

The Role of Visual Perception in the Hopping Locomotion of *Rhinella marina*

By Doug Werry '18

A Study Presented to the Faculty of Wheaton College in Partial Fulfillment of the
Requirements for Graduation with Departmental Honors in Biology

Norton, Massachusetts

May, 2018

Table of Contents

Introduction	3
Methods	8
Results	15
Discussion	29
Works Cited	37

A special thanks to my friends, family, biomechanics lab, and advising committee (Professors Ekstrom, Dyer, and Collins) for helping me out along the way!

Introduction:

Hopping is a saltatory form of locomotion utilized by a diverse range of species throughout the animal kingdom. This form of terrestrial movement requires a large amount of force to be exerted and dissipated by an organism. To first create an initial leap, an animal must generate substantial forces in two vectors, both to distance itself from the ground and to propel itself forward. This is accomplished through the concentric contraction of muscles in the legs, which pull upon the skeleton across a fulcrum (joint), causing it to act as a lever against the ground (Vogel, 2013). For terrestrial organisms, the ability to hurl oneself through the air is not enough however, and selection pressures have pushed for the development of mechanisms to dissipate the stress upon impact with the ground. Impact forces, or ground reactive forces, can cause damage to multiple tissues, including bone, ligaments, and muscles (Aerts et al., 2013). A form of locomotion that causes significant trauma upon utilization is not particularly evolutionarily advantageous. To combat the ground reaction forces of terrestrial hopping, species have evolved diverse coping mechanisms to dissipate these intense forces. Turkeys will extend their legs farther in correspondence with higher drop heights, enabling them to decelerate over a longer distance, reducing the force exerted on their bodies (Konow and Roberts, 2015). Species of kangaroo and wallaby have been shown to absorb kinetic energy from impact forces through elastic storage in the gastrocnemius and plantaris tendons of their hind legs (Alexander and Vernon, 2009). This energy storage reduces the cost of continued hopping, particularly at high speeds, as well as aids in force dissipation. At higher drop heights (typically during falls of over 100 milliseconds), humans have been shown to

increasingly flex their legs (Ford et al., 2011; Hsu and Huang 2002; Melvill Jones and Watt, 1971; Peng et al., 2011; Santello et al., 2001).

Cane toads, *Rhinella marina*, are often used to examine hopping locomotion (Akella and Gillis, 2011; Azizi et al., 2014; Cox and Gillis, 2015; Ekstrom and Gillis, 2015). The landing mechanism utilized by this species share commonalities with many other organisms, including those previously mentioned. Like the kangaroo, the cane toad uses hopping as a continuous form of locomotion. Sustained hopping over high repetitions requires immense coordination. Controlled landings are not only beneficial to dissipate impact forces but are also necessary in order to prepare for the next leap. The cane toad accomplishes this through anticipatory muscle contractions, specifically in the forelimbs, that enable it to safely lower the rest of its body to the ground (Akella and Gillis, 2011; Azizi et al, 2014; Cox and Gillis, 2015). These anticipatory contractions are often referred to as “tuning” the muscles in response to an imminent impact. In the cane toad, the magnitude of tuned contractions has been shown to vary directly with hop distance, indicating that these contractions are intended to predict the magnitude of the force upon landing (Gillis et al, 2010; Azizi et al, 2014; Cox and Gillis, 2015).

In similarity to the increased leg extension of turkey at higher drop heights, the cane toad will increase its humeral-elbow excursion in correspondence to farther hop distances, allowing for more time to decelerate (Cox and Gillis, 2015). In addition to fluctuations of the humeral-elbow excursion, investigations of the cane toad forelimb have depicted other anticipatory mechanisms prior to impact. This includes tuning in the forearm/wrist prior to impact (Ekstrom and Gillis, 2015), as well as in the anconeus muscle in preparation for landing (Azizi and Abbott, 2012). Muscle tuning prior to landing is vital

to prevent damage to the muscles of the cane toad (Azizi and Abbott, 2012). Although compressive and shearing forces are the main damaging forces on bones during a ground impact, the muscles of the cane toad are at risk of damage through eccentric contractions during landing. In contrast to a concentric muscle contraction, an eccentric contraction occurs when a muscle contracts against an overwhelming load, resulting in net sarcomere lengthening despite the muscle being active (Herzog et al, 2015). If an eccentric contraction occurs when a muscle is extended past optimal length, extensive tissue damage can result, reducing the potential contraction force of the muscle for future hops (Proske and Morgan, 2001). In the cane toad, a key muscle at risk of this effect is the anconeus, which is homologous to the tricep in humans. It faces potentially damaging extension when the humeral-elbow excursion (Figure 2, Section C) is reduced rapidly upon a ground impact. In order to reduce these damaging effects upon landing, anticipatory muscle contractions shorten sarcomere length, which have been shown to reduce the length of eccentric contractions (Azizi and Abbott, 2012; Butterfield and Herzog, 2006; Gosselin and Burton, 2002; Talbot and Morgan, 1998). As an additional benefit, tuning contractions increases joint stiffness in local areas, reducing the likelihood for limb crumpling and deformation (Azizi and Abbott, 2012).

The ability of the cane toad to dissipate ground reaction forces by incorporating anticipatory muscle contractions prior to landing is a complex process with multiple mechanisms at play. The tuning necessary to synchronize muscle contractions in the anconeus and forearm/wrist muscles requires immense precision. This raises the question of how it is accomplished, specifically in regard to what sensory feedback is most necessary to successfully complete a hop. Liebermann and Goodman (2007) examined

the role of vision in pre-landing muscle activity of falling humans at three different drop heights. The rectus femoris muscle was found to initiate contraction consistently earlier in blindfolded subjects, however these results were not particularly substantial. Although Liebermann and Goodman's conclusions were modest, other studies have also shown that non-visual sensory information does not fully compensate for the need for visual cues in anticipatory muscle tuning in human falling (Santello et al., 2001). Despite this, Santello et al. (2001) and Liebermann and Goodman (2007) both agree that controlled landings can still be accomplished with a lack of vision at drop heights of up to 0.8 meters in humans. Additionally, subjects showed significant adaptability when blindfolded (Santello et al. 2001). When height was kept constant for multiple drops, like the 10 consecutive drops from each height that Santello et al. (2001) used, non-visual sensory information was essential to cue anticipatory pre-landing muscle tuning. Over consecutive repetitions at the same height, participants learned from non-visual sensory information (such as time from initial drop to ground contact) to synchronize muscle tuning, and thus anticipatory muscle activity began to more closely resemble the non-blindfolded subjects. It is hypothesized that when vision is available, both visual and nonvisual sensory information are used in unison in anticipatory muscle-tuning, but when vision is lacking, other neurological pathways account for its absence (Santello et al., 2001).

These studies (Santello et al., 2001; Liebermann and Goodman, 2007) confirm that both visual and non-visual sensory information are components of a controlled, tuned landing in falling humans. It is important to note however, that the landing mechanics of a hop exhibited by the cane toad are far different than the unidimensional falls of these human

experiments. Visual sensory information is potentially far more important in the tuned landings of a hop, which includes vectors in both the fore-aft and vertical directions. Additionally, toads have developed acute binocular vision in order to hunt terrestrial prey (Roster et al, 1995; Taylor, 2001). This vision is precise enough to distinguish between insects of high resemblance, such as the bumblebee, *Bombus americanorum*, and its robberfly mimic, *Mallophora bomboides* (Brower, 1960). It is quite possible that this highly evolved sense of vision could develop important roles in other areas of the cane toad's physiology, such as in locomotion. In an experiment examining cane toad landing patterns on asymmetric surfaces lead by Cox and Gillis (2016), it was suggested that vision does not take priority over other sensory systems in anticipating a fall. It was instead suggested that proprioception, particularly through the hindlimbs, in combination with the vestibular system is most essential for coordinating a tuned hop. In their experiment, toads would either hop from a rolling surface onto a flat landing platform (requiring integration of the proprioceptive system), or from a stationary platform onto an angled surface (requiring integration of the vision). By using these particular layouts, Cox and Gillis (2016) intended to eliminate the influence of one specific sensory system in each setup. Although ingenious, the design of this experiment leaves room for interpretation, specifically whether vision was ever truly eliminated. Since the toads were always sighted, it is difficult to be truly confident that visual information was still not integrated into certain dynamics of the hop. Cox and Gillis admit that their experiment looked at the role of vision through a narrow set of conditions, and that more variation in contrast and landing features is recommended for future trials. Due to this, and in combination with the fact that toads have been reported to incorporate vision into other

aspects of their locomotion, such as navigating terrain (Collet, 1982) and locating prey (Brower, 1960; Vallortigara et al, 1998; Robins and Rogers, 2006), it seems that the role of vision in cane toad hopping should be reexamined in more detail.

This experiment examined how a lack of vision influences the cane toad's forelimb kinematics, through the duration of a single-hop sequences. We hypothesize that if visual perception is vital to cane toad forelimb landing mechanics, there will be significant disparities in the elbow extension/flexion angle, humeral protraction/retraction angle, and humeral elevation/depression angle between blind and sighted toads.

Methods and Materials:

Note: While the methods and materials section of this paper includes procedures on how cane toads were blinded and recorded hopping, this was the work of past thesis students. This specific thesis investigation involved the digitization of these recordings, as well as their subsequent statistical analysis.

Specimens

Five cane toads, *Rhinella marina*, were the investigated species for this study. All toads were obtained from a vendor in Florida. The cane toads were kept at the Wheaton College animal care facility for the duration of this experiment. The toads were kept in large plastic tubs, on a rotation of twelve hours of light, and twelve hours of dark. They were given food and water daily. All procedures were completed under IACUC protocol 71-2013.

