

I give permission for public access to my thesis and for copying to be done at the discretion of the archives' librarian and/or the College library.

---

Signature

---

Date

Does Height Matter? The Effects of Take-off Elevation on Forelimb Movements during  
Jumping and Landing in Cane Toads

by

Eleni Electra Karagiannis

A Paper Presented to the  
Faculty of Mount Holyoke College in  
Partial Fulfillment of the Requirements for  
the Degree of Bachelors of Arts with  
Honor

Department of Biological Sciences  
South Hadley, MA 01075

May 2013

This paper was prepared  
under the direction of  
Professor Gary Gillis  
for eight credits.

I dedicate my thesis to my family who has been so supportive of me and my educational endeavors.

## **ACKNOWLEDGMENTS**

First and foremost, thank you to Professor Gary Gillis for being a supportive thesis advisor and giving me the opportunity to be a part of his research lab and conduct my study. Funding from the National Science Foundation grant IOS-1051603 made this project possible. Thank you to Pamela Matheson for all her help on the statistical analysis on my results and for everything she has taught me. Thank you to my lab mates Flynn Vickowski, Nell Maynard, and Sufiya Shaikh for all their help during my experiments. And thank you to my family and friends who have been amazing and so supportive of me during my senior as I completed my study.

## TABLE OF CONTENTS

	Page
List of Figures .....	vii
List of Tables .....	viii
Introduction .....	1
Materials and Methods .....	10
Results .....	20
Discussion .....	45
Literature Cited .....	53

## LIST OF FIGURES

	Page
Figure 1. Diagram of landing in a jump.....	5
Figure 2. Diagram of parts of a jump.....	7
Figure 3. Diagram of arciferal pectoral girdle.....	7
Figure 4. Demonstration of label application on an animal.....	11
Figure 5. Picture of a Cane toad with numbered labels.....	14
Figure 6. Picture of digitizing with Matlab.....	15
Figure 7. Diagram of humeral protraction/retraction and elevation/depression.....	17
Figure 8. Example of elbow angle, humeral protraction/retraction, and humeral elevation/depression plotted against time for level hops.....	22
Figure 9. Plots of preparatory extension vs. hop distance in four cane toads.....	25
Figure 10. Plots of impact flexion vs. hop distance in four cane toads.....	26
Figure 11. Example of elbow angle, humeral protraction/retraction, and humeral elevation/depression plotted against time for elevated hops.....	28
Figure 12. Box and whisker plot of preparatory extension.....	31
Figure 13. Linear regression of preparatory extension versus hop distance for level and elevated hops.....	32
Figure 14. Box and whisker plot of impact flexion.....	34
Figure 15. Linear regression of impact flexion versus hop distance for level and elevated hops.....	35
Figure 16. Box and whisker plot of minimum elbow angle.....	37
Figure 17. Linear regression of minimum elbow angle versus hop distance for level and elevated hops.....	38
Figure 18. Box and whisker plot of preparatory protraction.....	40

Figure 19. Box and whisker plot of impact retraction.....	41
Figure 20. Box and whisker plot of preparatory depression.....	43
Figure 21. Box and whisker plot of impact elevation.....	44



## LIST OF TABLES

	Page
Table 1. Forelimb movement variables and their averages in level hops.....	23
Table 2. Forelimb movement variables and their averages in elevated hops.....	29
Table 3. Forelimb movement variables and their grand averages in level and elevated hops.....	49

## ABSTRACT

Past research has shown the significance of the musculoskeletal system in enabling deceleration during certain actions, including landing from a step, hop or jump. For instance, in toads, landing forces increase with hop distance, and pre-landing forelimb muscle activity is greater in longer hops. Toads prepare for landing differently depending on hop distance by altering forelimb muscle recruitment patterns prior to impact. In this study I tested whether differences in underlying muscle activity translate into differences in toad forelimb movements depending upon the length of the hop.

Using high-speed video I characterized elbow joint angular excursions and humeral movements from toads hopping on flat surfaces and from elevated platforms. I found that the animals extended their arms in mid-air while protracting and depressing their humerus until they landed. Upon landing, the forelimbs flexed as the humerus retracted and elevated. On flat surfaces, extension and flexion increased with greater hop distance, indicating a compensatory relationship between the two that helps maintain a consistent “final” elbow angle after landing. Elbow movements were greater in elevated hops, but hop distance no longer influenced these variables. Thus, arm movements do appear to depend on hop distance, but this dependence is eliminated during elevated hops.

## INTRODUCTION

During most forms of terrestrial locomotion, periodically impacting the ground is a common phenomenon found across a wide variety of species, including those that hop and jump to get around as well as those that walk and run. For instance, as humans we perform simple walking movements daily, and for each step we initiate, we must land, or impact the ground, at the end of that step. In order to keep walking smoothly we have to land properly for each step, and this is done by preparing the limb for the forces that will be encountered with every landing.

To control the limb at impact, muscles involved in stabilizing joints and counteracting the forces of impact must be recruited appropriately. It was initially thought that stretch reflexes controlled lower limb movements during locomotor impacts such as those involved in stepping, jumping and falling (Jones and Watt 1971a). Studies in the 1970's tried to determine the importance of stretch reflexes in controlled landing. A stretch reflex refers to the contractile response of an innervated skeletal muscle to stretching to regulate muscle length, as seen in routine neurological exams on the knee, ankle, or triceps, for instance (Purves et al. 2001). Studies investigating the importance of such stretch reflexes in the 1970's involved electromyography (EMG), in which electrodes were placed on the surface of the lower limbs of human subjects to measure electrical activity in the gastrocnemius muscle, an ankle extensor (Jones and Watt 1971a). The subjects wore a hinged plate to determine flexion of the ankle joint, and were asked to step down or hop on a force plate. It was found that electrical activity in

the gastrocnemius began prior to landing and thus prior to any impact-related foot and leg movements that could lead to muscles being lengthened and cause a stretch reflex. Therefore, the stretch reflex could not be the only muscle activation route involved in controlled landing, and because the force development associated with the stretch reflex after impact was shown to be so small as to be insignificant, it was concluded that it has no useful contribution to landing (Jones and Watt 1971a).

These initial studies involved voluntary movements (stepping, hopping) of experimental subjects and later work began to focus on landing during unexpected falls in humans. In studies of this nature, subjects were suspended from a bar at different heights and released unexpectedly onto a force plate. EMG data was again recorded for the gastrocnemius muscle. Results indicated that the subjects changed how they prepared for landing depending on the height of the fall (Jones and Watt 1971b). In particular, recruitment intensities were higher before landing during drops from higher heights. The authors concluded that that these adjustments in preparation likely involved an anticipatory response that might be “an accurately timed burst of pre-programmed muscle activity” (Greenwood and Hopkins 1975).

To test this idea of a pre-programmed response, Greenwood and Hopkins (1975) measured EMG activity in the same muscle, but at a greater range of fall heights. Subjects were suspended in a harness, and released unexpectedly from varying heights. Two bursts of EMG activity occurred: the first was a startle

reaction, and the second occurred consistently 200-300 ms after release and prior to landing. It was concluded that this second burst of activity was “timed to occur before landing” (Greenwood and Hopkins 1975). It is therefore likely that the second peak of activity in this study acts as the timed pre-programmed activity to which Jones and Watt referred. The intensity of the activity was also directly related to the distance the subjects fell, and the conclusion was that this activity was involved in the control of landing (Greenwood and Hopkins 1975). In addition, the study also examined landing when subjects were blindfolded, and found that similar bouts of anticipatory limb muscle activity still occurred, indicating that vision was not necessary for recruiting leg muscles in order to stabilize landing during a fall or hop (Greenwood and Hopkins 1975).

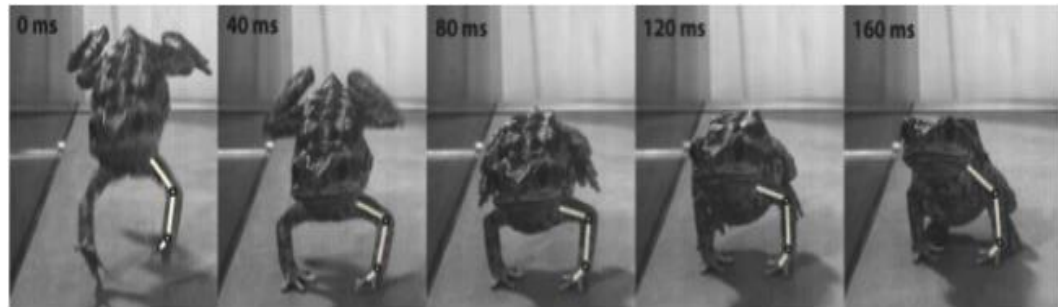
Modulation of pre-landing muscle recruitment during jumps of different magnitude is also seen in other mammals. For instance, pre-landing muscle activity is observed in the gastrocnemius muscle in forelimbs of cats jumping down onto a surface, and this activity is similarly scaled in intensity to jump height to help decelerate and stabilize the body at impact (Prochazka et al. 1977). Similarly, pre-landing muscle recruitment intensity in the triceps muscles of monkeys jumping down onto a platform was also found to increase with increasing vertical jump heights (Dyhre-Poulson and Laursen 1983). Recently, work in the Gillis lab has demonstrated that members of the order Anura (frogs and toads) have also been shown to modulate pre-landing muscle recruitment

patterns in preparation for landing from hops of different distance (Gillis et al., 2010).

Anurans are a lineage within the class Amphibia that includes all extant frogs and toads. Most of these animals live in or near water in habitats varying from tropical rainforests and mountaintops to swamps (Cogger and Zweifel, 1998), which may contain rivers, lakes, and ponds (Noble, 1931). Some species of anurans live in a much more terrestrial setting, even including deserts (Cogger and Zweifel, 1998). All anurans develop into quadruped adults, but different species develop different methods of moving about. Most terrestrial anurans perform saltatory locomotion (jumping and hopping), and most species of anurans that perform saltatory locomotion have elongated toe and tarsal bones in the hind limbs to “provide a broad platform for jump takeoffs” (Wells, 2007). Moreover, unlike other vertebrate tetrapods, frogs have longer hind limbs (with a fused tibio-fibula) than forelimbs (with a fused radio-ulna), and a shortened vertebral column. These features enhance a frog’s ability to jump (Wells 2007).

Because jumping and hopping are the most common forms of movement in frogs and toads, they have been studied most often. Most work done on the neuromuscular control of anuran jumping has focused on the role of the hind limbs during take-off. We now know that all major hind limb muscles are active as anurans generate the forces that propel them into the air during a jump (Marsh 1994; Olson and Marsh 1998; Gillis and Biewener 2000). Longer jumps presumably lead to greater impact forces, but considerably less work has focused

on the landing phase of the jump, in which the forelimbs are generally used to initially decelerate the body at impact (Fig. 1) (Peters et al. 1996; Nauwelaerts and Aerts 2005).



