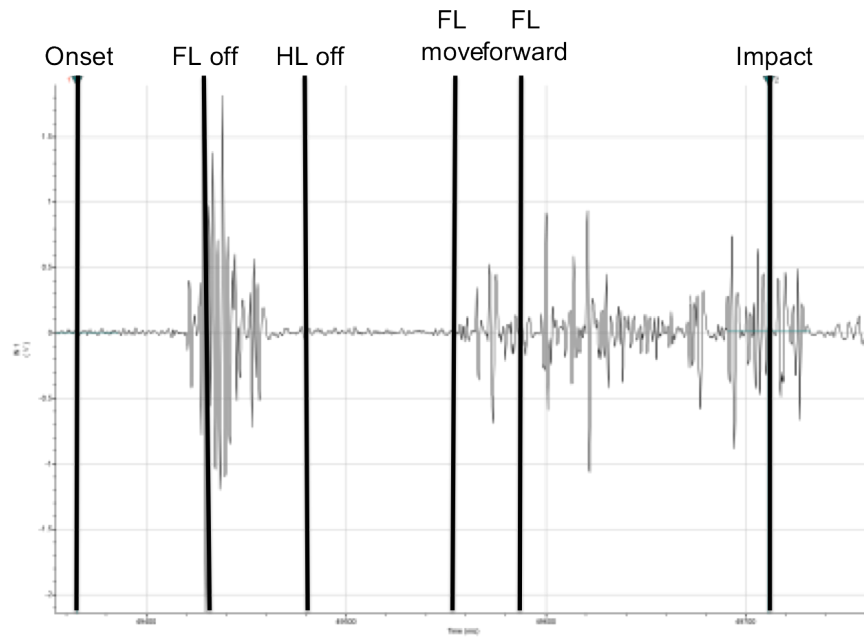


Figure 8. The phases of the jump labeled with the average durations identified for each type of hop in this study.

### *Onset Latency Analysis*

Vertical hops exhibited a variety of relationships between aerial duration and onset latency. Positive significant relationships were seen in two animals for the deltoideus scapularis and in one animal for the coracoradialis, but all other implantations showed no such relationship. In regard to horizontal hops, consistent significant positive relationships between aerial duration and onset latency were observed in most animals in both muscles (Figures 15 and 16).

a)



b)

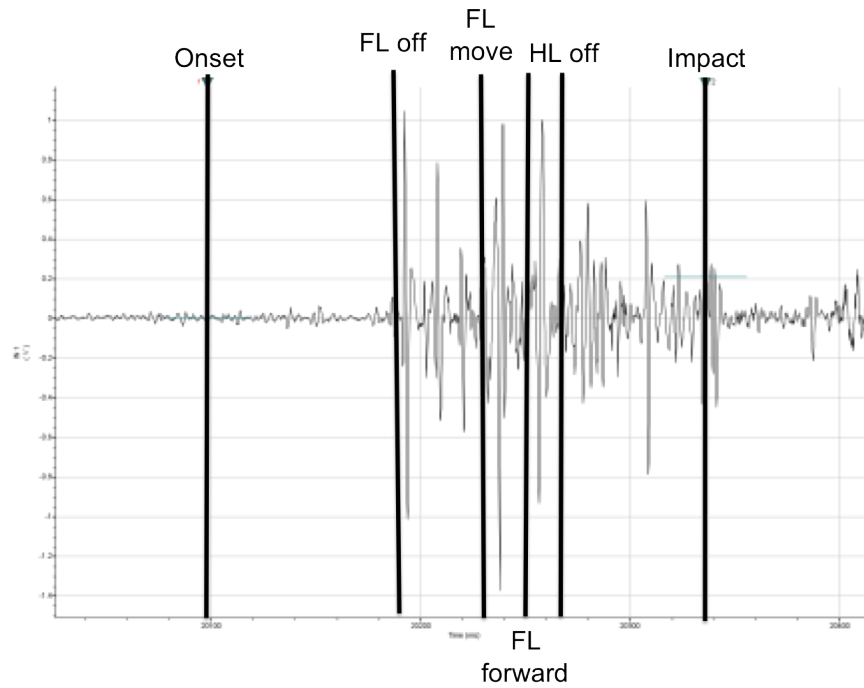
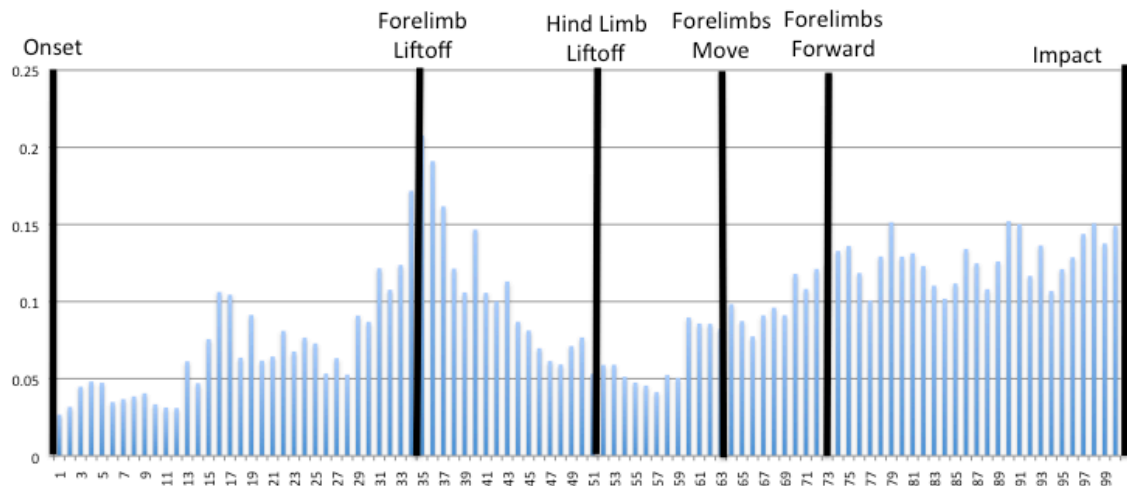