Experimental Layout

Each cane toad hopped a total of approximately 50 times for this experiment. These 50 hops were split equally between sighted and blind trials, with the sighted trials occurring first. After recording and data collection, many of the hops were not usable for kinematic analysis, mainly due to the trajectory of the hop. If the hop was too far off to the side of the landing platform, some of the key identification markers necessary for kinematic analysis would become obscured. This is why a portion of the total reported hops were excluded from the results. Despite this, each toad for each condition of the experiment had at least 14-15 hops analyzed. Hops took place on a flat runway strip of graded surface, which enabled the toads to achieve sufficient traction for hop takeoffs. If the cane toad specimens did not initiate a hop within the first 3 seconds of placement on the runway, they were incentivized to do so using a combination of stimuli. These stimuli included a series of loud claps, a gust of air, or a finger stroke to the posterior end of the toad.

Eliminating Vision

Specimens were anesthetized using a mixture of 1L freshwater, 1.4g tricaine methanesulfonate (MS-222), and 1.4g sodium bicarbonate (baking soda). Cane toads were placed into this solution until rendered unresponsive (approximately 15-30 minutes). Once the toads were fully anesthetized, a small incision was made on the soft palate of the toad. This enabled access to the ventral surface of the optic nerves, which could then be cut. The initial incision was then sutured shut (6.0 silk) and the toads given time to recover. Once the cane toad's mobility was fully restored, it was then recorded performing its series of blind hops. Following completion of hops, the toads were

ethanized through submersion in anesthetic solution and placed in the lab refrigerator for 24 hours. Toads were then dissected to confirm optic nerve severance.

Video Analysis

Each cane toad hop was recorded on two HiSpec Lite cameras at 500fps. Cameras were placed on flat ground at approximately a 45-degree angle in respect to one another, with the one camera positioned perpendicular to the right of the specimen, and the other 45 degrees away, in the intended direction of the toad hop.

In order to produce consistent anatomical landmarks for later kinematic analysis of the high-speed footage, each cane toad was marked with a series of iridescent paint markers on specific body locations. These markers were placed at the elbow, wrist, and midway along the humerus of the right forelimb. Additionally, all specimens were given three more markers along their back. Two of these points were midline along the back, with the anterior marker about two centimeters behind the eyes of the toad, and the posterior marker approximately three centimeters behind the previous, lying slightly behind the shoulder girdle. The third back marker was placed to make a right angle with the line created by the two midline markers. It lay on the same transverse plane as the posterior midline marker. These back markers were essential for monitoring the humeral movements of the toad's forelimb in both the vertical and horizontal planes of motion.

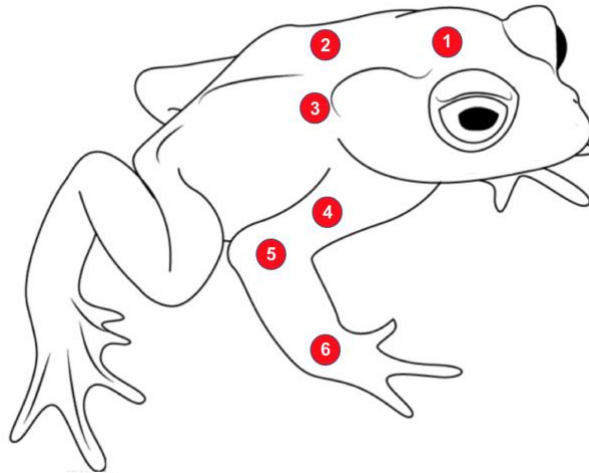


Figure 1. The anatomical landmarks used for marker placement on the cane toad specimens. It's important to note that points 1, 2, and 3 form a right angle along the midline of the specimen's back.

The three-dimensional positioning of each marker was digitized using DLTdataviewer5, a MatLab script designed to digitize and point-track video feeds (Hedrick, 2008). Prior to each the hopping trials of each toad, a cube with known dimensions was placed onto the runway into the field of view of the two high speed cameras. With values of depth, length, and width known, DLTdateviewer5 calibrated the angle and space between the cameras. This enables the digitized points obtained from both camera feeds to be combined to form a single set of x, y, and z coordinates for each marker of each hop, which are then compiled onto an excel spreadsheet. To ensure accuracy and consistency of point digitization, each marker was manually highlighted using DLTdataviewer5 on both video feeds for every frame of the all hopping trials recorded.

Each hop spreadsheet was cropped and combined into larger spreadsheets consisting of multiple trials, which were separated based upon specific specimen as well as sight status

(sighted or blinded). Under these spreadsheets, formulas were derived to calculate angles of elbow flexion/extension, humeral protraction/retraction, and humeral elevation/depression for each frame of each hop. Elbow flexion/extension angle was calculated by using points 4, 5 and 6 shown on Figure 1. Using the law of cosines, the length of line segments from points 4 to 5, 5 to 6, and 6 to 4 were obtained.

$$\text{Segment A (4 to 5)} = \text{SQRT}(((\text{Pt4_x} - \text{Pt5_x})^2) + ((\text{Pt4_y} - \text{Pt5_y})^2) + ((\text{Pt4_z} - \text{Pt5_z})^2))$$

$$\text{Segment B (5 to 6)} = \text{SQRT}(((\text{Pt5_x} - \text{Pt6_x})^2) + ((\text{Pt5_y} - \text{Pt6_y})^2) + ((\text{Pt5_z} - \text{Pt6_z})^2))$$

$$\text{Segment C (6 to 4)} = \text{SQRT}(((\text{Pt6_x} - \text{Pt4_x})^2) + ((\text{Pt6_y} - \text{Pt4_y})^2) + ((\text{Pt6_z} - \text{Pt4_z})^2))$$

Using segment lengths, the flexion angle was calculated by using the second law of cosines equation. The direction of elbow extension was defined as the positive vector.

$$\text{Elbow Flexion/Extension} = \text{ARCCOS}(((C^2) - (A^2) - (B^2)) / (-2 * A * B))$$

To calculate humeral angles, a similar method involving the law of cosines was used. In order to obtain the humeral protraction/retraction angle however, line segment 4 to 5 had to first be projected onto the same horizontal plane as segment 1 to 2. To accomplish this, point 4 was set equal to point 2, and the difference between point 4 and point 2 was calculated and applied to point 5. Using this method, the humeral protraction/retraction angle could be found as the complement to the angle created by 4 to 5 and 1 to 2. A similar mechanism was incorporated in the work of Cox and Gillis (2015). The direction of protraction was defined as the positive vector.

$$\text{Pt5_x}' = \text{Pt_5} + (\text{Pt2_x} - \text{Pt4_x}) \quad \text{Pt5_y}' = \text{Pt_5} + (\text{Pt2_y} - \text{Pt4_y}) \quad \text{Pt5_z}' = \text{Pt_5} + (\text{Pt2_z} - \text{Pt4_z})$$

$$\text{Segment D (1 to 2)} = \text{SQRT}(((\text{pt1_x} - \text{pt2_x})^2) + ((\text{pt1_y} - \text{pt2_y})^2) + ((\text{pt1_z} - \text{pt2_z})^2))$$

$$\text{Segment E (2 to 5')} = \text{SQRT}(((\text{pt2_x} - \text{pt5'_x})^2) + ((\text{pt2_y} - \text{pt5'_y})^2) + ((\text{pt2_z} - \text{pt5'_z})^2))$$

$$\text{Segment F (5' to 1)} = \text{SQRT}(((\text{pt5'_x} - \text{p1_x})^2) + ((\text{pt5'_y} - \text{p1_y})^2) + ((\text{pt5'_z} - \text{p1_z})^2))$$

$$\text{Complement of Humeral Pro/Ret} = \text{ARCCOS}(((F^2) - (D^2) - (E^2)) / (-2 * D * E))$$

$$\text{Humeral Protraction/Retraction} = 180 - \text{Complement of Humeral Pro/Ret}$$

The same technique used to create humeral protraction/retraction angle was used to derive the humeral elevation/depression angle, with the only substitution being that the line segment 4 to 5 was instead projected on to the same vertical plane as segment 2 to 3. To accomplish this, point 4 was set equal to point 3, and the difference between point 4 and point 3 was calculated and applied to point 5. The direction of elevation was defined as the positive vector.

$$\mathbf{Pt5_x''} = \mathbf{Pt_5} + (\mathbf{Pt3_x} - \mathbf{Pt4_x}) \quad \mathbf{Pt5_y''} = \mathbf{Pt_5} + (\mathbf{Pt3_y} - \mathbf{Pt4_y}) \quad \mathbf{Pt5_z''} = \mathbf{Pt_5} + (\mathbf{Pt3_z} - \mathbf{Pt4_z})$$

$$\text{Segment G (2 to 3)} = \text{SQRT}(((\mathbf{pt3_x} - \mathbf{pt2_x})^2) + ((\mathbf{pt3_y} - \mathbf{pt2_y})^2) + ((\mathbf{pt3_z} - \mathbf{pt2_z})^2))$$

$$\text{Segment H (3 to 5'')} = \text{SQRT}(((\mathbf{pt3_x} - \mathbf{pt5''_x})^2) + ((\mathbf{pt3_y} - \mathbf{pt5''_y})^2) + ((\mathbf{pt3_z} - \mathbf{pt5''_z})^2))$$

$$\text{Segment I (5' to 2)} = \text{SQRT}(((\mathbf{pt5''_x} - \mathbf{p2_x})^2) + ((\mathbf{pt5''_y} - \mathbf{p2_y})^2) + ((\mathbf{pt5''_z} - \mathbf{p2_z})^2))$$

$$\text{Humeral Depression/Elevation} = \text{ARCCOS}((((\mathbf{I}^2) - ((\mathbf{H}^2) - ((\mathbf{G}^2)))/(-2 * \mathbf{G} * \mathbf{H})))$$