**Figure1.** Cane toad using its forelimbs to decelerate and control the body at impact.

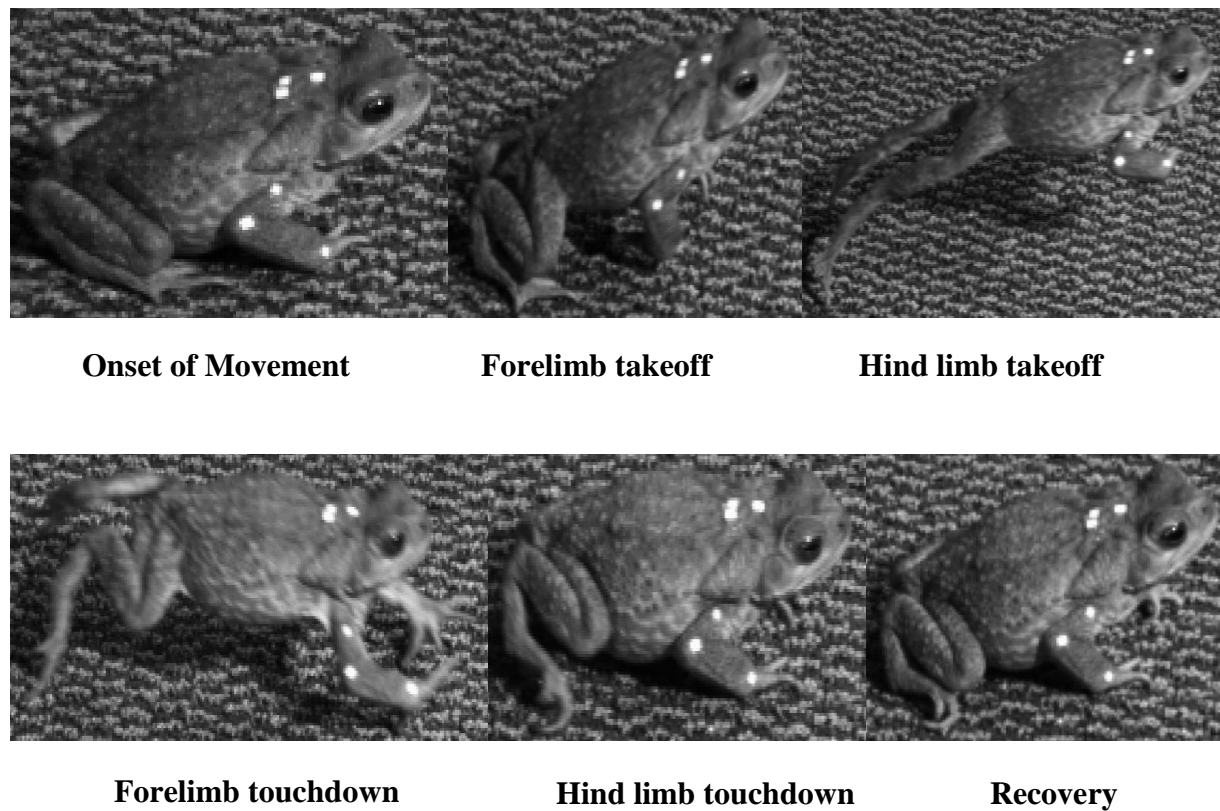
#### *Anuran Locomotion*

In anuran locomotion, a jump is defined as a leap that is at least eight times as long as the animal's body length; hops on the other hand are shorter (Zug 1985). Jumps and hops are made up of four intervals: propulsion, flight, landing and recovery (Fig. 2) (Nauwelaerts and Aerts 2005). Propulsion occurs following onset of movement, during which ground reaction forces slowly increase as the animal pushes against the takeoff surface while extending its hind limbs. Once the hind limbs leave the ground, the animal is in the flight phase until it touches down or lands, generally with its forelimbs, on the ground again. The end of landing occurs when the horizontal ground reaction forces are minimal and the vertical forces are equal to the animal's weight. Recovery begins at the end of landing,

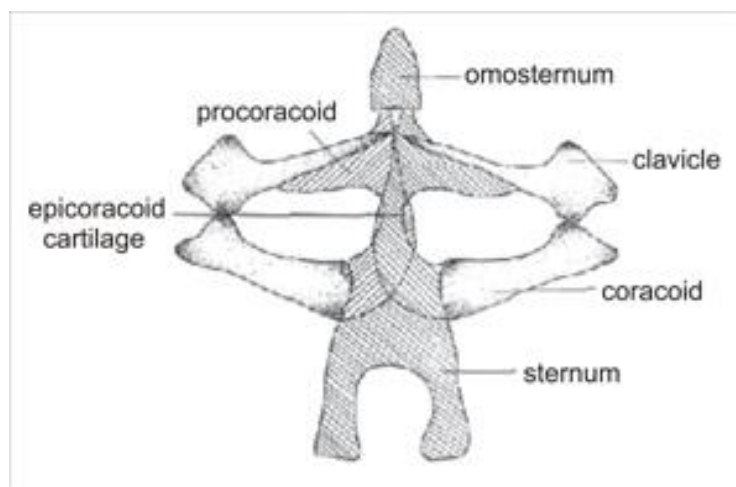
during which the animal repositions itself to prepare for the next jump or hop (Nauwelaerts et al. 2005).

Studies in primitive anurans have shown that forelimb use in landing is a derived behavior. For instance, animals within the family Leiolopomatidae, or “tailed frogs,” lack forelimb preparation prior to landing and instead make initial ground contact with their head (Essner et al. 2010). In most anurans, however, forelimb landing is common, and the landing forces are typically high. For example in *Rana esculenta*, the forces experienced by the forelimbs at impact are greater than those generated by the much bigger legs during takeoff (Nauwelaerts and Aerts, 2005). Because there are great forces experienced in landing, the muscles of the arms and pectoral region must generate appropriate forces to control and decelerate the body. The longer the hop, the greater the forces the forelimbs must encounter. In addition to using muscle activity to manage impact forces, the forelimbs can also handle landing in some species because during impact, the arciferal girdle (Fig. 3) undergoes compression, resulting in compression of the coracoids and tension of the epicoracoid cartilage. The compression of the arciferal girdle allows for deceleration over a greater distance (Emerson 1988).





**Figure 2.** A series of images outlining propulsion, flight, landing, and recovery in a jump or hop.



**Figure 3.** Diagram of arciferal pectoral girdle.

Among anurans, cane toads (*Bufo marinus*) have recently been used as a model to study landing behavior because they control and decelerate their bodies smoothly after impact (Gillis et al., 2010). Studies using electromyography to examine recruitment patterns in various chest and forelimb muscles in cane toads have been performed to understand the neuromuscular control of the cane toad's landing behavior. It has been found that electrical activity occurs in toad forelimb muscles as animals extend their arms toward the ground in preparation for landing (Gillis et al. 2010; Akella and Gillis 2011), and the intensity of this pre-landing muscle activity is positively correlated to hop distance (Gillis et al. 2010). Moreover, onset timing of muscle activity changes with increasing hop distance, so that onset occurs later in longer hops and thus at a nearly fixed interval prior to impact, suggesting the likely importance of the underlying forelimb muscle activity in preparing for and coordinating landing (Gillis et al. 2010).

Given that pre-landing forelimb muscle recruitment in toads predictably changes with hop distance and impact force, the question arises as to whether differences in underlying muscle activity patterns translate into differences in the ways the forelimbs are moved in preparation for landing, and if so, how. Further, because landing forces can be manipulated by altering the height of the takeoff surface, one can also address the flexibility of landing preparatory movements in these animals (i.e., will they prepare for landing differently between level hopping and when takeoff and landing heights are offset?).

### *Hypotheses*

It is clear from past studies that the intensity of pre-landing muscle recruitment increases with increasing hop distance. I hypothesize that for level jumping, the differences in pre-landing muscle recruitment patterns will translate into differences in forelimb movements, so that toads will undergo greater degrees of arm movement before and after impact in longer hops.

For the elevated jumps, since the additional vertical height in take-off will result in greater vertical and horizontal distances traveled for a given amount of effort during takeoff, the animals will spend more time in the air and land with greater impact forces. I hypothesize that animals will extend their arms farther, prior to impact, than they would during a level jump, and that they will flex more after impact as well.

## MATERIALS AND METHODS

Two different studies were performed to characterize forelimb movements during hopping. One was completed over the summer and involved studying landing during level hops. The second was completed during the school year and focused on comparing landing between elevated and level hops.

### *Animals*

For the summer study, four cane toads (*Bufo marinus*) were used, while the comparative study involved the use of five cane toads. The first set of animals ranged in size from 137-213 grams, and the second set of animals ranged in size from 149 to 263 grams. All were purchased from the same commercial supplier in Florida. They were kept in glass or plastic tanks in groups of one to three. All animals were provided a bowl of water for hydration and were fed crickets three times a week. They were kept in a room with a constant temperature of 24°C and a schedule of 12 hours of light followed by 12 hours of dark.

### *Jumping Trials*

To measure forelimb movements during hopping, three white cardboard squares (0.3 cm X 0.3cm) were glued onto the skin of the forelimb using Elmer's crazy glue close to the shoulder, on the elbow and on the wrist in each toad. These marked points represented the outline of the elbow angle. I also used three

cardboard points to mark a right angle on the back of the toads to have a reference x-y-z coordinate system with which to measure humeral movements relative to the body (see Figures 4 and 5 for marker placement) and distances covered by the animal during each hop. This marking process was utilized in both studies.



**Figure 4.** Demonstration of applying the markers on the back and the forelimb of a cane toad.

For the summer study, the animals were placed in a glass tank measuring 45cmX90cmX43cm after they were marked up and were hopped only off the flat, bottom surface of the tank. In the second study the animals were placed in a carpeted hopping arena that measured 61cmX183cm. Half of the hopping arena was framed using grey foam poster boards with a height of 30.5 cm fortified by duct tape. This served as a back drop for the jumping arena and maximized lighting for video recordings. Animals were hopped in two conditions, either taking off of the flat surface of the carpeted jumping arena, or off of a 7.6 cm carpeted elevation pad. The elevation was created using three vhs tapes bound together with duct tape. This elevation was chosen because preliminary work showed that using heights much higher than this led to unsuccessful landings.

In both studies, lighting was provided by 600-watt Lowel Omni Total-lights. To initiate hops, animals were set down onto the hopping surface by hand. Sometimes the animals would hop without stimulation a few seconds after release. If not, clapping and hitting the surface of the hopping arena were used to stimulate the animals to hop. In the first study, 8-13 hops were recorded for each animal studied. In the second study, 15 hops were recorded for both level and elevated hops for each animal studied. All hops were recorded at 500 frames per second using two high speed video cameras (Fastec Imaging, model Hispec1 2G Mono) positioned above the hopping arena and perpendicular to one another. Only hops that were relatively straight and in which all the marker points were visible at all times were saved as uncompressed avi files.