Figure 9. a) A representative EMG signal of a deltoideus scapularis during a vertical hop with kinematic event timing plotted. b) A representative EMG signal of a deltoideus scapularis during a horizontal hop with kinematic event timing plotted.

a)



b)

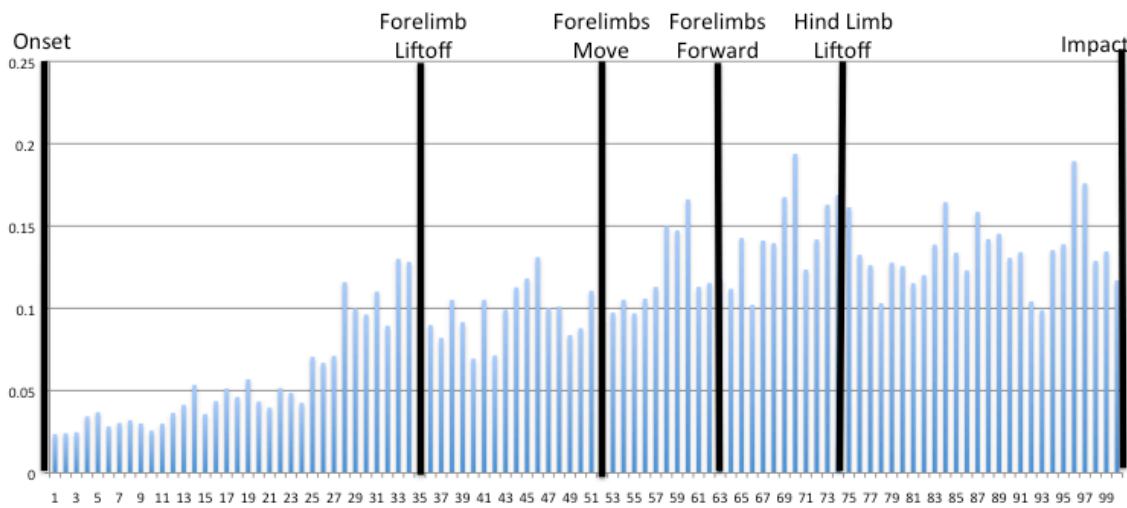


Figure 10. a) A cumulative binning figure for all four animals for vertical hops for the deltoideus scapularis with the kinematic event timing plotted. b) A

cumulative binning figure for all four animals for horizontal hops for the deltoideus scapularis with the kinematic event timing plotted.