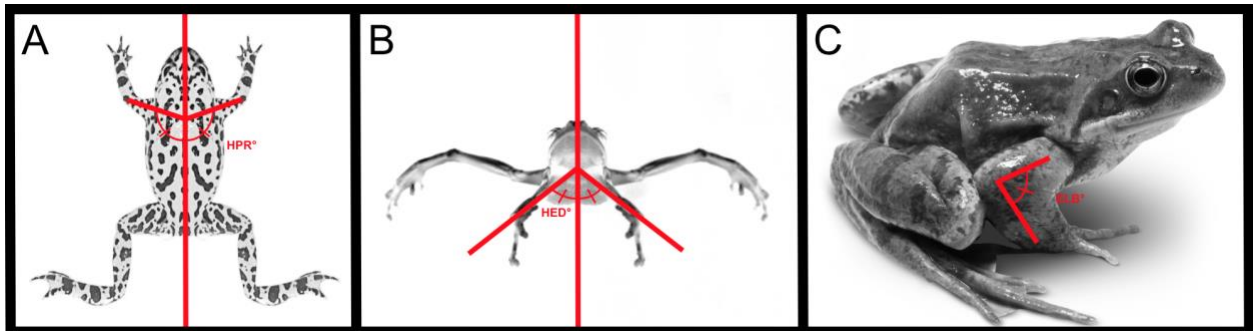


Figure 2. The angle defined as the Elbow Flexion/Extension Angle, Humeral Elevation/Depression Angle, Humeral Protraction/Retraction Angle are represented in sections A, B, and C, respectively. Elbow extension was defined as the straightening of the arm, humeral elevation was defined as arm movement towards the back, and humeral protraction was defined as arm movement towards the head. All of these movements resulted in an angle increase. The complementary movements of flexion, depression, and retraction resulted in angle decreases.

Statistical Analysis

As Cox and Gillis (2015) describe, there are 4 distinctive phases incorporated into the hop of the cane toad. In sequential order, this includes initiation (Figure 5 Section A - initiation of hindlimb extension until the end of elbow extension), forelimb liftoff (Figure 5 Section B - beginning of elbow flexion until the end), impact preparation (Figure 5 Section C - end of elbow flexion until forelimb-ground contact), and landing (Figure 5 Section D - initiation of ground contact until the end of elbow flexion). Additionally, each phase is illustrated on Figure 3. These phase definitions were used in this experiment in order to compare the forelimb kinematics between sighted and blind toads more extensively.

Mixed generalized linear models (GLM), with sight status as the factor and hop length (time duration) as a covariate, were constructed in respect to various measurements of Elbow Flexion/Extension, Humeral Protraction/Retraction, and Humeral Elevation/Depression angle measurements for each of the four phases of the hop. Specific measurements included initial angle, final angle, minimum angle, maximum angle, and mean angle for each phase of each forelimb angle. Using the initial and final measurements, angular excursions (net changes in angle across the phase) were obtained, which subsequently lead to measurements of angular velocity. The p-value for the fixed effect of sight status was used to indicate the influence of blinding on each of the dependent variables. An alpha level of 0.05 was used to determine significance for all tests. All statistical analyses were carried out using Statistics Package for Social Sciences (SPSS) software by IBM. All confidence intervals are of 95% confidence and are written

as mean \pm standard deviation of the combined trials, with the sighted confidence interval preceding the blinded condition.

Results

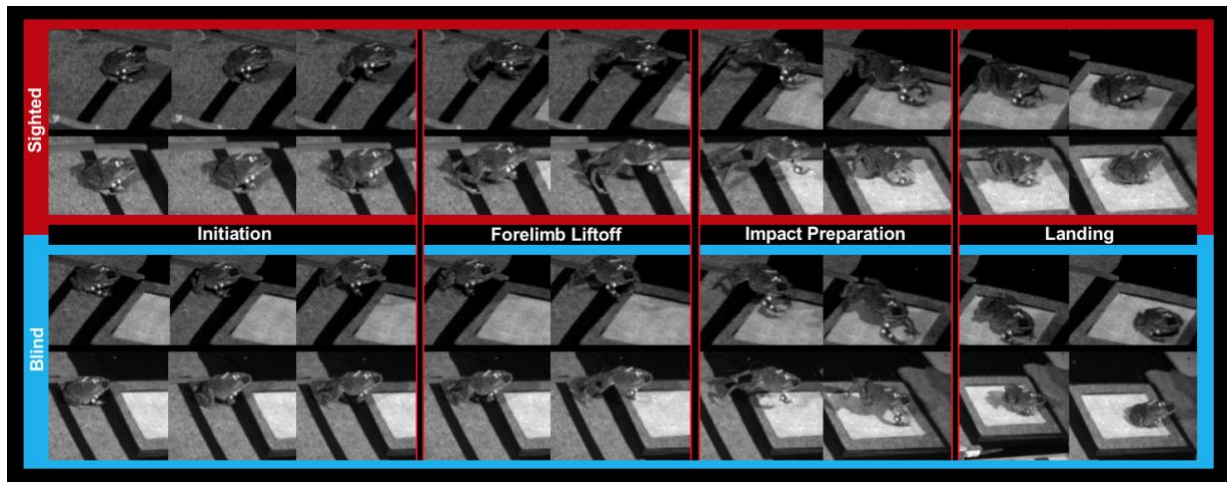


Figure 3. Frames from both a sighted and blind cane toad hop. These frames are intended to illustrate the specific motions incorporated into each phase of the hop. As the figure shows, the impact preparation phase is the only true aerial phase of the hop. It is also important to note that despite an absence of vision, blind toads still exhibit all hop phases.

A total of 143 hops were performed for this experiment. Each toad performed 14-15 hops under sighted and blinded conditions. Hop duration ranged from 298ms to 882ms in sighted toads, and 312ms to 722ms in blind toads. Overall, sighted cane toad hops (443 ± 126 ms) were significantly longer than blind hops (418 ± 69.6 ms) ($p < 0.05$). Further, in the forelimb liftoff phase, the sighted toad time durations (95 ± 31 ms) were significantly longer than blinded (82 ± 28 ms) ($p < 0.05$). This was likewise the case for the impact preparation phase (124 ± 63 ms and 116 ± 53 ms) ($p < 0.05$). The initiation (102 ± 47 ms

and $106 \pm 39\text{ms}$) and landing ($112 \pm 39\text{ms}$ and $113 \pm 45\text{ms}$) phases both showed no significant difference between the sighted and blind hops ($p=0.747$ and $p=0.592$, respectively). These times are depicted in Figure 4.

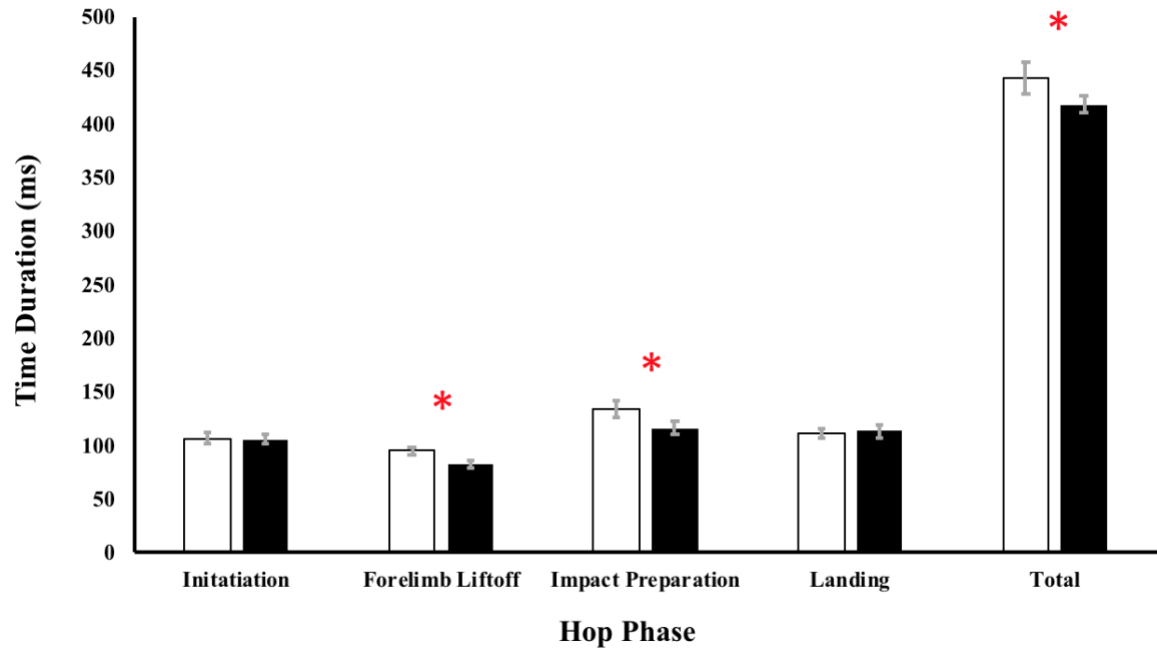


Figure 4. The variation in the time duration of each hop phase, separated by sight status. White bars represent toads with sight while black bars represent the blinded counterparts. Both the forelimb liftoff and the impact preparation phase showed significant differences in time duration between the sighted and blind conditions ($p<0.05$). Additionally, the overall time duration between sighted and blind toads was significantly different ($p<0.05$).