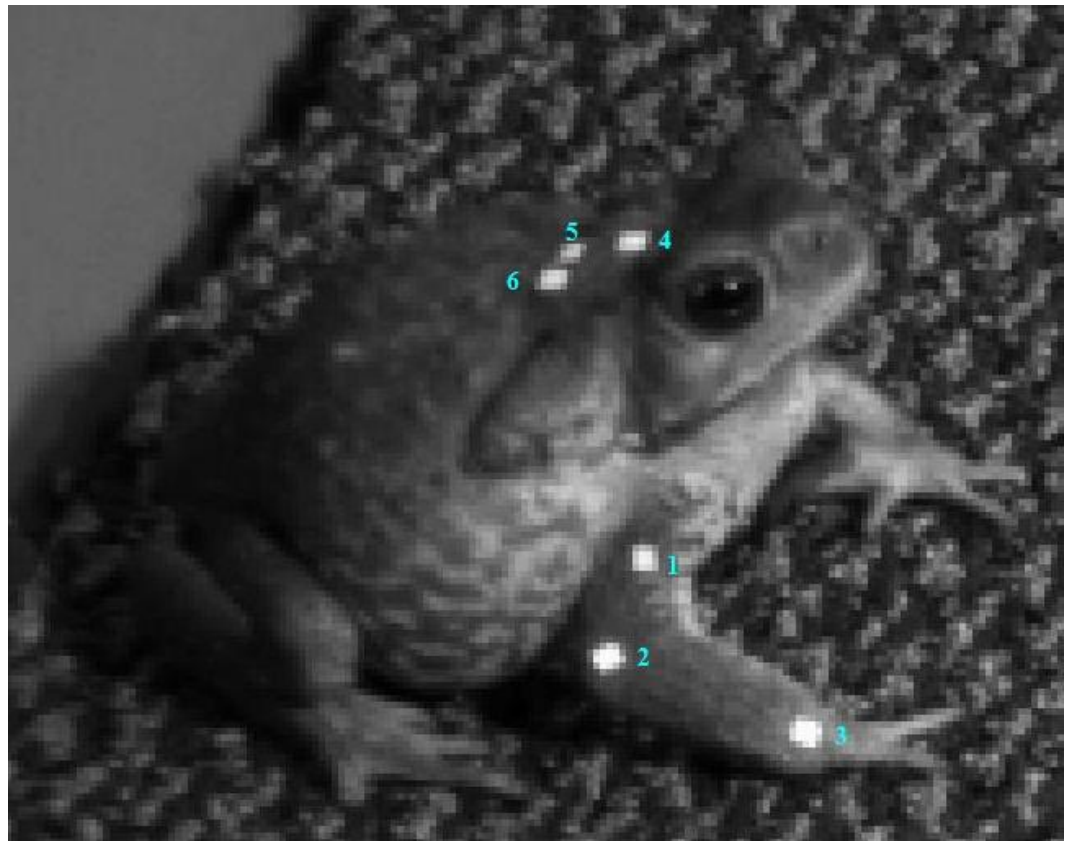
### *Data analysis*

For both studies, calibration of the jumping area was completed in the same way. Calibration was completed prior to recording hops in order to define the scale in all three dimensions in the videos. A 64 point cube with a side length of 21.6 cm was placed in the jumping arena and positioned in a way so that from both cameras, almost all of the points were visible. A jpeg image from each camera view of the cube was taken from a frame of a video recording. The calibration was performed by uploading one of the camera images through the Matlab routine `DLTcal5.calib`, and labeling all of the visible points. Then the second image was uploaded and the points were labeled again.

Once the hopping trials for an animal were completed, the video recordings were used to determine the timing of important, readily identifiable events during a hop, including the onset of movement, forelimb take off (when the forelimbs left the ground), hind limb take off (when the hindlimbs left the ground) and forelimb landing. The timings of these events were recorded in milliseconds. The videos were also used to digitize the markers on the toads using a custom-designed Matlab routine named `DLTdv5` (Hedrick 2008).

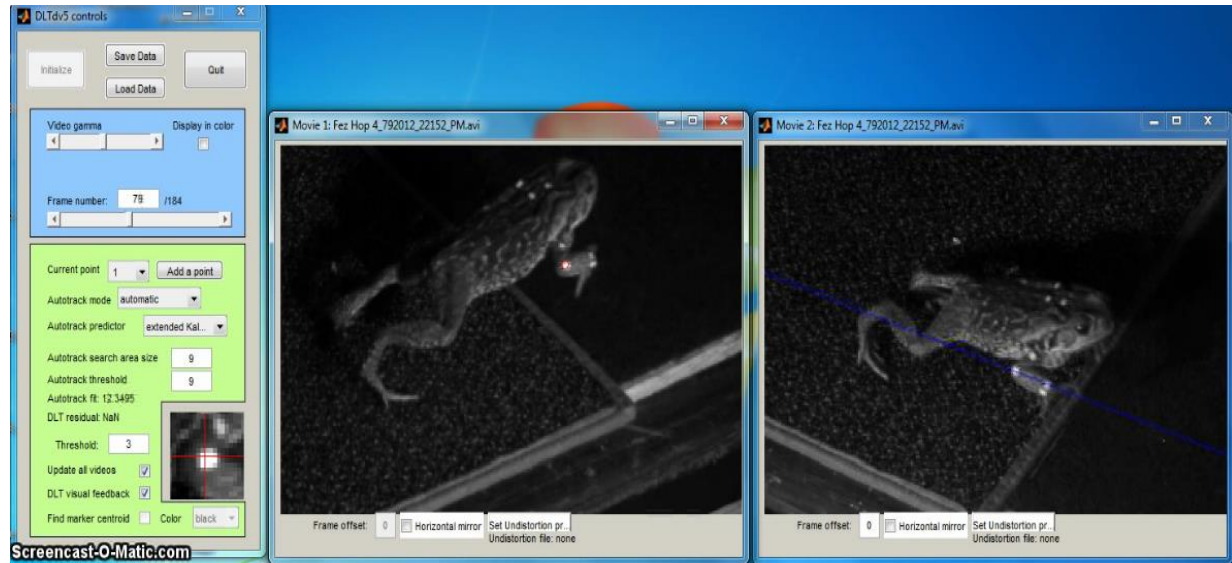
To digitize arm and body points, videos from both cameras of each hop were uploaded, followed by the appropriate calibration file. To digitize, I used the computer's mouse to locate and click on the first point in one of the videos (Fig. 5). I would then use an auto track function, which allowed the computer to find that point in each of the remaining frames of the video (Fig. 6). Occasionally auto

tracking would miss the actual point and manual correction was required. After digitizing point one from every video frame from one camera's view, I would digitize the next point in the same manner, proceeding in order until all the points were completed from that camera's vantage point. All six points were then digitized using the same procedure in the second video in the same manner.



**Figure 5.** Image of animal with all six points labeled 1-6.

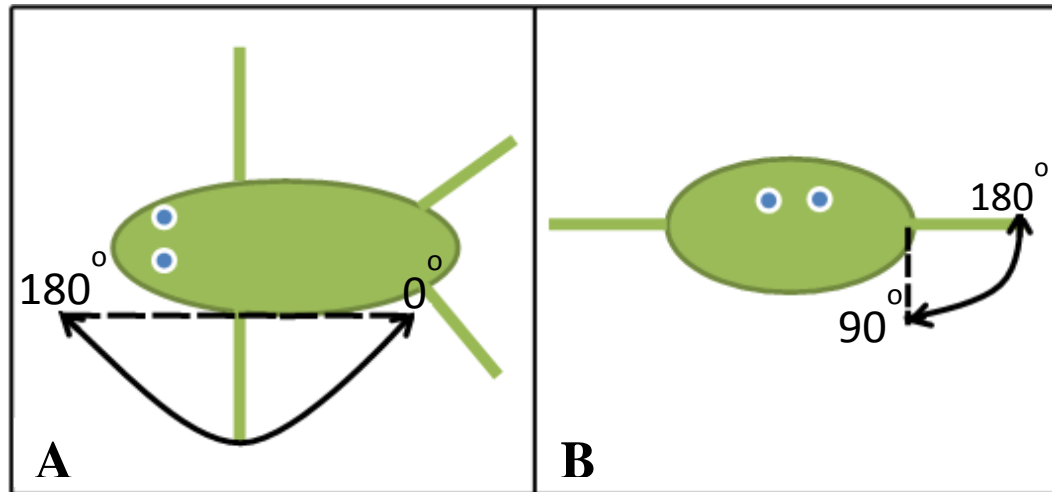




**Figure 6.** Image of matlab program DVLt5 tracking the location of a point in one video, while showing the blue line through the other video to demonstrate the precision of the calibration.

After all six points were digitized from both cameras' vantage points, the Matlab routine created a spreadsheet of the x, y and z coordinates for each point frame by frame in a hop. These coordinate data were then used by additional Matlab routines to calculate the following variables, 1) elbow flexion/extension angle, 2) humeral protraction/retraction angle, and 3) humeral elevation/depression angle, during each frame of every video (Fig. 7). Coordinate data were also used to determine the horizontal distance achieved in each hop.

For elbow angle, extension is defined as an increase in elbow angle, while flexion is defined by a decrease in elbow angle. Protraction and retraction refer to humeral movements that occur parallel to the plane of the animal's back in the anterior (protraction) and posterior (retraction) directions. Full protraction was defined by a value of 180 degrees while full retraction was defined by a humeral angle of zero degrees (Fig. 7A). Elevation and depression refer to humeral movements that are perpendicular to the long axis of the body. Elevation occurred when the animals lifted their humerus up towards the level plane defined by the back. An angle of 180 degrees meant the humerus was parallel to the back itself. In contrast, depression occurred when the animals lowered their humerus below the level plane of the back (Fig. 7B).



**Figure 7.** Panel A shows a sketch of protraction and retraction in a cane toad. Panel B shows a sketch of elevation and depression.

### *Statistics*

For the first study, means and standard deviations for preparatory extension and impact flexion were collected. Linear regression was used to look at the effects of hop distance on these variables. For the second study, means and quartiles for seven variables (preparatory extension, impact flexion, minimum elbow angle, preparatory protraction and depression, and impact retraction and elevation) were determined through excel and were used to design error bars for the plotted results. Descriptive stats were calculated for level and elevated hops, and then the effects of elevation were examined.

Research was conducted as a two-way within-subjects design, with a time varying covariant. The primary independent variable was elevation (level or elevated take off), the 15 repetitions or trials were also treated as an independent variable and considered (although not part of the hypothesis testing) in the analysis as an effect of time. The Statistical Packages for the Social Sciences (SPSS, version 19) was used to evaluate the effect of elevation on seven separate dependent variables (preparatory extension, impact flexion, minimum elbow angle, preparatory protraction, impact retraction, preparatory depression, and impact elevation). For each trial, hop distance was recorded in order to evaluate it and control for this possible confounder. Due to the complexity of the research design, the mixed linear model procedure in SPSS was used to conduct the seven separate analyses of covariance (ANCOVA). Prior to conducting the analyses, tests of linearity and homogeneity of regression slopes were conducted for each

ANCOVA. For dependent variables that did not result in homogeneity of regression (as indicated by a significant interaction between the dependent variable and hop distance), protected pair-wise comparisons were performed (Bonferroni method) (Bland and Altman 1995) by examining the mean differences of the dependent variable between elevated and level hops at three different hop distances; low (mean -1sd), middle (mean), and high (mean+1sd) (Engqvist 2005). Apriori alpha was set at 0.05.

All of the values for hop distance and the angle variables were inserted into an excel spreadsheet in separate columns. In order to better plot and analyze the angle variables, a five point smoothing equation was used:

$$\text{Angle} = (\text{Angle1} + 3*\text{Angle2}+4*\text{Angle3}+3*\text{Angle4}+\text{Angle5})/12$$

In the smoothing equation, each angle involved in the calculation represents an angle measurement for a different frame. For instance, for smooth elbow angle, I took the five angle measurements from the first five frames of a recorded hop to produce a “smoothed” elbow angle value.