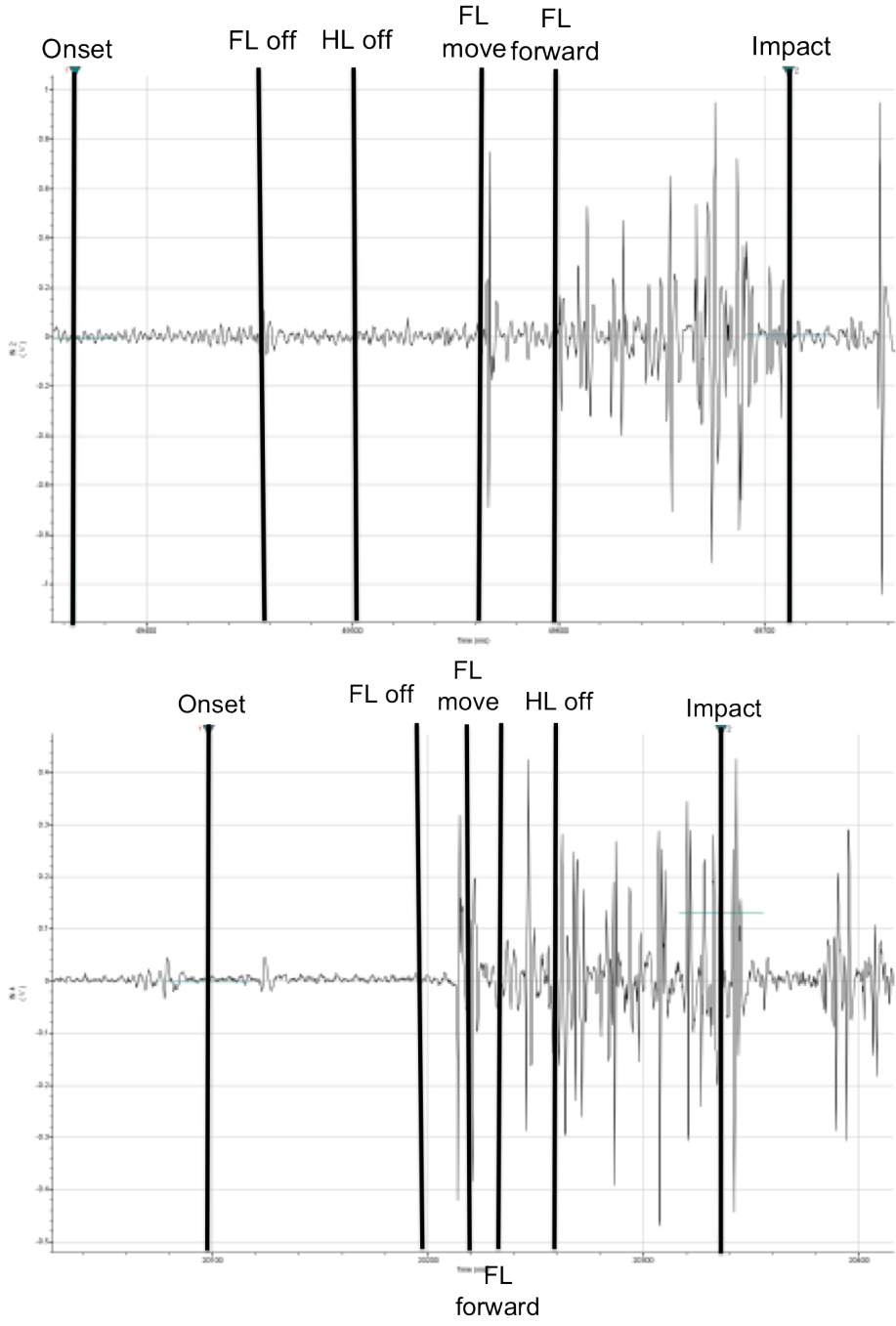
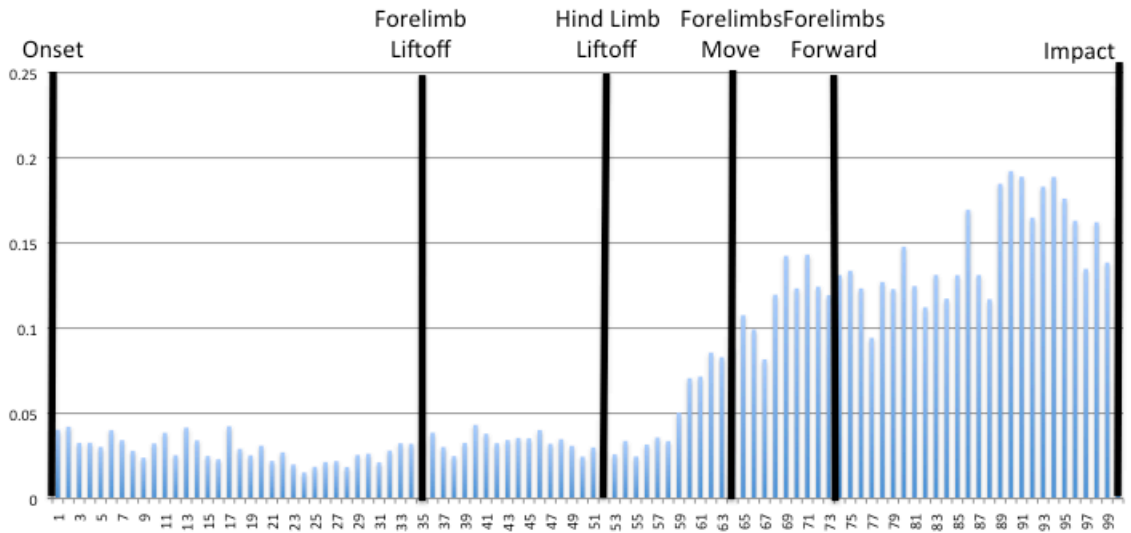


Figure 11. a) A representative EMG signal of a coracoradialis during a vertical hop with kinematic event timing plotted. b) A representative EMG signal of a coracoradialis during a horizontal hop with kinematic event timing plotted.

a)



b)

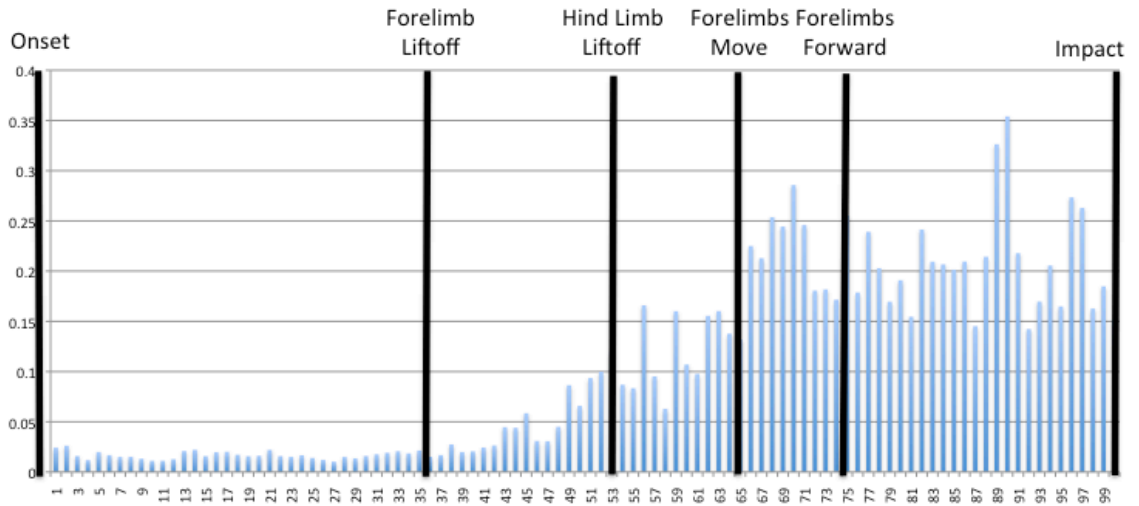


Figure 12. a) A cumulative binning figure for all four animals for vertical hops for the coracoradialis with the kinematic event timing plotted. b) A cumulative



binning figure for all four animals for horizontal hops for the coracoradialis with the kinematic event timing plotted.