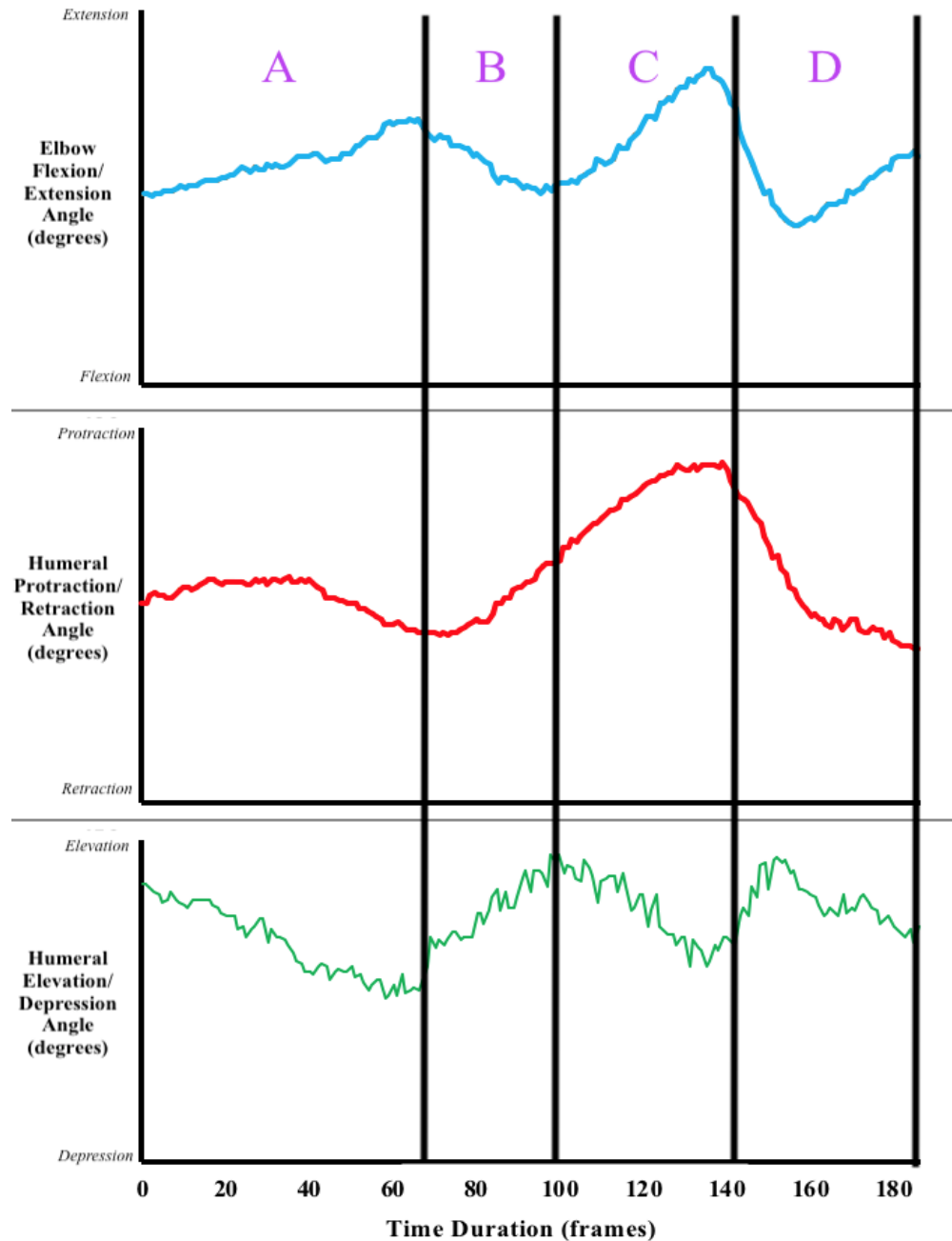


Figure 5. The typical angles of Elbow Flexion/Extension, Humeral Protraction/Retraction, and Humeral Elevation/Depression across all phases of one representative cane toad hop. Initiation, Forelimb Liftoff, Impact Preparation, and Landing phases are represented by the letters A, B, C, D, respectively. The strongest

slopes can be seen in the impact preparation phase, where elbow extension and humeral protraction increase, but elevation decreases.

Initiation

Sighted toads begin their hops from approximately a right angle of elbow flexion (76.61 ± 13.73 deg). Sighted toads started with their arms slightly retracted (52.52 ± 15.62 deg) and highly elevated, splaying the forelimbs apart in a wide stance (127.41 ± 8.63 deg). This can be seen in Figure 5 Section A. In comparison, blinded toads typically had slightly more elbow flexion upon takeoff (69.44 ± 15.87 deg), with similar angles of humeral retraction (50.85 ± 15.07 deg) and elevation (126.02 ± 7.91 deg). Both the initial angle of elbow flexion and humeral elevation were found to be significantly different between sight conditions, with sighted toads having a more extended elbow and more elevated humerus upon initiation of the hop ($p < 0.05$). Mean elbow extension and humeral elevation were also found to be significantly larger in sighted toads during this phase ($p < 0.05$) (Figure 6). Substantial elbow extension occurs through this phase (blue line of Figure 5 Section A, Figure 3), resulting in final angles of 90.00 ± 17.42 deg and 90.51 ± 15.50 deg, with maximum extensions of 95.43 ± 17.32 deg and 95.56 ± 12.27 deg, and maximum flexions of 74.45 ± 13.22 deg and 67.81 ± 15.37 deg. Blind toads were found to have significantly higher angles of maximum flexion during this phase ($p < 0.05$). The elbow excursion, or change in elbow flexion seen during this phase, was found to not be significantly different between sighted and blind conditions (13.39 ± 13.90 and 21.07 ± 17.58 deg) ($p = 0.065$). Humeral retraction also ended in a similar range as it began, with angles of 42.34 ± 15.20 deg and 41.36 ± 16.69 deg. Maximum protraction during the initiation phase was insubstantial (57.22 ± 15.82 deg and 55.93 ± 15.47 deg), so it is

evident that a retracted humerus is a typical characteristic of this phase, regardless of sight status (red line of Figure 5 Section A, Figure 3). Since the humeral protraction/retraction angle remains consistent throughout initiation, there were no significant differences in excursions of humeral protraction between sighted and blind conditions (-10.17 ± 11.40 deg and 9.50 ± 8.19 deg) ($p=0.420$). The humerus ended the phase in a similarly splayed stance in both sight conditions, with angles of 120.29 ± 5.69 deg and 117.70 ± 8.26 deg. The maximum elevations for toads during this phase were 131.52 ± 15.82 deg and 129.48 ± 7.82 deg, while the maximum depressions were 113.33 ± 7.29 deg and 112.21 ± 7.90 deg. Sighted toads were found to have significantly higher angles of maximum elevation ($p<0.05$). Excursions in humeral depression did not vary significantly between sight groups (-7.11 ± 9.27 deg and -8.32 ± 7.76 deg) ($p=0.245$).

The angular velocity of elbow flexion was found to be significantly higher in blind toads than sighted, with average flexion rates of -134.98 ± 143.52 deg/sec and -190.30 ± 148.61 deg/sec ($p<0.05$). Both of these average velocities contain extremely large standard deviations, however, and must be taken into account when interpreting these results. From these ranges, it's obvious that there is extensive variation in elbow flexion velocities within both groups, even when hop duration is accounted for. The same phenomena is present in both average humeral protraction velocity (94.09 ± 85.57 deg/sec and 104.33 ± 122.48 deg/sec) and average humeral elevation velocity (78.89 ± 75.94 deg/sec and 75.75 ± 93.72 deg/sec).

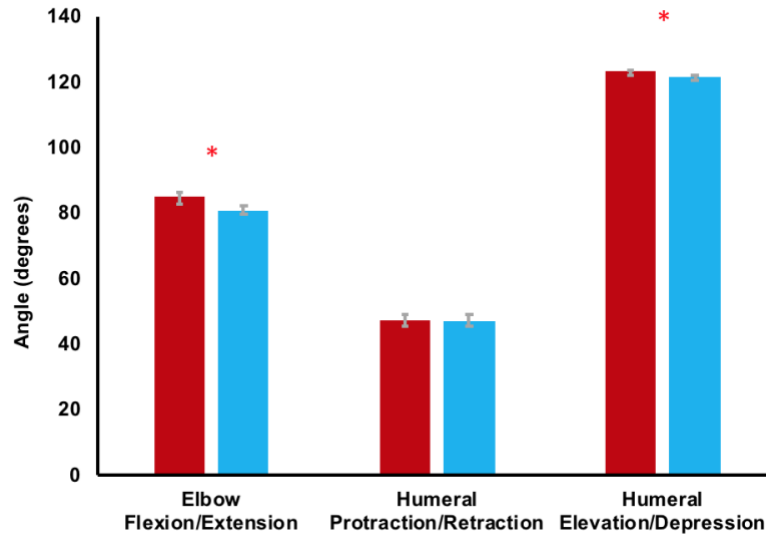


Figure 6. The variation in the mean value for all three forelimb angles separated by sight status for the initiation phase. Red bars represent toads with sight while blue bars represent the blinded counterparts. Both the mean elbow flexion/extension angle and the humeral elevation/depression angle had significant differences in time duration between the sighted and blind conditions ($p < 0.05$). Sighted toads exhibited both more extension and more elevation, on average, than blind toads.