From elbow angle, five angle measurements were calculated: rest angle, maximum extension 1, maximum flexion1, maximum extension 2, and maximum flexion 2. These were then used to determine preparatory extension and impact flexion. A spreadsheet was made for every hop of an experiment, and a cumulative spreadsheet was also made to create box and whisker plots and examine the angle variables in elevated versus level hops.

## RESULTS

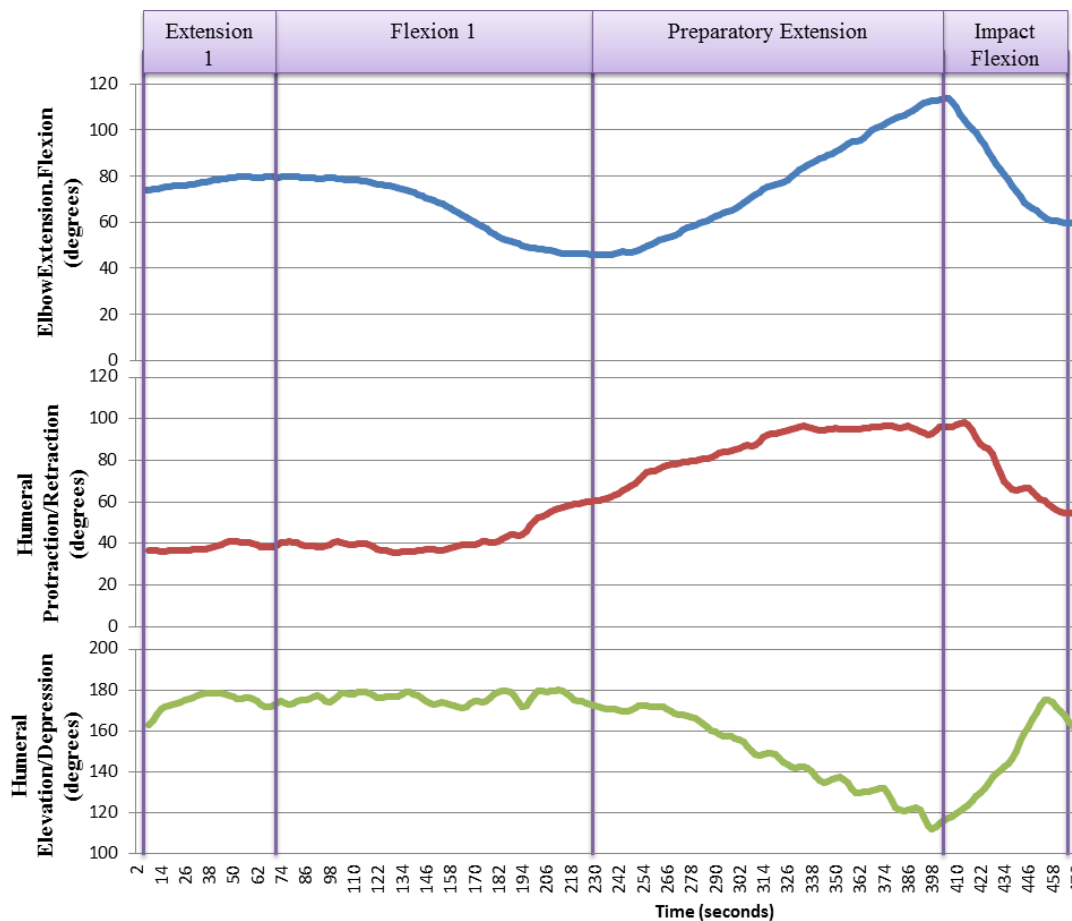
### *Patterns in level hopping*

All four animals from the summer demonstrated similar forelimb movements during hopping. Using excursions of the elbow angle I divided hops into four phases (Fig. 8). Phase 1, termed extension one, began with the onset of movement and was typically characterized by a small degree of elbow extension as the forelimbs pushed against the ground in the early stages of the hop (Fig. 8). Amounts of elbow extension during this phase ranged from  $0.8^{\circ}$ - $12^{\circ}$ , averaging  $6^{\circ}$  (Table 1).

Phase 2, termed flexion one, began when an animal lifted its forearms toward the body by flexing its elbows during take-off (Fig. 8). Amounts of elbow flexion during this phase ranged from  $25^{\circ}$ - $36^{\circ}$ , averaging  $30^{\circ}$  (Table 1). Phase 3, termed preparatory extension, was characterized by the animal extending its arms in mid-air to prepare for impact (Fig. 8). Amounts of elbow extension during this phase ranged from  $32^{\circ}$ - $53^{\circ}$ , averaging  $40^{\circ}$  (Table 1). Phase 4, termed impact flexion, began with ground impact and was characterized by elbow flexion as the forelimb came into contact with the ground and the arm responded to the force of impact (Fig. 8). Amounts of elbow flexion during this phase ranged from  $31^{\circ}$ - $42^{\circ}$ , averaging  $35^{\circ}$  (Table 1).

Humeral movements varied among hops in each toad within the early phases of the hop (extension one and flexion one). However, the movements of the humerus were consistent during preparatory extension and impact flexion.

During preparatory extension, the animals typically underwent substantial humeral protraction and depression in preparation for impact. Such preparatory humeral protraction averaged  $71^{\circ}$  (range =  $63^{\circ}$ - $87^{\circ}$ ) while preparatory humeral depression averaged  $29^{\circ}$  (range =  $12^{\circ}$ - $38^{\circ}$ ) (Table 1). After impact, while the elbow flexed, the humerus underwent impact-related retraction and elevation. Amounts of retraction ranged from  $53^{\circ}$ - $86^{\circ}$  (average =  $64^{\circ}$ ) and amounts of elevation ranged from  $18^{\circ}$ - $34^{\circ}$  degrees (average =  $26^{\circ}$ ) (Table 1).



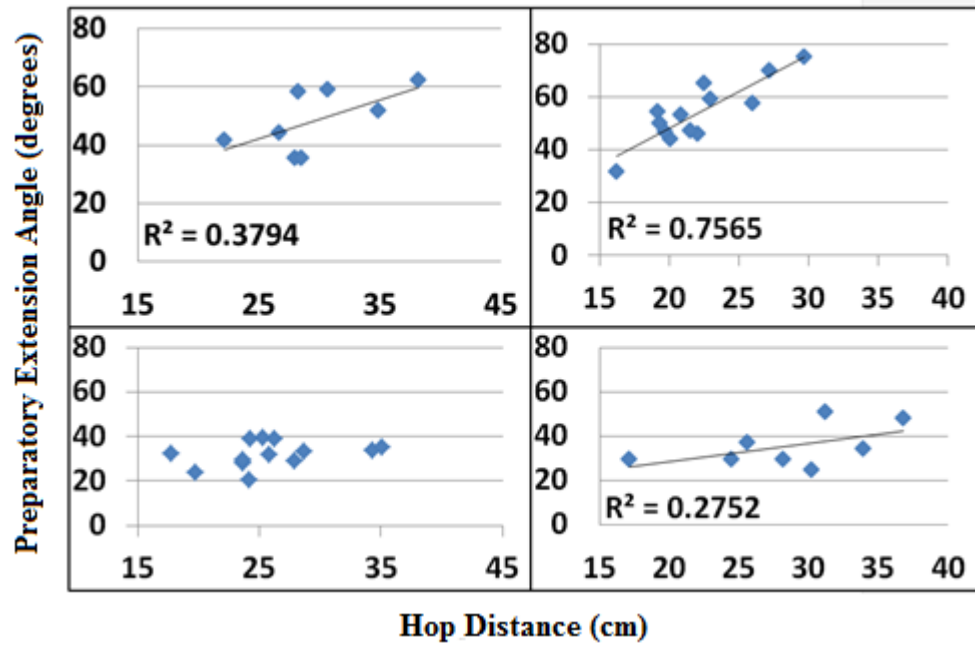
**Figure 8.** Plots of elbow extension/flexion, humeral protraction/retraction, and humeral elevation/depression vs. time for a representative level hop.



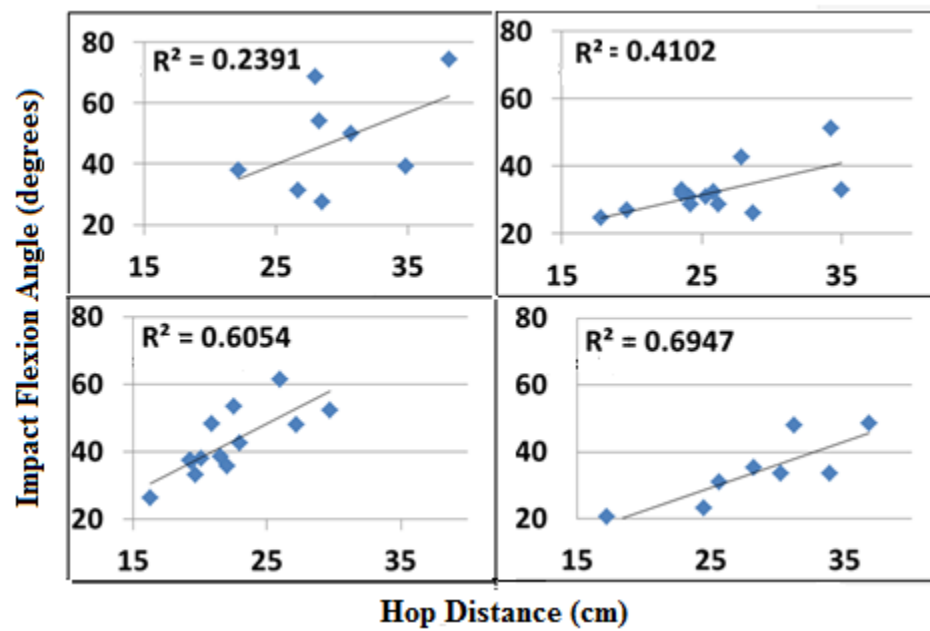
**Table 1.** Forelimb movement variables and their averages.

<b>Angle Variable</b>	<b>Angle range (degrees)</b>	<b>Average angle (degrees)</b>
Elbow Extension 1	0.8-12	6
Elbow Flexion 1	25-36	30
Preparatory Extension	32-53	40
Impact Flexion	31-42	35
Preparatory Protraction	63-87	71
Impact Retraction	53-86	64
Preparatory Depression	12-38	29
Impact Elevation	18-34	26

Preparatory extension and impact flexion were plotted against hop distance for each toad (Figs. 9 and 10). Three of the four animals demonstrated a significant positive relationship between the amount of preparatory extension and hop distance (Fig. 9). All four animals demonstrated significant positive relationships between impact flexion and hop distance (Fig. 10). Thus, both the degree of impact flexion and the amount of preparatory extension tended to increase with increasing hop distance.