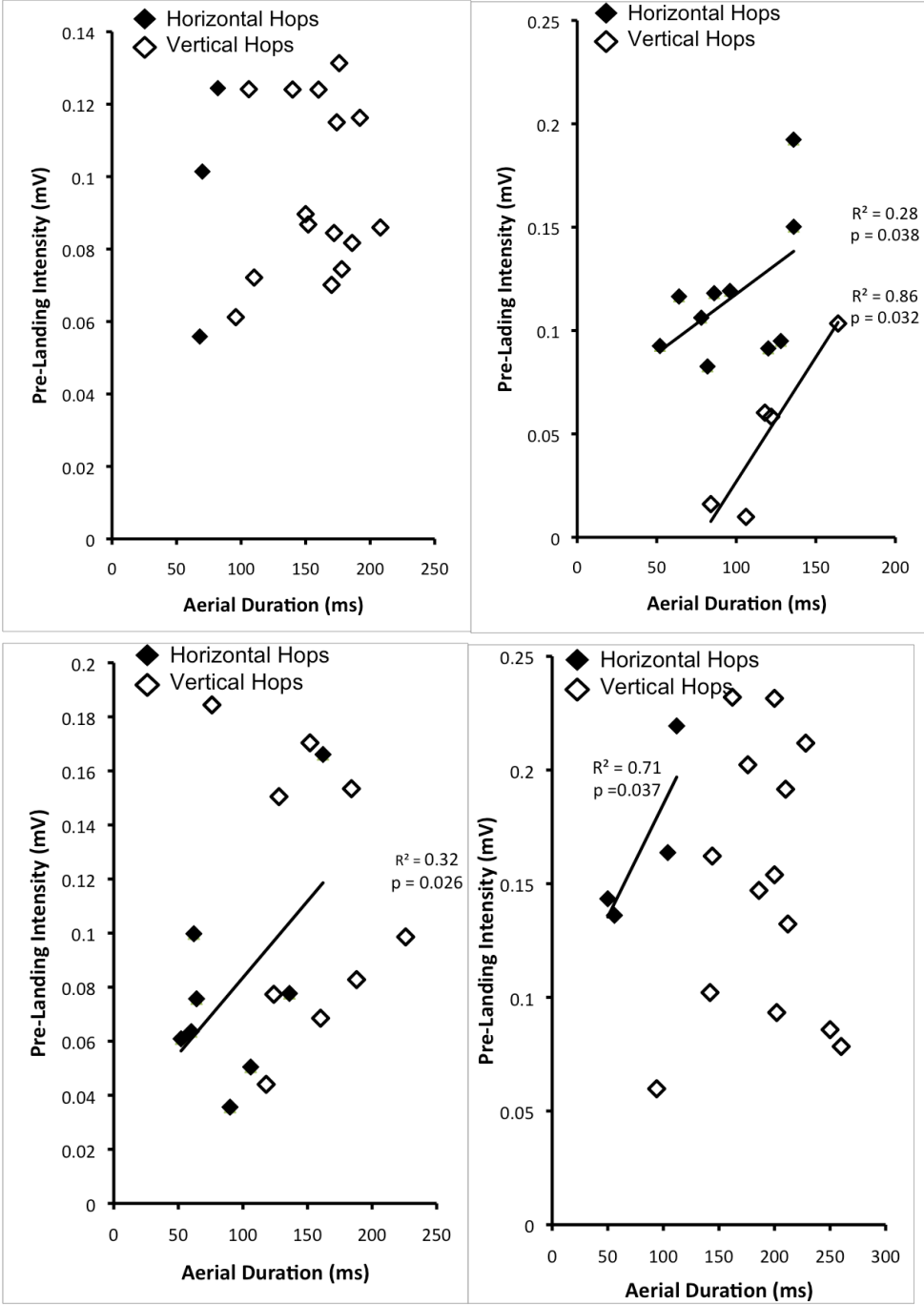


Figure 13. Scatter plots of aerial duration versus pre-landing intensity for each of the four individuals implanted exhibiting horizontal hops in black and vertical hops in white for

the deltoideus scapularis. Trend lines are present for those relationships that were significant.