Forelimb Liftoff

In continuation to the end of initiation, the elbow began extended, with angles of 89.01 ± 17.04 deg and 90.29 ± 14.18 deg (Figure 3). The elbow flexes through the forelimb liftoff phase, however, creating excursions of -21.02 ± 12.94 deg and -20.27 ± 12.94 deg, and resulting in ending angles of 90.07 ± 16.91 deg and 90.42 ± 13.68 deg (blue line of Figure 5 Section B). These excursions were not significantly different between sighted and blind toads ($p = 0.804$). During this excursion, maximum elbow flexion was 63.17 ± 13.01 deg and 65.73 ± 10.16 deg, and maximum extension was 90.08 ± 16.91 deg and 90.42 ± 13.68 deg. Cane toads exhibit general humeral protraction during forelimb liftoff,

starting at angles of 42.001 ± 16.69 deg and 42.04 ± 16.84 deg, and ending with angles of 70.25 ± 17.95 deg and 67.10 ± 14.76 deg (red line of Figure 5 Section B, Figure 3). This protraction created excursions in of 28.25 ± 14.12 deg and 25.07 ± 14.23 deg. The maximum protractions were 74.34 ± 16.86 deg and 69.70 ± 15.14 deg, while the maximum retractions were 36.72 ± 15.54 deg and 36.59 ± 16.42 deg for the phase. Although the humeral protraction excursion angle didn't vary substantially between sighted and blind ($p=0.154$), both the final and maximum angle of protraction were significantly larger in sighted toads ($p<0.05$). Cane toads began this phase with humeral elevation angles of 120.83 ± 6.45 deg and 118.83 ± 8.42 deg, but splayed their arms further to end with angles of 141.48 ± 10.01 deg and 136.49 ± 8.35 deg (green line of Figure 5 Section B, Figure 3). This resulted in humeral elevation excursion angles of 20.65 ± 8.89 deg and 17.66 ± 9.03 deg. They were no significantly different between blind and sighted toads ($p=0.088$). Elevation angles reached maximums of 146.76 ± 10.71 deg and 140.64 ± 9.86 deg, while maximum depression angles were 116.61 ± 5.90 deg and 114.85 ± 8.09 deg during forelimb liftoff. Similar to humeral protraction, both the final and maximum angle of humeral elevation were found to be significantly larger in sighted toads ($p<0.05$). Both the mean humeral protraction and extension of this phase were found to be significantly larger in sighted toads ($p<0.05$) (Figure 7). In combination with the force generated by the extending hindlimbs, the incorporation of a flexed elbow, and protracted and elevated arms enables the cane toad to lift off the ground and transition into the aerial phase of the hop (Figure 3). Similar to the initiation phase, the angular velocity of elbow flexion (241.03 ± 180.65 deg/sec and 257.26 ± 154.66 deg/sec), humeral protraction (-317.35 ± 202.81 deg/sec and -305.55 ± 177.42 deg/sec),

and humeral elevation (-236.65 ± 136.59 deg/sec and -221.01 ± 119.25 deg/sec) also showed extreme variation within each sight condition. In this phase, however, all confidence intervals stayed in the same directional vector at least, unlike in initiation.

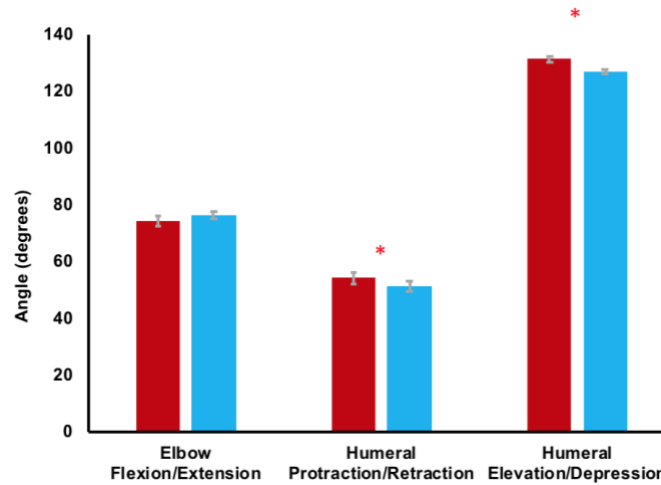


Figure 7. The variation in the mean angle for all three forelimb angles separated by sight status for the forelimb liftoff phase. Red bars represent toads with sight while blue bars represent the blinded counterparts. Both the mean humeral protraction/retraction and the humeral elevation/depression angle had significant differences in time duration between the sighted and blind conditions ($p < 0.05$). Sighted toads exhibited both more protraction and more elevation, on average, than blind toads.

Impact Preparation

In the only fully aerial phase of the hop, elbow flexion begins at angles of 68.51 ± 13.45 deg and 70.07 ± 9.73 deg and ends with increased angles of 97.45 ± 11.90 deg and 97.54 ± 10.96 deg (blue line of Figure 5 Section C). This creates elbow extension excursions of 28.94 ± 11.83 deg and 27.47 ± 12.33 deg, enabling the forearms to extend outwards in order to prepare for impact. These excursions were not significantly different between

sighted and blind toads ($p=0.379$). The maximum angles of elbow extension were 108.11 ± 14.83 deg and 104.12 ± 12.74 deg, with maximum flexions of 66.68 ± 13.02 deg and 68.42 ± 8.77 deg. Sighted toads were found to have significantly higher maximum extension angles than blind ($p<0.05$), however blind toads were found to have significantly more extended final elbow angles ($p<0.05$). The large discrepancy between maximum and final angles of elbow extension shows an interesting characteristic of the impact phase. In both the sighted and blind conditions, the toads exhibit significant elbow extension through the duration of this phase (Figure 5 Section C, Figure 3), however the peak of this extension does not occur at impact, but slightly prior. The humerus exhibits net retraction during this phase, starting at 72.16 ± 18.16 deg and 67.67 ± 14.79 deg, but ending at angles of 99.72 ± 21.31 deg and 96.99 ± 17.97 deg. This creates excursions of 27.56 ± 16.20 deg and 29.31 ± 12.43 deg. These were not significantly different ($p=0.912$). Sighted toads were found to be significantly more protracted at both the initial and final measurements of this phase ($p<0.05$). Maximum retraction peaked at 107.93 ± 22.26 deg and 101.48 ± 17.52 deg, with sighted toads having significantly larger maximum retraction angles ($p<0.05$). This dramatic increase in angle occurs as the toad swings its forelimbs forward, in order to align its arms in a position to make first contact with ground (Figure 3). The maximum protraction angles seen during this phase were 68.25 ± 17.39 deg and 66.14 ± 14.73 deg, with blind toads exhibiting significantly more retraction ($p<0.05$). Humeral elevation stayed relatively constant throughout this phase, starting at angles of 141.95 ± 8.62 deg and 134.96 ± 8.25 deg, and ending with angles of 132.50 ± 9.53 deg and 126.74 ± 8.97 deg. This created excursions of -9.46 ± 12.26 deg and -8.22 ± 12.42 deg. Sighted toads were found to have larger excursions in humeral

depression in this phase ($p < 0.05$). Maximum elevation angles were 151.25 ± 10.40 deg and 143.62 ± 8.16 deg, with maximum depression angles of 119.53 ± 9.83 deg and 117.35 ± 10.31 deg. For maximum elevation and depression angles, sighted toads showed significantly more elevated humeral positions than blind toads ($p < 0.05$). Additionally, sighted toads initiated the phase with a significantly more elevated position than blind. Sighted toads also exhibited larger mean extension and humeral protraction angles (Figure 7) ($p < 0.05$).

Like the other phases, angular velocities showed extreme variation for all forelimb angles within each sight condition. Elbow extension velocities (-240.75 ± 105.90 deg/sec and -250.69 ± 91.57 deg/sec) and humeral retraction velocities (-229.89 ± 136.89 deg/sec and -268.77 ± 91.44 deg/sec) were highly variable. Despite this substantial variation, sighted toads showed significantly faster humeral elevation velocities (89.92 ± 123.89 deg/sec and 64.75 ± 101.48 deg/sec) ($p < 0.05$).

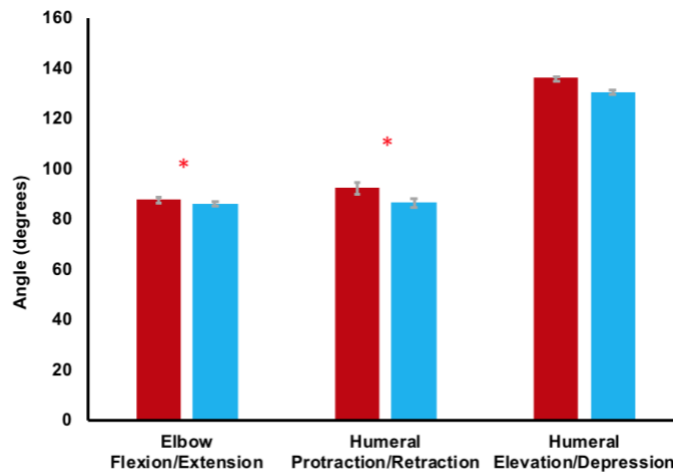


Figure 8. The variation in the mean angle for all three forelimb angles separated by sight status for the impact preparation phase. Red bars represent toads with sight while blue bars represent the blinded counterparts. Both the mean elbow flexion/extension angle and

the humeral protraction/retraction angle had significant differences in time duration between the sighted and blind conditions ($p < 0.05$). Sighted toads exhibited both more extension and protraction, on average, than blind toads.