**Figure 9.** Preparatory extension vs. hop distance for four cane toads.



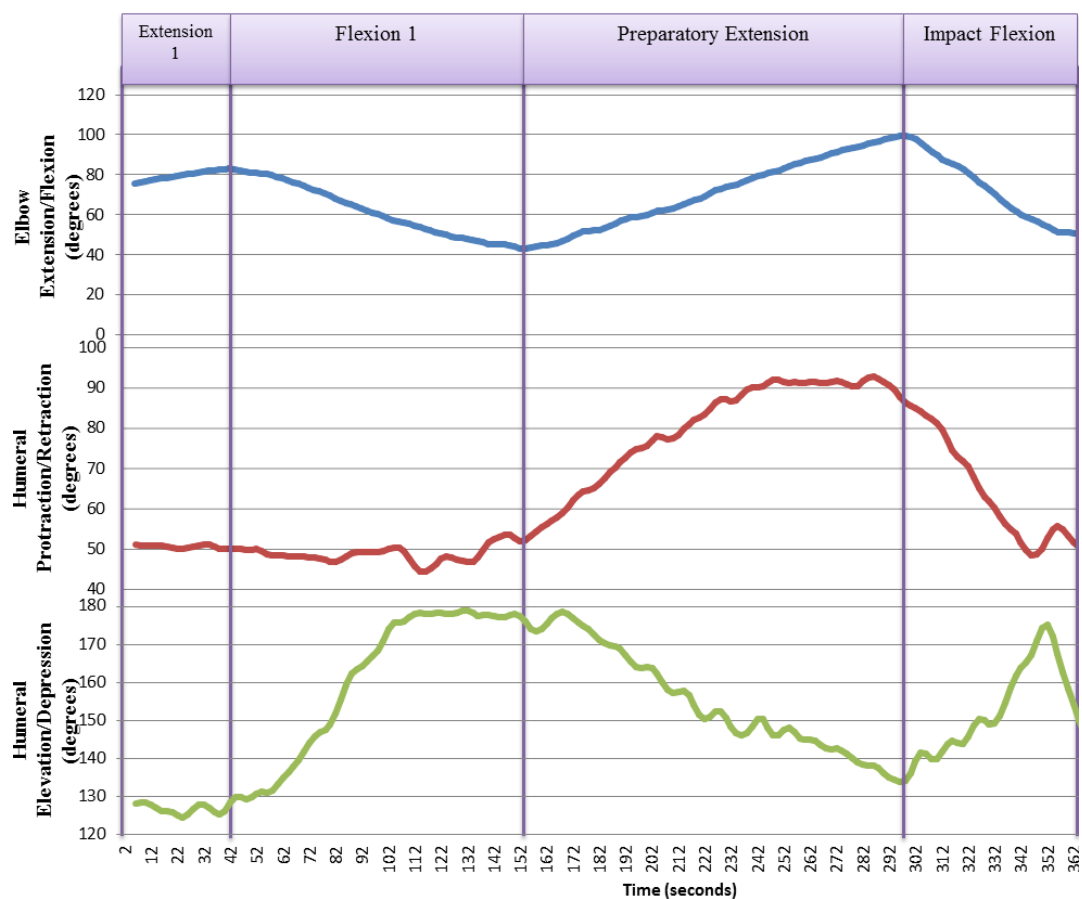
**Figure 10.** Impact flexion vs. hop distance for four cane toads.

### *Elevated hopping*

Five different toads were used to compare forelimb kinematics between level and elevated hopping. During elevated hops, toads exhibited qualitatively similar movements of the forelimb compared to level hopping. For example, the same four intervals could be identified using elbow excursions during elevated hopping (extension one, flexion one, preparatory extension, impact flexion) (Fig. 11).

During extension one, elbow extension ranged from 7°-25° degrees, averaging 18° (Table 2). During flexion one, elbow flexion ranged from 28°-42°, averaging 35° (Fig. 11). During preparatory extension, elbow extension ranged from 46°-66°, averaging 55° (Fig. 11, Table 2). During impact flexion, elbow flexion ranged from 52°-64°, averaging 57° (Fig. 11, Table 2). The animals also experienced minimum elbow angle, which is the last angle of impact flexion, and it was the smallest angle that the animals flexed to during landing. Minimum elbow angle ranged from 51°-64°, averaging 58° (Table 2).

Similar to during level hops, the animals experienced substantial humeral protraction and depression during preparatory extension. The humeral protraction averaged 62° (range = 53°-71°) while humeral depression averaged 64° (range = 31° -59°) (Table 2). After impact, while the elbow flexed, the humerus underwent impact-related retraction and elevation. Amounts of retraction ranged from 56°-77° (average = 47°) and amounts of elevation ranged from 30°-59° (average = 48°) (Table 2).



**Figure 11.** Plots of elbow extension/flexion, humeral protraction/retraction, and humeral elevation/depression vs. time for a representative elevated hop.

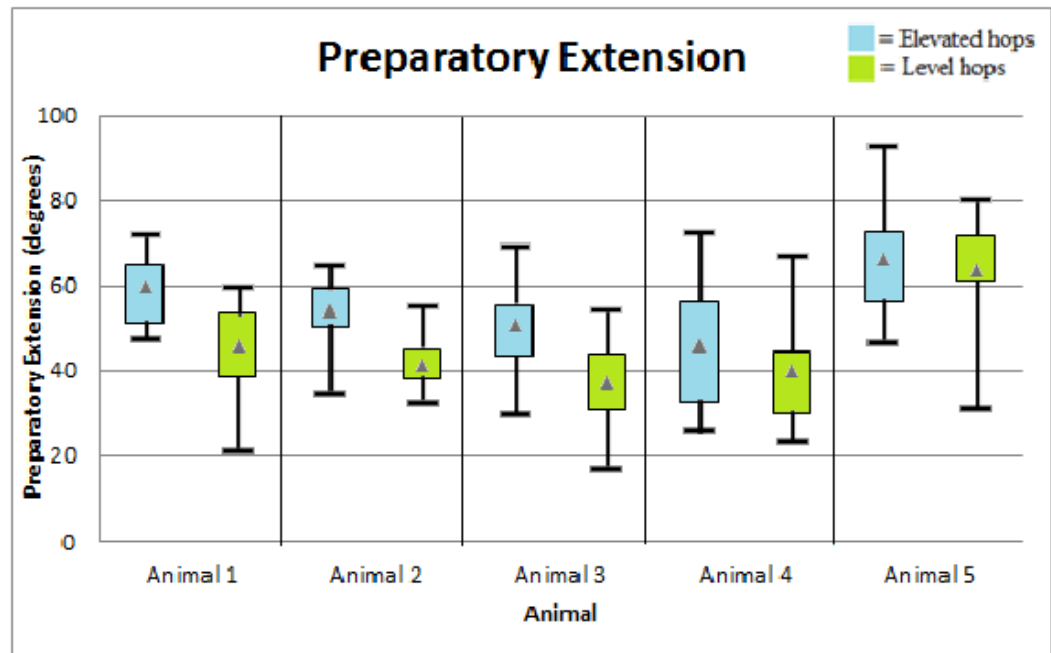
**Table 2.** Forelimb movement variables and their averages for elevated hops.

<b>Angle Variable</b>	<b>Angle range (degrees)</b>	<b>Average angle (degrees)</b>
Elbow Extension 1	7-20	18
Elbow Flexion 1	28-42	35
Preparatory Extension	46-66	55
Impact Flexion	52-64	57
Minimum Elbow Angle	51-64	58
Preparatory Protraction	53-71	62
Impact Retraction	56-77	65
Preparatory Depression	31-59	47
Impact Elevation	30-59	48

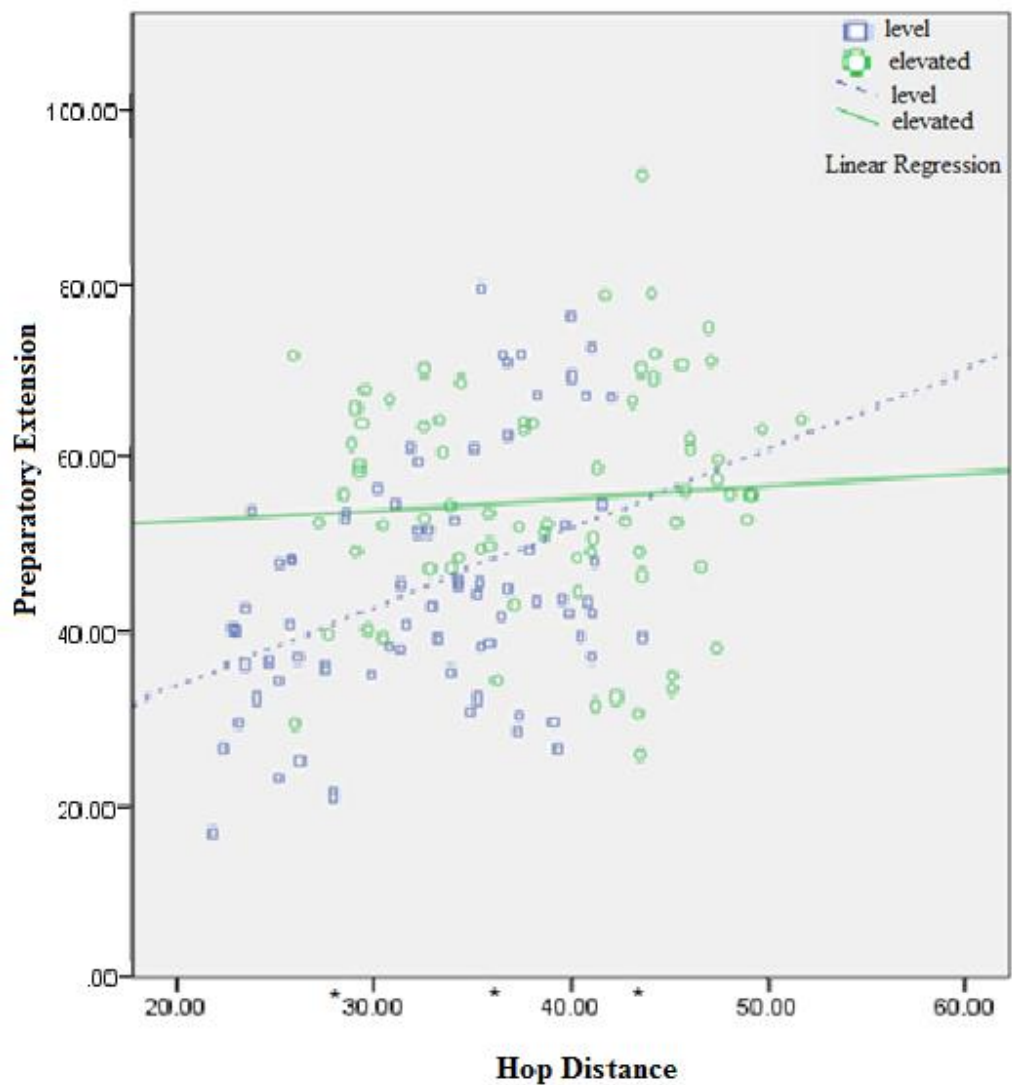
*Kinematic comparisons between level and elevated hopping*

All five animals underwent greater degrees of preparatory extension, on average, in elevated hops than in level hops (Fig. 12). The difference in preparatory extension between elevated and level hops was significant at the low hop distance of ~29 cm (one standard deviation below the mean hop distance), as well as at the average hop distance of ~36 cm (Fig. 13). The difference in preparatory extension was not significant at the high hop distance of ~43cm (one standard deviation above the mean hop distance) (Fig. 13). While there was a significant effect of hop distance on preparatory extension in level hops, no such influence was found for elevated hops (Fig. 13).