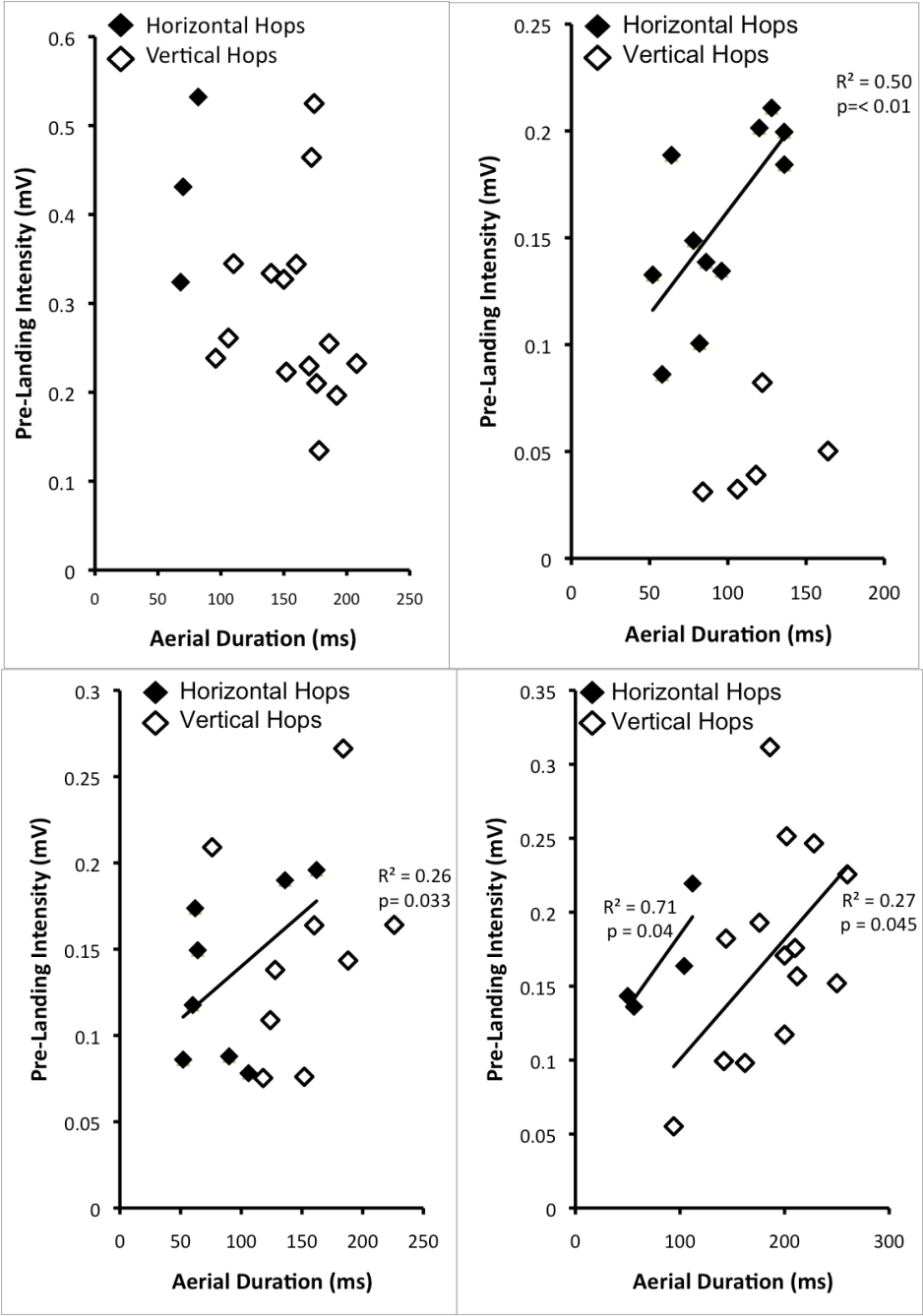


Figure 14. Scatter plots of aerial duration versus pre-landing intensity for each of the four individuals implanted exhibiting horizontal hops in black and vertical

hops in white for the coracoradialis. Trend lines are present for those relationships that were significant.