Landing

Upon initial ground contact, the elbows were extended, at angles of 96.60 ± 11.48 deg and 95.70 ± 10.84 deg but flexed to positions of 78.60 ± 19.64 deg and 74.86 ± 12.45 deg by the end of the hop (blue line of Figure 5 Section D, Figure 3). This created elbow flexion excursions of -18.00 ± 19.06 deg and -20.84 ± 15.43 deg. This flexion is incorporated into the hop in order to dissipate ground reaction force upon landing, as well as better position the cane toad for future hops. Maximum extension angles were 99.27 ± 14.16 deg and 97.50 ± 10.66 deg, and maximum flexion angles were 59.91 ± 12.25 deg and 56.63 ± 9.98 deg during the landing phase. Sighted toads were found to have more overall elbow extension during this phase. For all elbow flexion/extension angles measured (initial, final, maximum extension, and maximum flexion), sighted toads were found to have a significantly more extended elbow than their blind counterparts ($p < 0.05$). The mean angle of elbow extension was also significantly larger in sighted toads (Figure 9) ($p < 0.05$). The humerus started this phase protracted (red line of Figure 5 Section D), with angles of 99.58 ± 20.65 deg and 95.70 ± 10.84 deg but retracted to angles of 55.43 ± 22.16 deg and 52.57 ± 16.12 deg by the end of the hop. This created humeral retraction excursions of -44.15 ± 18.77 deg and -43.50 ± 12.71 deg. These were not significantly different ($p = 0.404$). Maximum retraction angles were 48.62 ± 20.59 deg and 48.91 ± 15.48 deg, and later maximum protraction angles were 101.91 ± 21.54 deg and $98.21 \pm$

16.82 deg. Sighted toads were found to have higher protraction than blind toads during this phase, with significantly more protraction in both the initial and maximum protraction angles ($p < 0.05$). The humerus started the landing phase highly elevated at angles of 132.73 ± 9.96 deg and 127.43 ± 10.42 deg and ended at angles of 126.20 ± 13.04 deg and 127.03 ± 8.22 deg, indicating a rather constant alignment (green line of Figure 5 Section D). Maximum elevation angles were 146.88 ± 7.36 deg and 145.21 ± 6.20 deg, and later maximum depression angles were 119.35 ± 11.00 deg and 119.88 ± 8.68 deg. Sighted toads were more depressed at the maximum depression angle ($p < 0.05$). It is important to note that all final forelimb angles of both sighted and blind conditions display a noticeable similarity to the first angles of the initiation phase. This similar positioning indicates that the hop has been fully completed, and that the toad is prepared and positioned for a potential second hop (Final frame of Figure 3). Angular velocities for elbow flexion (215.20 ± 197.79 deg/sec and 191.48 ± 238.12 deg/sec), humeral protraction (440.48 ± 248.36 deg/sec and 428.60 ± 172.85 deg/sec), and humeral elevation (56.10 ± 146.05 deg/sec and 0.75 ± 146.45 deg/sec) all showed considerable variation once again within both sight group, making them difficult to interpret.

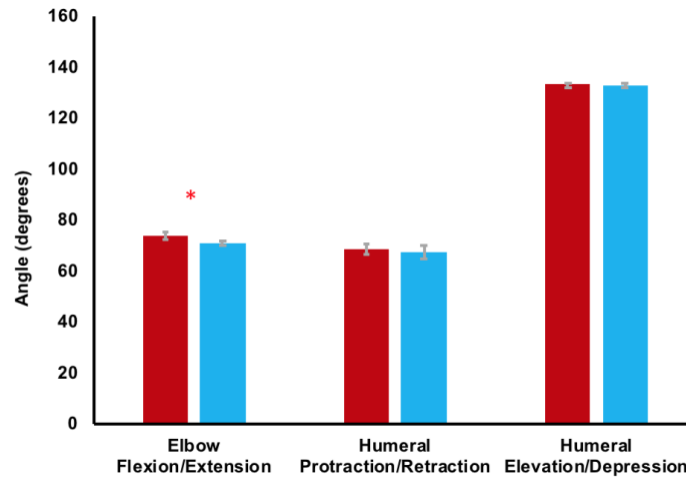


Figure 9. The variation in the mean angle for all three forelimb angles separated by sight status for the landing phase. Red bars represent toads with sight while blue bars represent the blinded counterparts. The mean elbow flexion/extension angle differed significantly between sighted and blind conditions. Sighted toads exhibited both more extension, on average, than blind toads.

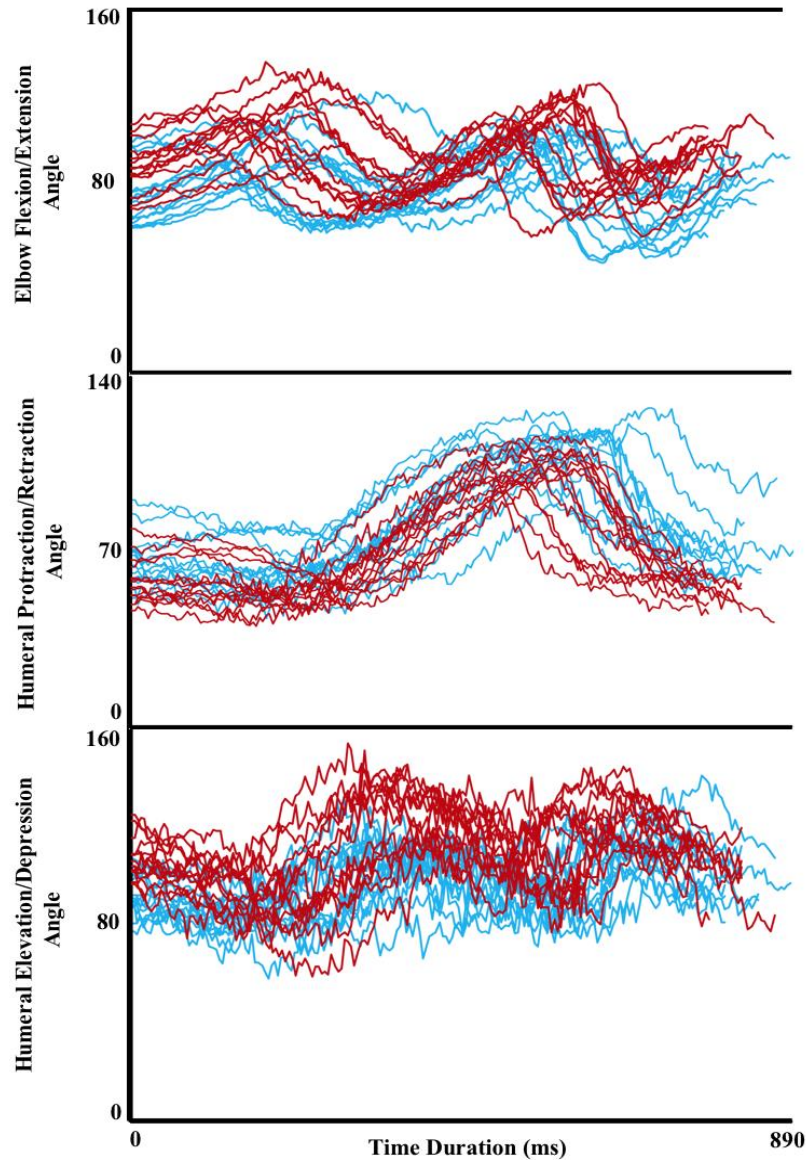


Figure 10. These graphs display the variation in elbow and humerus movements seen in a single toad's hopping trials. Each line embodies a separate hop, with red representing sighted and blue representing blind. General clustering can be seen across the two different sight conditions. For instance, sighted toads have increased elbow extension, particularly during the impact preparation phase (second hump in the Elbow Flexion/Extension section).

Discussion

The objective of this experiment was to determine whether visual feedback significantly influenced forelimb mechanics during the hopping locomotion of the cane toad, *Rhinella marina*. To this end, the kinematics of the forelimb, specifically in respect to the Elbow Flexion/Extension, Humeral Protraction/Retraction, and Humeral Elevation/Depression angles, were found to have some significant differences between sighted and blinded toads. In Cox and Gillis (2015), which described typical forelimb kinematics of sighted cane toads, toad forelimbs are significantly more extended and protracted at impact in longer hops, with these more exaggerated positions resulting in greater excursions of the elbow and humerus during the impact preparation phase. These mechanics are then mirrored by similarly distant-dependent amounts of elbow flexion and humeral retraction during landing phase. Due to these findings, hop duration was taken into account as a covariate upon analysis between sighted and blind toads.

To examine how vision influenced forelimb mechanics across an entire cane toad hop, both specific forelimb positions and forelimb excursions were taken into considerations. The only significant difference in forelimb excursion was during the impact preparation phase, where sighted toads had far larger changes in humeral depression during the phase. Far more variation between sighted and blind toads was found in the specific forelimb positioning during hop phases. For the elbow, the combination of initial, mean, and maximum flexion and extension angles show that sighted cane toads seem to be typically more extended during initiation (Figure 10). This effect does not carry on into forelimb liftoff, but differences in mean angle and maximum extension in the impact preparation phase, and initial, final, mean, and maximum extension and flexion angle in

the landing phases also depict a more extended forearm in these two final portions of the hop (Figure 10).

Sighted toad forelimbs were often found in positions of higher humeral protraction for the majority of the hop duration. Humeral protraction/retraction angle showed no variation between sighted and blind toads during the initiation phase, but final, mean and maximum protraction angles of the forelimb liftoff phase, the initial, final, mean, and maximum protraction and retraction angles of the impact preparation phase, and initial and maximum protraction angles of the landing phase all show that sighted toads typically depict positions of higher protraction.