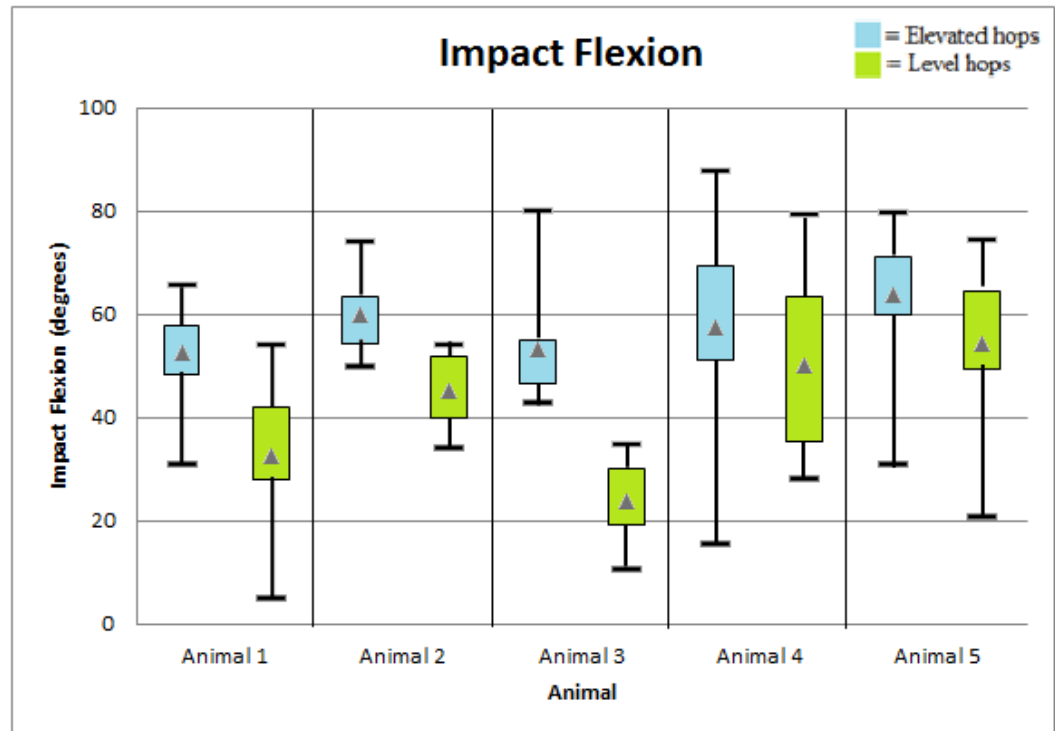


**Figure 12.** Box and whisker plot of preparatory extension in elevation trials vs. control trials for all five animals.

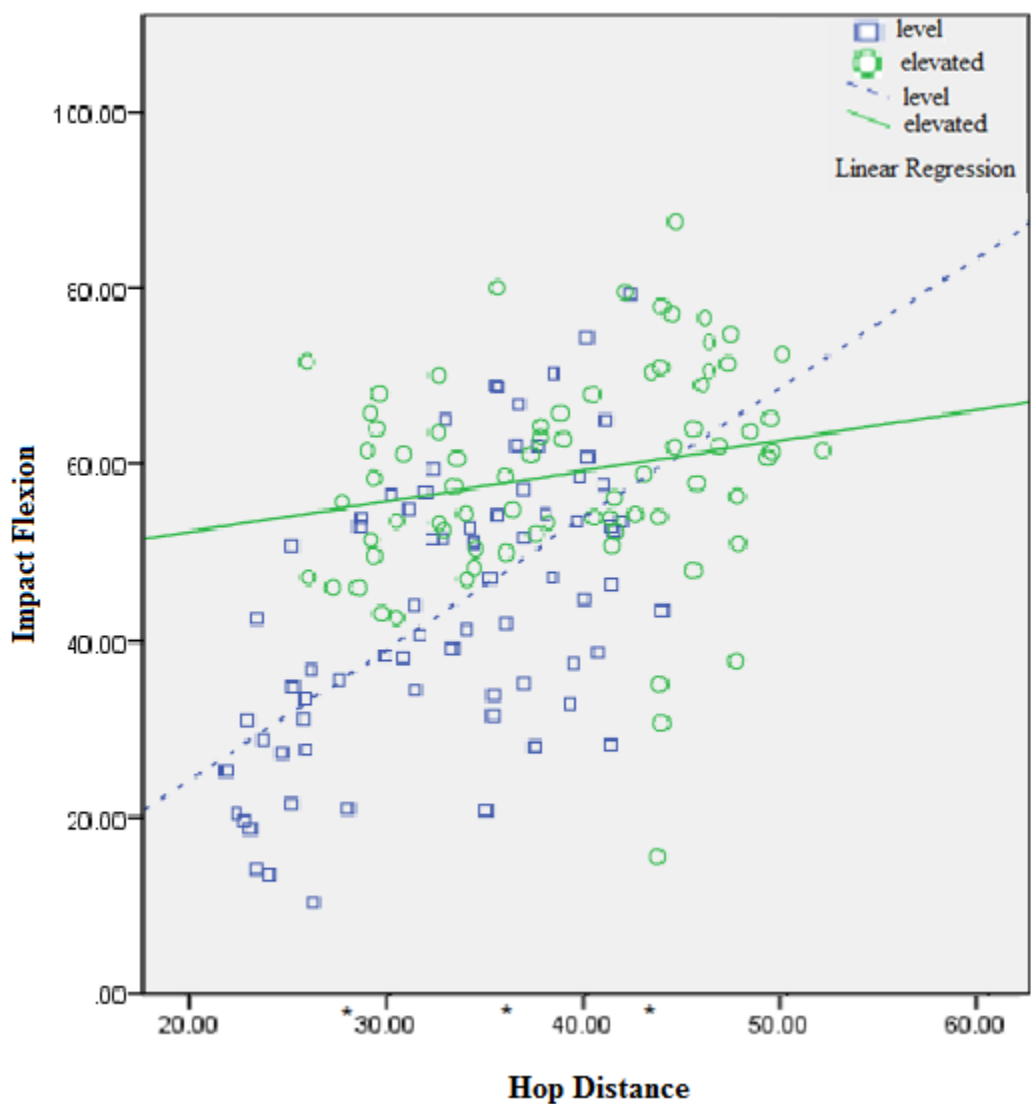


**Figure 13.** Linear regression of preparatory extension in two-way subject within-subjects design. \* indicates a hop distance at which marginal mean difference in preparatory extension was examined.  $P = 0.0001$  for low mean,  $0.016$  for main average, and  $0.858$  for high mean.

All five animals also experienced greater impact flexion, on average, in elevated hops than in level hops (Fig. 14). The difference in impact flexion between elevated and level hops was significant in short hops of ~29 cm ( $P = .0001$ ) and for hops at the average distance of ~36 cm ( $P = 0.0001$ ) (Fig. 15). During the longest hops, there was no difference in impact flexion at a high mean of ~43 cm (Fig. 15). Hop distance had a significant effect on impact flexion during level hops, but not elevated hops (Fig. 15).