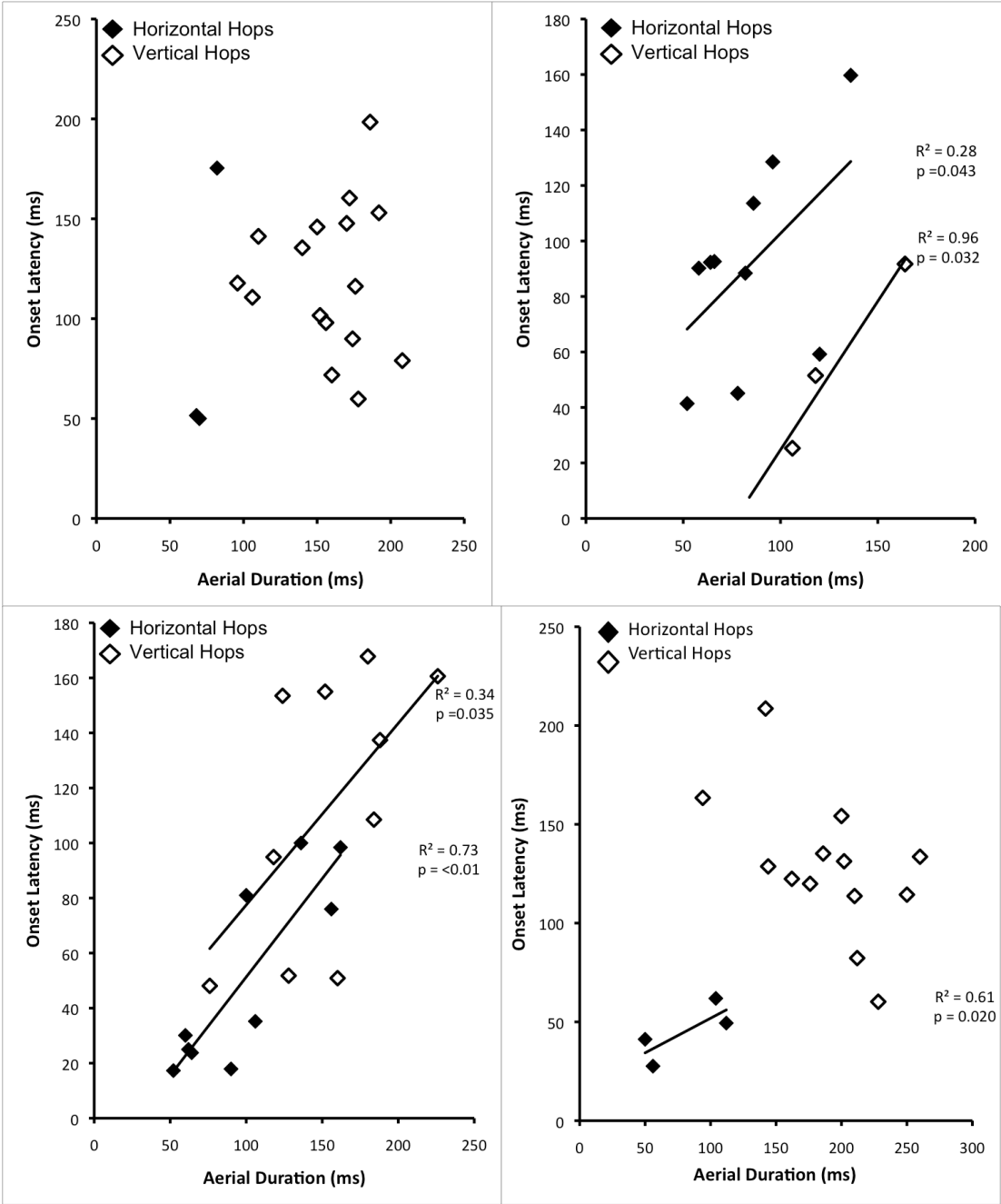


Figure 15. Scatter plots of aerial duration versus onset latency for each of four individuals implanted exhibiting horizontal hops in black and vertical hops in

white for the deltoideus scapularis. Trend lines are present for those relationships that were significant.

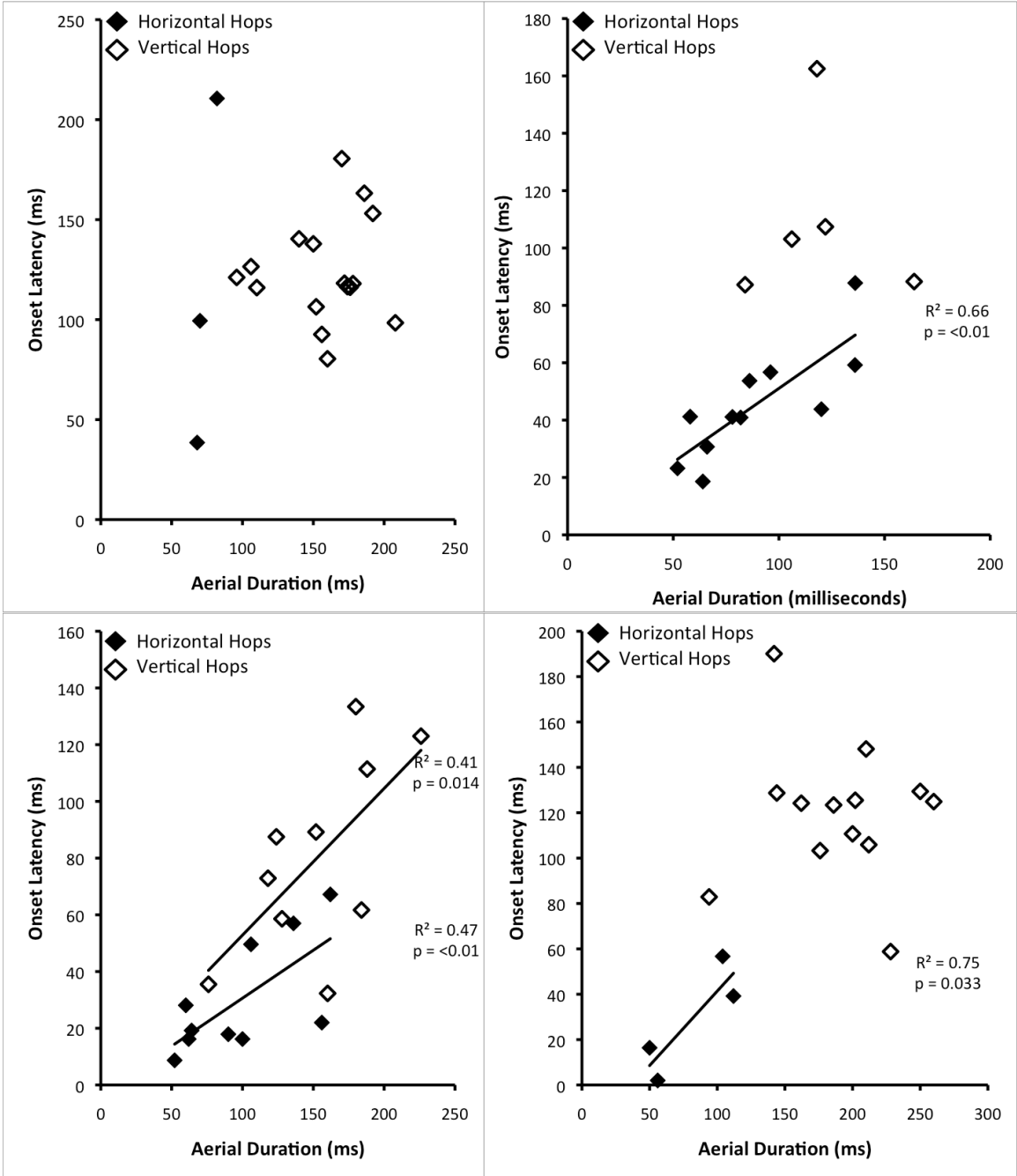


Figure 16. Scatter plots of aerial duration versus onset latency for each of four individuals implanted exhibiting horizontal hops in black and vertical hops in

white for the coracoradialis. Trend lines are present for those relationships that were significant.