To accompany the other two trends in forelimb position, sighted toads were often found in positions of higher humeral elevation. The initial, mean, and maximum elevation angles of the initiation phase, the mean, final, and maximum elevation of the forelimb liftoff phase, the initial, and maximum elevation and depression angles of the impact preparation phase, and the maximum depression angle of the landing phase all show sighted toads typically depict positions of higher elevation.

Takeoff Surface

A flat takeoff surface was chosen for several key reasons. If some type of unpredictable or variable landing surface were to be incorporated into this experiment, such as that sloped terrain used in Cox and Gillis (2016), it is a likely possibility that some of these phases would be interrupted or even removed in entirety. For future directions, this possibility could be an interesting effect to examine. For this preliminary research, however, it seems advantageous to compare and incorporate the role vision for every single hop phase across its entire intended duration. Once this phase-specific data is

obtained, it will be far easier to incorporate the influence of other variables, such as habitat-specific variable terrain.

Additionally, using varying terrains, such as a landing platform with varying slopes, in a repetitive method could potentially lead to course adaptation. In a situation of course adaptation, the subject begins to “learn” how to maneuver the course more effectively, resulting in significant changes in anticipatory muscle activity across trials of identical conditions in response to how many times it’s been attempted. This would introduce confounding bias into an experiment, rendering highly questionable results. A similar situation is mentioned with human subjects in Santello et al. (2001). Although the adaptive capability of humans vastly outweighs toads, learning in Bufonidae has been observed and recorded. Brzoska and Schneider (1978) reported that the species *Bufo b. bufo* could be trained to discriminate auditory stimuli, as well as exhibit prey-catching behavior after learning specific visual cues. For future experiments involving variable terrain, it will be essential to create an experimental layout in which this learning effect is minimized. For this procedure, using a flat surface controls for this effect as much as possible by keeping the influence of the runway completely constant.

Examining the Normalcy of the Cane Toad Hops

The trends in elbow and humerus movement throughout the hop resemble the results of Cox and Gillis (2015). Sighted toads initiated their hops from approximately a right angle of elbow flexion/extension and had similar excursions of elbow flexion/extension, humeral protraction/retraction, and humeral elevation/depression positions. These similarities continued on into the forelimb liftoff, impact preparation, and landing phases. The only discrepancy noted between the two experiments is in regard to the

measurements of humeral elevation/depression excursions across the final three phases of the hop. For example, in the impact preparation phase, Cox and Gillis report excursions of -37 ± 10 deg, which are dramatically larger changes in humeral depression than those seen in this experiment (approximately 30 degrees upon comparison of sample means). These trends are similar in the forelimb liftoff and landing phases as well. The most likely explanation for this discrepancy is a combination of the differences in hop length between the two experiments (Cox and Gillis (2015) reported longer hop lengths), as well as differences in defining phase transition points. Upon close examination of the humeral elevation/depression portion of Figure 6 (green line), it can be noted that the transition from humeral depression to elevation slightly proceeds the switch from the impact preparation phase to the landing phase (phase change occurs at initial forelimb contact with the ground). Due to this slight delay, both the humeral depression excursion of the impact preparation phase and the elevation excursion of the landing phase do not represent the full angular change seen during these last two phases. This slight delay can also be seen on Figure 6 for elbow flexion/extension and humeral retraction/protraction, but far more insignificantly. For this experiment, first ground contact was literal, with the transition to landing phase occurring as soon as the first digits of the forelimb touched ground. For Cox and Gillis (2015) and Gillis, Ekstrom, and Azizi (2014), it's suspected that initial ground contact was defined as a later interaction, where a more substantial portion of the forelimb made contact, such as the entire hand of the toad. This would explain the absence of delay, since a larger amount of forelimb-ground contact ensures a better landing platform for integrating the eccentric contractions that dissipate the ground reaction force of landing. When this phenomenon is disregarded, the humerus and elbow

movements of Cox and Gillis (2015) and Gillis, Ekstrom, and Azizi (2014) are approximately the same as those seen by the sighted cane toads of this experiment.

Implications of Vision for Landing

If the trends in increased elbow flexion, humeral retraction, and humeral depression are assumed to be universal occurrences in cane toads who lack vision, the important next step is to examine how these alterations influence the effectiveness of their hop mechanics. With the specific directionality of these forelimb changes in mind, a more flexed, retracted, and depressed forelimb may result in an increased likelihood of muscular damage. This potential damage would be induced by the eccentric contractions that accompany the landing phase of the hop, as the cane toad must dissipate the impact forces upon contact with the ground (Gillis, Akella, and Gunaratne, 2010; Azizi and Abbott, 2012; Griep et al, 2013). In an examination of anconeus muscle activity in cane toads prior and post-landing, Azizi and Abbot (2012) observed that due to anticipatory muscle tuning before impact, the anconeus was never stretched past optimal length. Since eccentric contractions past optimal length have been shown a high potential for significant muscle damage (Proske and Morgan, 2001), the ability to limit the amount of extension the anconeus endures past this threshold is extremely evolutionarily advantageous to avoid muscular injuries. The anconeus is an elbow extensor (Azizi and Abbott, 2012), meaning that the increased elbow flexion, like that of the blind cane toads, creates a more lengthened anconeus prior to impact. This limits the change in sarcomere length possible before extension stretches past optimal length during an eccentric contraction. Since passing optimal length induces muscle damage, having a reduced range of potential extension increases the likelihood of transitioning into tissue-damaging

lengths of extension, subsequently causing injury. Additionally, the proper positioning of the elbow and function of the anconeus is essential to a successful cane toad landing, as landing impacts have been shown to be absorbed in entirety by the forelimbs (Griep et al, 2013). By using solely the forelimbs, *R. marina* can complete a hop without chest impact. This benefits the toad by decreasing the distance the body must be lifted for proceeding hops (Griep et al, 2013), and by creating a shorter recovery phase after initial landing (Essner et al, 2010). The effectiveness of this ability may be reduced in blind toads, since elbow flexion is the main method of impact force dissipation, and these animals start their landing from a more flexed position. This reduces the range over which impact force can be minimized, leading to potentially more forceful landings. Increased elbow flexion exhibited in blind toads, particularly during the impact preparation and landing phases of the hop, may be detrimental to a proper landing by both potentially increasing the likelihood for muscle damage as well as causing improper ground reaction force dispersal by the forelimbs. In combination, these two changes could result in the reduced effectiveness of continuous hopping, creating a lack of mobility in the cane toad. For *R. marina*, versatile mobility is paramount, and is major reason why it has become such an effective invasive species worldwide (Philips et al, 2006; Urban et al, 2008).

The muscles responsible for elevating/depressing and protracting/retracting the humerus have also been hypothesized to be necessary for proper loading of impact forces onto the pectoral girdle through the suprascapular-scapular synchondrosis (Griep et al, 2013). This includes the use of the rhomboideus, serratus and levator scapulae muscles (Emerson, 1983). Griep et al (2013) state that the correct orientations of these girdle components are necessary to avoid shearing forces across the bone, which is a particularly hazardous type

of impact force (Ford and Keaveny, 1996; Liu et al, 2003). Changes in humeral positions, particularly in the direction of protraction and retraction, have been correlated with differences in pectoral girdle rotations. In addition to the negative effects of increased elbow flexion on hop landing, increased retraction and depression in blind toads has the potential to throw off force loading over consecutive hops, resulting in further potential injury and imbalance.

Summary

Since a clear relationship between forelimb anticipatory activity prior to landing and hop distance has been established (Akella and Gillis, 2011; Aziz and Abbot, 2013; Azizi et al, 2014; Cox and Gillis, 2015; Ekstrom and Gillis, 2015; Cox and Gillis, 2016), it is obvious that this complex interaction requires the integration of a sensory system.

Without this integration, coordinating muscle contraction intensity and forelimb position with the distance and velocity of a respective hop would be impossible. Cox and Gillis (2016) emphasize the role of proprioception and the vestibular system for this task, and the implication of these two systems has been confirmed to have significant importance in order to sufficiently perceive both verticality and motion in humans (Brandt et al 2002). The results of this study suggest that visual input also plays a role in the landing process, due to the changes in humeral and elbow forelimb positions seen between sighted and blinded toads across the duration of the hop. In both sight conditions, toads were still able to complete a hop successfully, and each phase of the hop was still easily distinguishable. Since this is the case, we suggest that vision is necessary for “fine tuning” the forelimbs during the hop, with the vestibular system and proprioception still being the largest nervous system components at play. Upon examination of the specific

forelimb positions of blind toads, it was identified that the more flexed, retracted, and depressed orientations of the elbow and humerus may result in less effective hops through imbalanced landings and increase the likelihood for injury through both the anconeus muscles and of the bones of the pectoral girdle. For future studies, it is suggested that the influence of vision be tested on consecutive hop sequences, rather than the using the singular hop method of this study. If a lack of vision really does influence hopping kinematics, these effects will most likely be amplified across multiple hops, leading to greater data disparities between sighted and blind toads. Additionally, a beneficial future direction will be to examine how the impact of vision changes on variable terrain rather than the flat surface of this experiment. This direction could yield useful results in regard to how particular phases of the hop are altered when terrain is unknown.