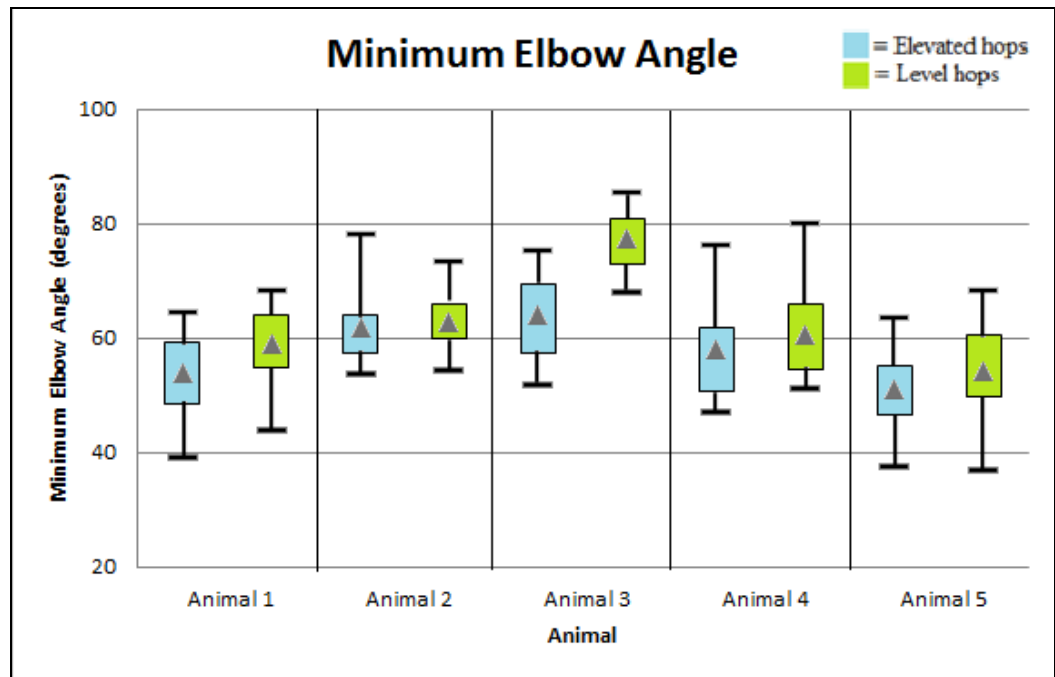


**Figure 14.** Box and whisker plot of impact flexion in elevation trials vs. control trials for all five animals.

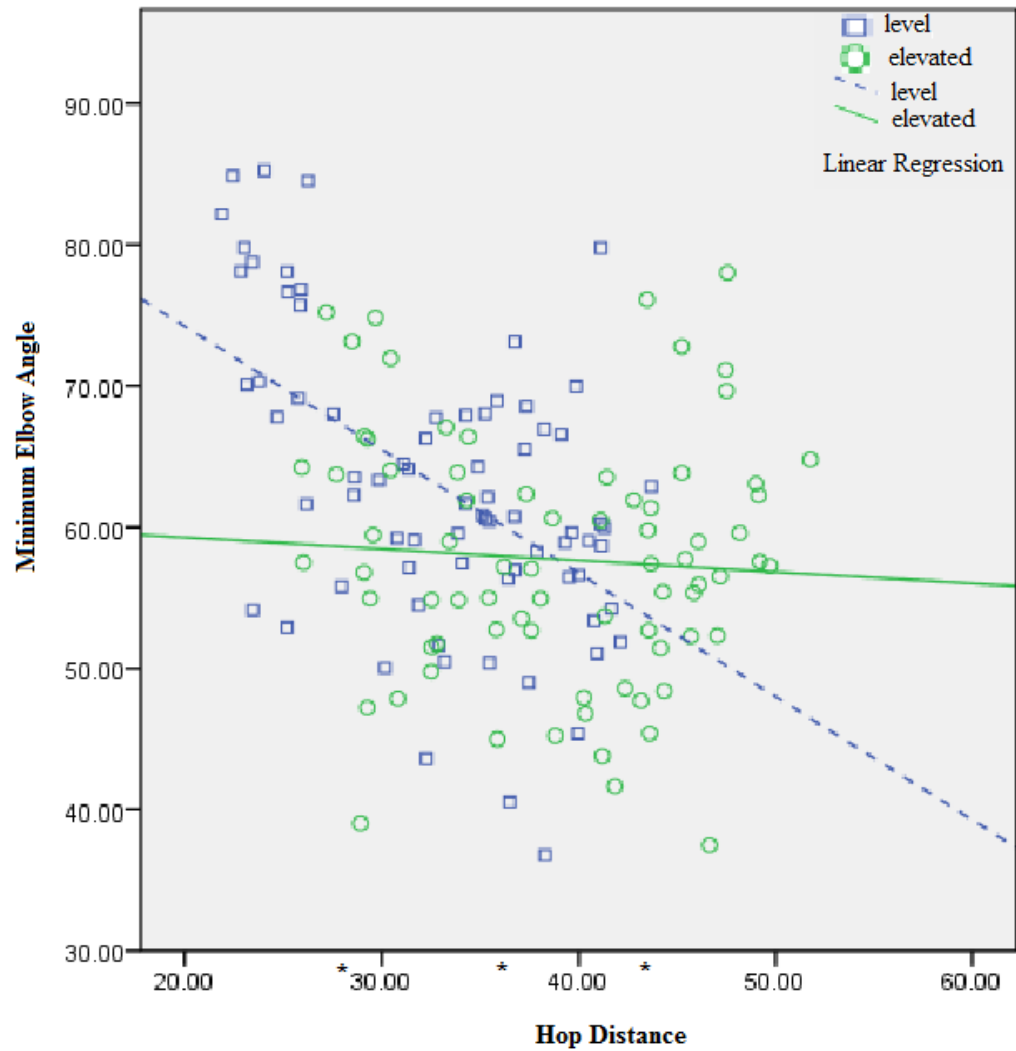


**Figure 15.** Linear regression of impact flexion in two-way subject within-subjects design. \* indicates a hop distance at which marginal mean difference in impact flexion was examined.  $P = 0.0001$  for low mean,  $0.0001$  for main average, and  $0.378$  for high mean.

All five animals also experienced lower average minimum elbow angles after impact during elevated hops (i.e., the elbow was flexed to a greater degree) (Fig. 16). As with the previous two variables, this difference was significant in short hops of ~ 29 cm ( $P = 0.0001$ ) and at the average hop distance of ~36 cm ( $P = 0.013$ ) (Fig. 17). During the longest hops, there was no difference in impact flexion a high mean of ~43 cm (Fig. 17). There was a significant negative effect of hop distance on minimum elbow angle during level hops, but no such effect during elevated hops (Fig. 17).



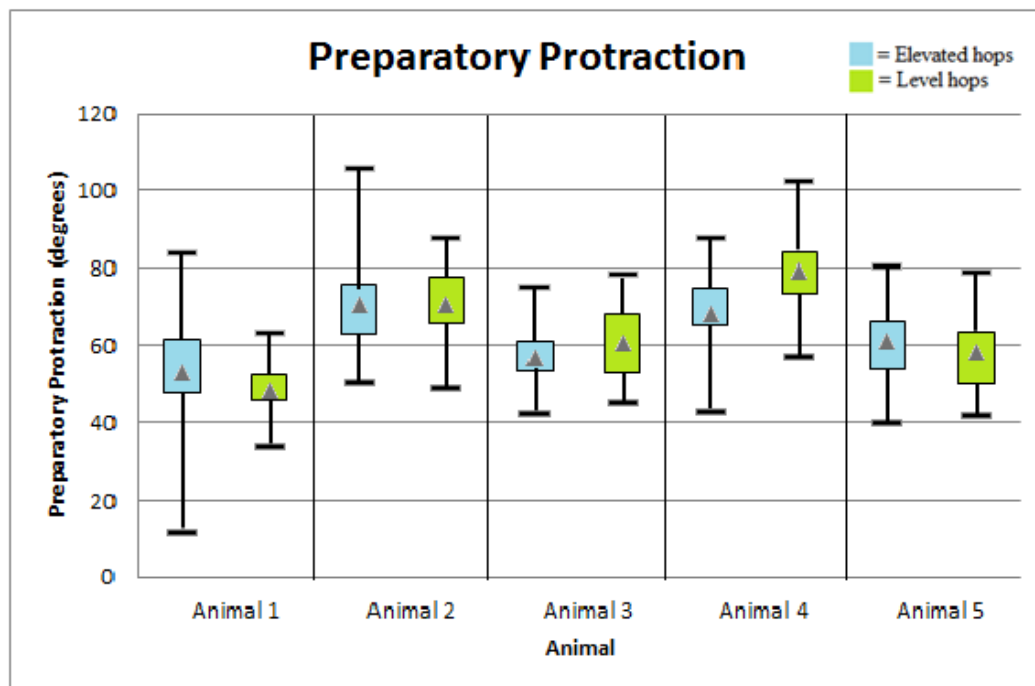
**Figure 16.** Box and whisker plot of minimum elbow angle after impact for each animal in the elevation trial and control trial.



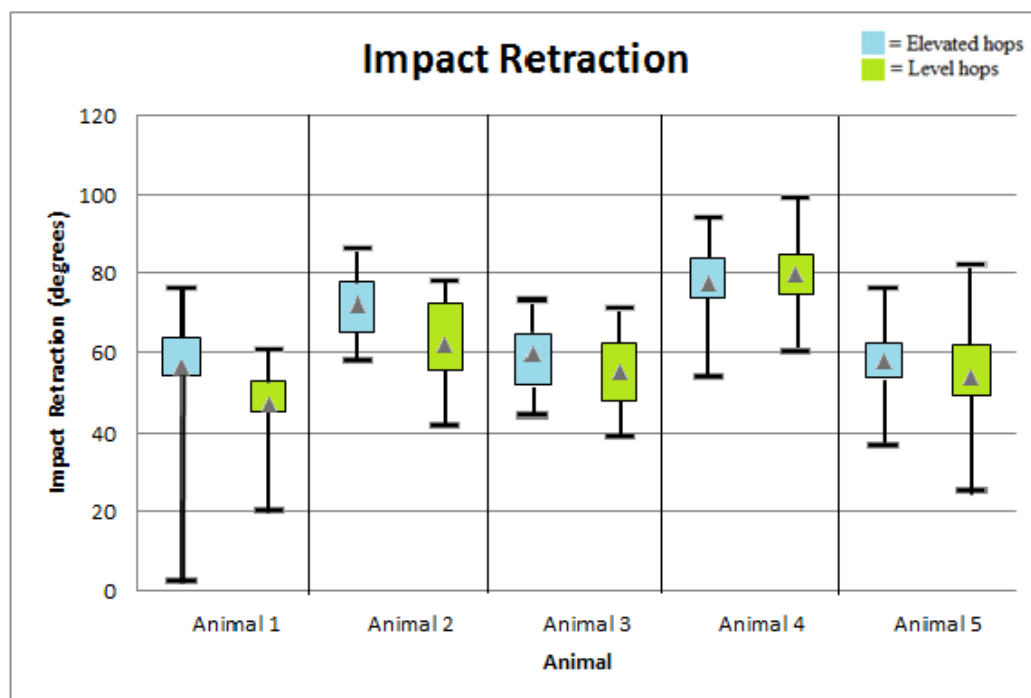
**Figure 17.** Linear regression of minimum elbow angle in two-way subject within-subjects design. \* indicates a hop distance at which marginal mean difference in minimum elbow angle was examined.  $P = 0.0001$  for low mean, 0.013 for main average, and 0.857 for high mean.



The degree of preparatory humeral protraction did not follow a clear pattern. In some animals, average values were higher during elevated hops, while the opposite was true in other animals (Fig. 18). Statistical analysis showed no significant difference in protraction between elevated and level hops ( $P=0.408$ ). Four of the animals experienced greater average amounts of humeral retraction in elevated hops than in level hops (Fig. 19), but statistical analysis showed no significant difference in retraction between elevated and level hops ( $P=0.149$ ).