## DISCUSSION

The goal of this study was to explore potential differences in forelimb muscle function in relation to landing during hopping onto horizontal versus vertical surfaces. I hypothesized that, when hopping horizontally, these animals would use their forelimbs to prepare for landing in a manner much like toads, which commonly hop on horizontal surfaces. In contrast, given the different arm positioning prior to landing, I expected the vertical hops of tree frogs to present with muscle activity patterns that were distinctive from those of the horizontal hops.

When hopping horizontally, the tree frogs did, in fact, demonstrate similar forelimb kinematics in preparation for landing and muscle recruitment patterns to those observed in the toad. Furthermore, when muscle variables were regressed against aerial phase duration, I found that the intensity and timing of pre-landing muscle recruitment were tuned when these tree frogs hopped horizontally. When vertically hopping, the tendency toward abduction of the forelimbs from the sagittal plane of the body and additional bursts of activity in congruence with additional arm motion were observed. Unlike during horizontal hops, the animals did not tune forelimb muscles based on hop distance to prepare for landing.

*Anuran Evolution*

The earliest amphibians possessed well-developed limbs but maintained many of the traits of sarcopterygian (lobe-finned) fishes. During the Devonian period, it is suspected that amphibians began to transition to more terrestrial environments due to the competitive nature of the aquatic environment, which left no niches for the amphibians to fill. This transition from the water led to the development of more robust limbs that could support the body without the water's aid (Carroll *et al.*, 1999). Later, during the Permian era, amphibians became dominant terrestrial predators. Over the last 300 million years, amphibian success waxed and waned as adaptive morphological and behavioral transitions occurred to enhance the likelihood of survival (Carroll *et al.*, 1999). A major, and unique body plan to evolve was that of anurans, whose relatively long legs, fused limb bones, and oddly shaped pelvis all facilitate the locomotor mode common to most species in the group: hopping and jumping. Among more derived anurans is a wide variety of morphologically similar clades. Relationships between some of the most studied anuran clades, like ranids (true frogs), hylids (tree frogs), and bufonids (true toads) indicate that they are quite distantly related to one another, despite many morphological similarities (Figure 17). However, they exploit different environmental niches, which, in turn, have led to the development of some environment-specific locomotor behaviors (Figure 17) (Essner *et al.*, 2010). Bufonids typically hop short distances in a terrestrial environment; many ranids are capable of longer jumps, which can be onto land but are often into water, and tree frogs can climb, jump onto and cling to vertical surfaces.

## *Anura (abbreviated)*

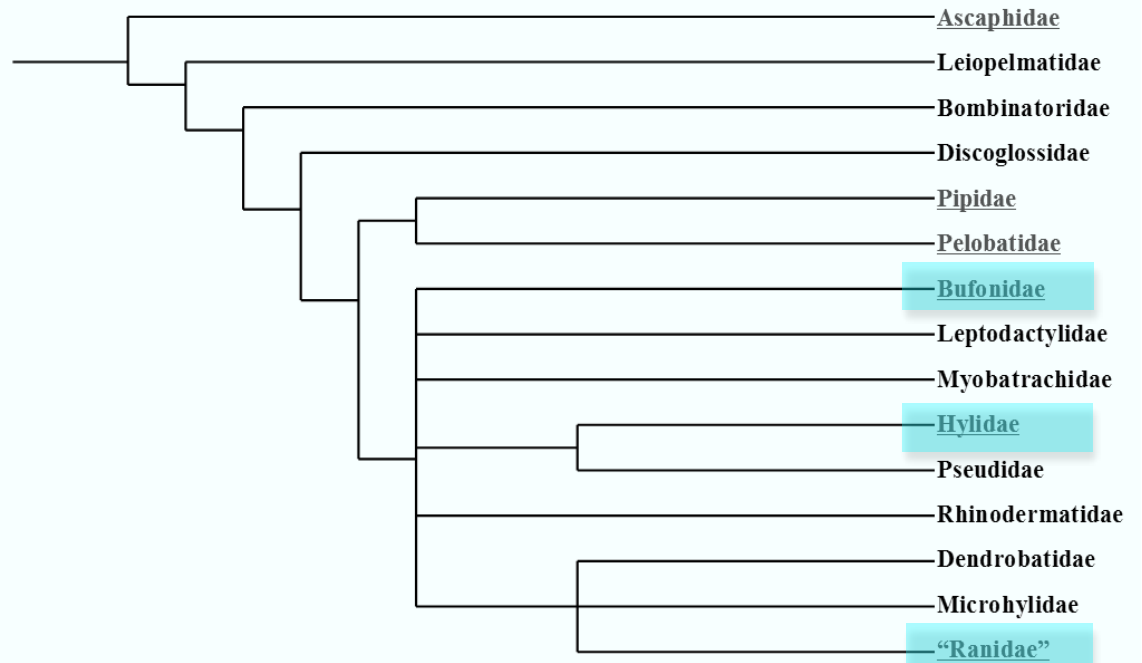


Figure 17. A phylogenetic tree highlighting Bufonidae (true toads), Hylidae (tree frogs), and Ranidae (true frogs).

(<http://bio.research.ucsc.edu>)

Lauder (1991) has studied functional evolution and explored correlated patterns of change in structure, muscle function, and behavior. He has established that an adaptive change in behavior does not require simultaneous changes in both the morphology and motor pattern driving the behavior. For example, Lauder *et al.* (1991) demonstrated that the evolution of an irregular feeding behavior in some sunfish species was brought about by morphological changes to the jaws, but the motor patterns driving the new jaw morphology were conserved between the normal and irregular feeders. Lauder (1991) states, “Motor patterns used to produce behavior may be very conservative in ontogeny and phylogeny. It thus is not necessary to alter all levels of biological design to affect a novel behavior”.