The forelimb kinematics of toads exhibit an exciting instance of adaptation to the terrestrial world. *Leiopelmatidae*, the most primitive family of frogs, incorporate no forelimb tuning in preparation for a landing impact, and instead typically crash-land onto their torsos (Essner et al, 2010). *Leiopelmatidae* frogs are suspected to use hopping solely as a mechanism of escaping into water and thus experience no real physical repercussions from their uncoordinated hops (Gans and Parsons, 1966), which would explain the lack of tuned landing mechanics. In contrast, the more forceful impacts of landing on solid ground seem to present a strong selection pressure on newer frog lineages. In larger anurans who began to spend more time in the terrestrial environment, being able to cope with the stronger ground reactive forces on solid substrates is a necessity. This challenge was answered with the incorporation by extending the forelimbs in the direction of a

landing, allowing frogs to now decelerate over longer distances, ultimately reducing landing forces. While toads possess a strong adaptation to terrestrial landscapes, their origins remain clear, as they still utilize water to host their offspring (Crossland et al, 2008). With this in mind, understanding how a cane toad lands from a hop can provide useful insight into the adaptations developed by other terrestrial beings. Gravity is a constant obstacle for terrestrial animals, and the mechanisms used to combat its effects create one of the largest instances of convergent evolution known to the animal kingdom. By examining the cane toad's specific phylogenetic path to terrestrial existence, we come closer to understanding the evolutionary paths taken by other terrestrial organisms.

Works Cited

Aerts, I., Cumps, E., Verhagen, E., Verschueren, J. and Meeusen, R. (2013). A systematic review of different jump-landing variables in relation to injuries. *J. Sports Med. Phys. Fitness* 53, 509-519

Akella, T. and Gillis, G. B. (2011). Hopping isn't always about the legs: forelimb muscle activity patterns during toad locomotion. *J. Exp. Zool. A* 315, 1-11.

Alexander, R. M., & Vernon, A. (2009). The Mechanics of Hopping by Kangaroos (Macropodidae). *Journal of Zoology*, 177(2), 265-303. doi:10.1111/j.1469-7998.1975.tb05983.x

Azizi, E., & Abbott, E. M. (2012). Anticipatory motor patterns limit muscle stretch during landing in toads. *Biology letters*, 9(1), 20121045. doi:10.1098/rsbl.2012.1045

Azizi, E., Larson, N. P., Abbott, E. M. and Danos, N. (2014). Reduce torques and stick the landing: limb posture during landing in toads. *J. Exp. Biol.* 217, 3742-3747. doi:10.1242/jeb.108506

Brandt, T. , Glasauer, S., Stephan, T., Bense, S., Yousry, T., Deutschlander, A., and Dieterich, M. (2002). Visual-Vestibular and Visuovisual Cortical Interaction. *Annals of the New York Academy of Sciences*, 956, 230-241. doi:10.1111/j.1749-6632.2002.tb02822.x

Brower, L.P., J.V.Z Brower, and P.W Westcott. (1960). Experimental studies of mimicry 5. The reactions of toads (*Bufo terrestris*) to bumblebees (*Bombus americanorum*) and their robberfly mimics (*Mallophora bomboides*), with a discussion of aggressive mimicry. *Amer. Nat.* 94:343- 356.

Butterfield TA, Herzog W. (2006) Effect of altering starting length and activation timing of muscle on fiber strain and muscle damage. *J. Appl. Physiol.* 100, 1489 – 1498. (doi:10.1152/japplphysiol.00524.2005)

Brzoska, J., & Schneider, H. (1978). Modification of prey-catching behavior by learning in the common toad (*Bufo b. bufo* [L], Anura, Amphibia): Changes in responses to visual objects and effects of auditory stimuli. *Behavioural Processes*, 3(2), 125-136. doi:10.1016/0376-6357(78)90039-6

Collett S. (1982) Do toads plan routes—a study of the detour behavior of *Bufo viridis*. *J. Comp. Physiol.* 146, 261–271

Cox, S. M., & Gillis, G. B. (2015). Forelimb kinematics during hopping and landing in toads. *Journal of Experimental Biology*, 218(19), 3051–3058. doi:10.1242/jeb.125385

Cox, S. M., & Gillis, G. B. (2016). Sensory feedback and coordinating asymmetrical landing in toads. *Biology Letters*, 12(6), 20160196. doi:10.1098/rsbl.2016.0196

Crossland, M. R., Brown, G. P., Anstis, M., Shilton, C. M., & Shine, R. (2008). Mass mortality of native anuran tadpoles in tropical Australia due to the invasive cane toad (*Bufo marinus*). *Biological Conservation*, 141(9), 2387–2394. doi:10.1016/j.biocon.2008.07.005

Ekstrom, L. J. and Gillis, G. B. (2015). Pre-landing wrist muscle activity in hopping toads. *J. Exp. Biol.* 218, 2410–2415.

Emerson, S. (1983). Functional analysis of frog pectoral girdles. The epicoracoid cartilages. *Journal of Zoology* 201, 293–308

Essner, R., Suffian, D., Bishop, P.J., Reilly, S. (2010). Landing in basal frogs: evidence of saltatorial patterns in the evolution of anuran locomotion. *Naturwissenschaften* 97, 935–939

Ford, C.M., Keaveny, T.M. (1996). The dependence of shear failure properties of trabecular bone on apparent density and trabecular orientation. *Journal of Biomechanics*. 29, 1309–1317.

Ford, K. R., Myer, G. D., Schmitt, L. C., Uhl, T. L. and Hewett, T. E. (2011). Preferential quadriceps activation in female athletes with incremental increases in landing intensity. *J. Appl. Biomech.* 27, 215–222.

Gans C, Parsons TS. (1966). On the origin of the jumping mechanism in frogs. *Evolution*. 20, 92–99

Gillis GB, Akella T, Gunaratne R. (2010) Do toads have a jump on how far they hop? Pre-landing activity timing and intensity in forelimb muscles of hopping *Bufo marinus*. *Biol. Lett.* 6, 486 – 489. (doi:10.1098/rsbl.2009.1005)

Gillis G, Ekstrom L, Azizi E. (2014). Biomechanics and Control of Landing in Toads, *Integrative and Comparative Biology*. 54(6) 1136–1147
<https://doi.org/10.1093/icb/icu053>

Gosselin LE, Burton H. 2002 Impact of initial muscle length on force deficit following lengthening contractions in mammalian skeletal muscle. *Muscle Nerve* 25, 822–827. (doi:10.1002/mus.10112)

Griep, S., Schilling, N., Marshall, P., Amling, M., Hahne, L. M. and Haas, A. (2013). Pectoral girdle movements and the role of the glenohumeral joint during landing in the toad, *Rhinella marina* (Linnaeus, 1758). *Zoomorphology* 132(3), 325-338. doi:10.1007/s00435-013-0189-0

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics*, 3(3), 034001. doi:10.1088/1748-3182/3/3/034001

Herzog, W., Powers, K., Johnston, K., & Duvall, M. (2015). A new paradigm for muscle contraction. *Frontiers in Physiology*, 6, 174. <http://doi.org/10.3389/fphys.2015.00174>

Hsu, T. and Huang, C. (2002). Influence of different heights on mechanics of children's landing. In *International Symposium on Biomechanics in Sports*, pp. 440-442.

Konow, N. and Roberts, T. J. (2015). The series elastic shock absorber: tendon elasticity modulates energy dissipation by muscle during burst deceleration. *Proc. R. Soc. B Biol. Sci.* 282, 1-8. doi:10.1098/rspb.2014.2800

Liebermann, D. G., & Goodman, D. (2007). Pre-landing muscle timing and post-landing effects of falling with continuous vision and in blindfold conditions. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, 17(2), 212–27. doi:10.1016/j.jelekin.2006.01.011

Liu, X., Wang, X., Niebur, G. (2003). Effects of damage on the orthotropic material symmetry of bovine tibial trabecular bone. *Journal of Biomechanics*. 36, 1753–1759.

Melvill Jones and Watt (1971). Muscle control of landing from unexpected falls in man. *J Physiol (Lond)*, 219 (1971), pp. 729-737

Peng, H.-T., Kernozek, T. W. and Song, C.-Y. (2011). Quadricep and hamstring activation during drop jumps with changes in drop height. *Phys. Ther. Sport* 12, 127-132. doi:10.1016/j.ptsp.2010.10.001

Phillips, B., Brown, G., Webb, J., Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature* 439(7078), 803

Proske U, Morgan DL. (2001) Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J. Physiol. Lond.* 537, 333 – 345. (doi:10.1111/j.1469-7793.2001.00333.x)

Santello, M., McDonagh, M. J. and Challis, J. H. (2001). Visual and non-visual control of landing movements in humans. *J. Physiol.* 537, 313-327. doi:10.1111/j.1469-7793.2001.0313k.x

Robins A, Rogers L. (2006) Complementary and lateralized forms of processing in *Bufo marinus* for novel and familiar prey. *Neurobiol. Learn Mem.* 86, 214–227. (doi:10.1016/j.nlm.2006.03.002)

Roster, N.O., D.L. Clark and J.C. Gillingham. (1995). Prey catching behavior in frogs and toads using video simulated prey. *Copeia* 1995:496-498

Talbot JA, Morgan DL. (1998) The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *J. Muscle Res. Cell Motil.* 19, 237–245. (doi:10.1023/A:1005325032106)

Urban, M., Phillips, B., Skelly, D., and Shine, R. (2008). A Toad More Traveled: The Heterogeneous Invasion Dynamics of Cane Toads in Australia. *The American Naturalist*, 171(3). doi:10.1086/527494

Taylor, M. R. (2001). The Role of Visual and Auditory Senses in Prey Detection by the Southern Toad, *Bufo terrestris*. *Beta Beta Beta Biological Society*, 72(3), 83-86.

Vallortigara G, Rogers LJ, Bisazza A, Lippolis G, Robins A. (1998) Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *Neuroreport* 9, 3341–3344. (doi:10.1097/00001756-199810050-00035)CrossRefPubMedWeb of Science

Vogel, S. (2013). *Comparative biomechanics: Life's physical world*. Princeton, NJ: Princeton University Press.