These ideas are very applicable when considering the kinematics and neuromuscular control of landing in tree frogs. These animals are capable of horizontal hopping, which is clearly a conserved behavior across most anurans. Additionally, evidence in this thesis supports that tree frogs are able to modulate muscle activation patterns integral to preparing for landing during horizontal hopping in a manner quite similar to distantly related toads and ranid frogs, suggesting conservation of a motor pattern (Gillis *et al.*, 2010; Hicks *et al.*, 2012). However, the tree frogs have also developed a novel behavior among anurans, hopping to a vertical surface, and have used this behavior to exploit an arboreal niche. In this case, they have obviously maintained their morphology, but developed new motor output which has resulted in this novel behavior.



### *Variation in Neuromuscular Control in Vertical Jumping*

The Cuban tree frog's extremely impressive vertical hops presented novel kinematic patterns. These animals did not demonstrate any bracing or stabilizing of the forelimbs upon landing. In the absence of the "push-up" like behavior, the frogs tended to attach with their toe pads as their bodies struck the surface. Rather than attempting to resist the impending impact forces, these animals appear to spread their limbs in mid-air to maximize the surface area available to make contact with and adhere to the vertical surface. Though the tree frogs generally exhibit these trends when hopping vertically, their speed and stickiness afford them the ability to vary these behaviors while still landing successfully.

When hopping vertically, the muscle activity patterns of the deltoideus scapularis correlate with the movements of the forelimbs. The bursts of activity occur during or just prior to forelimb liftoff and the swinging forward of the forelimbs in the aerial phase. The timing of both these kinematic events and their correlation with the onset of muscle activation implies their relation to one another. As an abductor of the shoulder joint, though the deltoideus scapularis may not be the main muscle responsible, the data implies that it participates in lifting the forelimbs off of the substrate and reaching forward in preparation for landing. In the coracoradialis, the frogs also exhibited a gradually increasing muscle recruitment pattern through the aerial phase when hopping vertically. In these bursts, the activity began consistently as the forelimbs moved forward

during the aerial phase of hop. As an elbow flexor, this muscle is responsible for lifting and moving the forelimbs forward. This function correlates with the muscle activity pattern, which implies its role in forelimb movement in the aerial phase of a hop before impact.

Limited evidence of any relationships between aerial phase duration and pre-landing intensity or onset latency in both the deltoideus and coracoradialis implies that these frogs do not consistently adjust forelimb preparation in advance of impact when hopping to a vertical surface. In both muscles, the irregularity of these results could be attributed to a variety of factors, including the large assortment of landing behaviors seen in these tree frogs when jumping to a vertical surface. For example, in some jumps, animals would land with their forelimbs hitting the wall first, in other jumps the hind limbs hit first, and in yet other jumps, both sets of limbs hit the wall at the same time. This lack of consistent landing kinematics highlights a major difference between horizontal and vertical hopping. How these animals land during vertical hops is seemingly less important than using their stickiness and speed to avoid predation and escape to a vertical surface. In vertical hops, neuromuscular control of the forelimbs is used less for bracing for landing and more for spreading out the limbs and maximizing the potential for body contact with the landing surface.

#### *Conservation of Neuromuscular Control in Horizontal Hopping*

In horizontal hops, the timing and intensity of muscle activity patterns in the deltoideus scapularis and the coracoradialis appeared to be tuned to aerial

phase duration. For example, in a longer hop, these animals activated their muscle more intensely and at a later time in preparation for landing. This is consistent with findings of several arm and chest muscles of toads (Gillis *et al.*, 2010).

This ability for these tree frogs to tune their muscle recruitment when hopping on a horizontal surface can allow us to draw conclusions about the conservation of this behavior among higher-order anurans. The most primitive modern frog, a leiopelmatid, exhibits a hopping behavior in which the animal shows no interest in using its forelimbs for landing (Essner *et al.*, 2010). This implies that during the course of anuran evolution, likely as toads and frogs habituated more terrestrial environments, the ability to use the forelimbs in landing developed. Furthermore, the demonstration of the additional adaptive ability to tune forelimb muscle recruitment to impending impact forces in tree frogs, and previously, toads (Gillis *et al.*, 2010) and other true frogs (Hicks *et al.*, 2012) emphasizes this evolutionary transition, after which this behavior was conserved.

### *Conclusion*

In the course of this study, I was able to observe and study the kinematics and neuromuscular control of the forelimbs of tree frogs jumping to horizontal and vertical surfaces. Animals prepare differently for these two types of jumps. When jumping vertically, these animals abduct their arms, maximizing the exposed sticky surface area likely to adhere to the landing surface. When hopping to a horizontal surface, they prepare for impact by moving their arms forward to

brace themselves for impact. In both types of hops, underlying muscles were recruited at times that indicated their role in preparatory forelimb movements. However, in vertical hops, the timing and intensity of muscle activation showed a wide range of variability, while in horizontal hops, predictable tuning of both the intensity and timing of muscle recruitment was observed. Specifically, later onsets and greater intensities of prelanding activity were characteristic of longer hops. This tuning is present in various other derived anuran species, indicating that it is likely a conserved ability in many anurans. As this ability to tune muscle activity prior to impact during jumping is also present in mammals, it would be interesting to study intermediate groups, such as jumping lizards to know if they too demonstrate this capability.

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