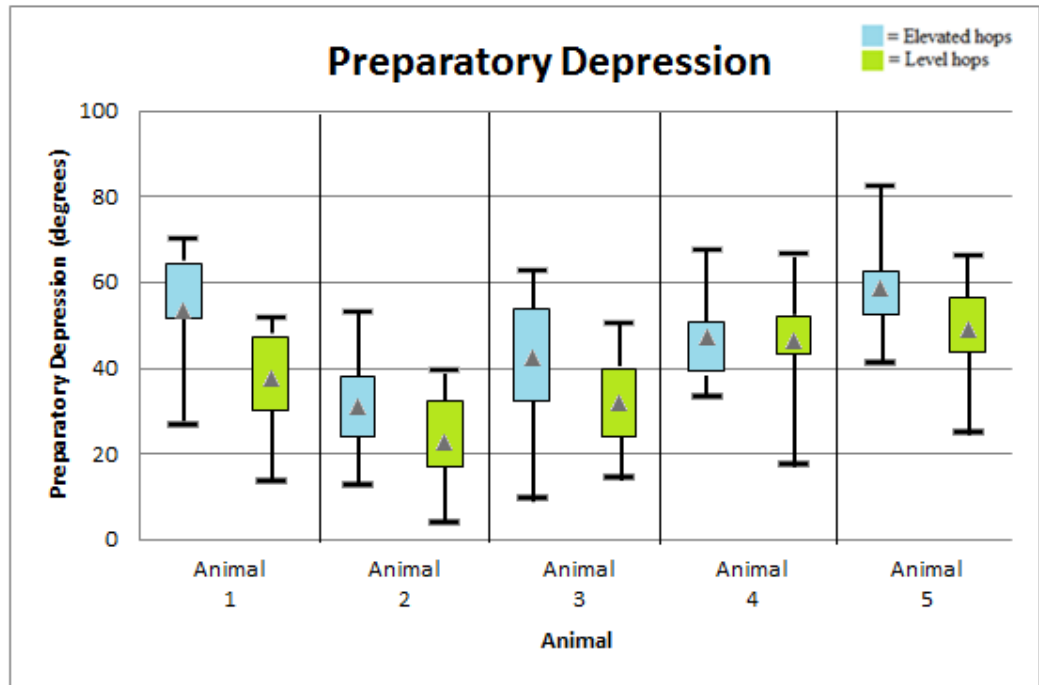


**Figure 18.** Box and whisker plot of protraction in elevation trials vs. control trials for all five animals.

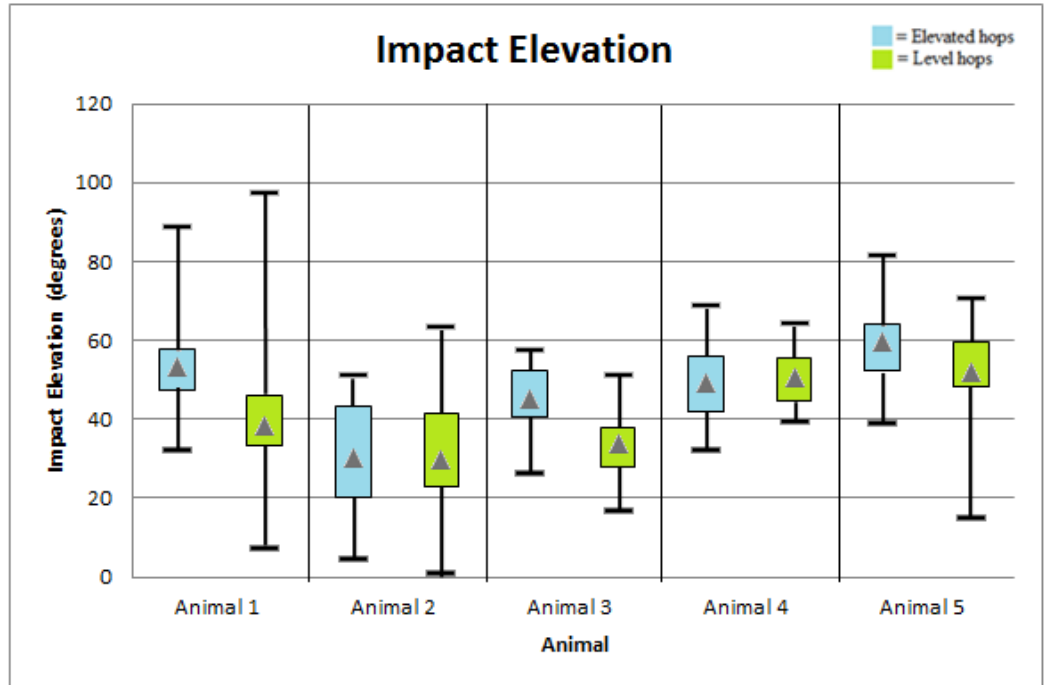


**Figure 19.** Box and whisker plot of retraction in elevation trials vs. control trials for all five animals.

Four of the five animals underwent greater average degrees of humeral depression in preparation for landing during elevated hops compared to control hops (Fig. 20). Statistical analysis showed that preparatory depression was significantly greater in elevated hops than in level hops ( $P=0.03$ ). Three out of the five animals experienced greater degrees of impact-related humeral elevation during elevated hops compared to control hops after impact (Fig. 21). Statistical analysis showed that impact elevation in elevated hops was marginally significantly greater than impact elevation in level hops ( $P=0.068$ ).



**Figure 20.** Box and whisker plot of depression in elevation trials vs. control trials for all five animals.



**Figure 21.** Box and whisker plot of elevation in elevation trials vs. control trials for all five animals.

## DISCUSSION

The goal of this study was to characterize forelimb movements during hopping and landing in cane toads to test whether kinematics change with respect to hop distance (as predicted by muscle activity differences) and takeoff elevation. I hypothesized that differences in pre-landing muscle recruitment intensity would result in greater arm movements before and during landing in longer hops. And due to the longer aerial durations and greater impact forces associated with elevated take-offs, I expected to see an even greater degree of forelimb movements in elevated hops.

### *Level Hopping*

Results from the first study showed that forelimb movements during hopping are qualitatively similar regardless of hop distance. For example, elbow kinematics can readily be broken down into four phases involving an initial small bout of extension that occurs as the forelimbs are lifted off the ground, followed by a more substantial bout of flexion as the animal pulls its arms up toward its body as its hindlimbs are taking off from the ground. After takeoff, the elbow then re-extends, pushing the hands toward the ground in a phase I've termed preparatory extension, and finally, at landing, the elbow flexes, in a phase I've called impact-related flexion as the ground reaction forces initially overwhelm the arm's muscles and the forelimb is compressed.

Both preparatory extension and impact flexion excursions tended to increase significantly with hop distance (Fig. 9 and 10). Moreover, the grand averages (i.e., typical amounts) of preparatory extension and impact flexion closely matched (Table 1). For example, if an animal underwent  $35^{\circ}$  of elbow flexion after impact, it typically prepared for that impact with approximately  $35^{\circ}$  of preparatory extension. These results indicate a possible compensatory relationship between preparatory extension and impact flexion. In short, the animals likely extend their forelimbs a certain amount prior to landing in order to control for and nearly match the amount of subsequent flexion that will occur after impact. Such matching of elbow excursions before and after landing implies the maintenance of a pretty consistent minimum elbow angle and forelimb posture after impact. It is important that the animals avoid too much flexion at the elbow during impact, as it could lead to over-stretching and even possible injury of the anconeus muscles (the equivalent of the triceps brachii complex in humans). Anconeus muscles act as elbow extensors, and thus when the elbow is flexed, they get stretched. It is well known that over-stretching of active muscle can lead to muscle damage (Butterfield and Herzog 2006; Talbot and Morgan 1998).

Matching amounts of preparatory extension and impact flexion, in the context of injury prevention, correspond well with the results of a recent study in which the length of the anconeus muscle was measured during cane toad hopping and landing (Azizi and Abbott 2013). During preparatory extension, they found that the anconeus shortened (range of ~5-20% of resting length), and it was stretched



after impact as the elbow flexed (range of ~5-25% of resting length). As would be predicted from my kinematic study, Azizi and Abbot (2013) found that the shortening strains of the anconeus associated with preparatory extension and stretching strains associated with impact-flexion both increased with increasing hop distance. Moreover, the two excursions closely matched, allowing them to conclude that the anticipatory excursion of pre-landing muscle shortening compensated for the subsequent amount of stretching that occurred after impact (Azizi and Abbott 2013). They also concluded that the consistency of the maximum length to which the muscle was stretched after landing was due to the changes in muscle shortening before impact. Muscle shortening prior to impact therefore prevented the muscle from stretching past its optimal length during landing, or else muscle damage could have been experienced by the animals (Azizi and Abbott 2013).

#### *Elevated Hopping Kinematics*

In the second study both elbow and humeral kinematics were examined to determine if manipulations of aerial durations and impact forces led to more extreme movements of the arm during hopping and landing. Results showed that in both level and elevated hops, all five animals presented the same basic forelimb movements during jumping and landing: initial bouts of elbow extension and flexion during takeoff followed in mid-air by preparatory elbow extension and then impact flexion in landing. Within the phases of preparatory extension and

impact flexion, four types of humeral movement were identified. During preparatory extension, the humerus protracted, moving toward the head (Fig. 7A) while simultaneously depressing downward toward the ground (Fig. 7B). When impact flexion began, the humerus retracted back toward the side of the torso (Fig. 7A), while simultaneously elevating in response to the ground reaction force (Fig. 7B).

In comparing forelimb movements between elevated and level hopping, all five animals underwent greater average preparatory extension (mean =  $55^{\circ}$ ) than during level hopping (mean =  $46^{\circ}$ ) and experienced greater degrees of impact flexion (mean =  $57^{\circ}$ ) during elevated hops compared to those on the level (mean =  $41^{\circ}$ ) (Figs. 12 and 14, Table 3). These results are consistent with my hypothesis that increased aerial durations would give animals more time to extend their arms (and thus result in more arm extension) in preparation for landing from elevated hops. They are also consistent with the hypothesis that greater impact forces associated with elevated hops would lead to greater degrees of elbow flexion after impact. In fact, results showed that average minimum elbow angle was greater in level hops for all five animals, indicating the increased levels of impact-related flexion present in elevated hops (Fig. 16).

**Table 3.** Grand averages of the different angle variables for elevated and level hops.

Variable	Average (degrees)	
	Elevated hops	Level hops
Preparatory Elbow Extension	55	46
Impact Elbow Flexion	57	41
Minimum Elbow Angle	58	63
Humeral Protraction	62	63
Humeral Retraction	65	59
Humeral Depression	47	39
Humeral Elevation	48	43

Human studies of impact-related flexion during drop-landing from different heights show similar results, in which greater impact forces lead to greater degrees of limb compression (McNitt-Gray, 1991; Yeow et al., 2010). Because humans land on their legs, the researchers measured knee flexion in these studies. Yeow et al., (2010) showed that humans experience greater ground reaction forces and knee flexion when dropping down from greater heights. For example, maximum knee flexion increased by 25% when drop height doubled from 0.3 m to 0.6 m. In another study, knee joint flexion significantly increased in recreational athletes and gymnasts by 71% and 33% respectively when drop height quadrupled from 0.32 m to 1.28 m, exhibiting shifts in the subjects' coordination to prepare for a changed landing situation (McNitt-Gray 1991).

A review by Marco Santello (2005) focusing on the neuromuscular control of human landing suggests that a “predictive” component is involved with managing impact forces to avoid injury upon landing. To account for the ground reaction forces from the impact, humans alter the intensity of leg muscle recruitment and

time it accordingly with changes in drop height and ground reaction forces (Santello et al. 1998). Similar alterations in preparatory muscle activity and movements occur in human arms before catching a ball. Not only does EMG activity of arm muscles increase with increasing drop height of the ball (Lacquaniti and Maioli 1989), but the wrist and elbow are also positioned closer to the body, and the arm is more flexed when waiting to catch the ball (Mazyn et al. 2006). Humans clearly anticipate impact forces of various kinds and modulate limb muscle and movements to coordinate the subsequent action and prevent injury.

Because hop distance has the potential to influence kinematics, it is important to account for it when comparing level and elevated kinematic variables. In my study impact flexion, preparatory extension, and minimum elbow angle were only affected significantly by elevation during hops of short and medium distances (Figs. 13, 15 and 17). In addition, in elevated hops, hop distance no longer affected how much an animal extended its arms prior to landing or flexed after impact. Data were widely variable during elevated hops and it is possible that these animals were unfamiliar with elevated surfaces due to the types of terrestrial environments they inhabit. So they might not have recognized that they were on an elevated surface during takeoff.

Nevertheless, as was found in the initial study last summer, level hops from the same animals still indicated clear positive relationships between distance and forelimb kinematics. Unlike in the summer animals, however, excursions at the

elbow before and after landing were not matched in magnitude during level hopping (or elevated hopping). It is unclear why the compensatory elbow movements observed within the animals studied last summer were not also observed in this subsequent academic year studies. It is possible that since the animals performed twice as many hops as animals during the summer, they became fatigued throughout the experiments and this could have potentially affected their performance. This possibility is reflected by the animals' random performance of either greater extension than flexion, or vice versa.

### *Conclusion*

In this study I examined forelimb kinematics of landing preparation in level and elevated hops. Preparation for landing was similar in both types of hops. The animals began to extend their forelimbs mid-air (preparatory extension) while protracting and depressing the humerus, followed by a period of flexion (impact flexion) along with humeral retraction and elevation upon impact. There were clear positive relationships between both preparatory extension and impact flexion, and hop distance in level hops from the first study. Such alterations in forelimb movement patterns match the differences previously observed in pre-landing muscle recruitment patterns (Gillis et al. 2010) and length changes (Abbot and Azizi, 2013). In elevated hops there was an overall increase in preparatory extension and impact flexion as predicted. However, animals could no longer account for changes in hop distance (Figs. 13, 15, 17). This inability to account

for changes in hop distance contrasts with the coordinated adjustments that other animals are capable of performing in landing, especially in humans.

## LITERATURE CITED

- Akella, T. and G.B. Gillis. 2011. Hopping isn't always about the legs: forelimb muscle activity, patterns during toad locomotion. *Journal of Experimental Zoology Part A-Ecological Genetics and Physiology*, 315A: 1-11.
- Azizi, E. and E.M. Abbott. 2013. Anticipatory motor patterns limit muscle stretch during landing in toads. *Biology Letters*, 9: 20121045.  
<http://dx.doi.org/10.1098/rsbl.2012.1045>
- Bland, J.M. and D.G. Altman. 1995. Multiple significance tests: the Bonferroni method. *BMJ*, 310: 170.
- Butterfield, T.A. and W. Herzog. 2006. Effect of altering starting length and activation timing of muscle on fiber strain and muscle damage. *Journal of Applied Physiology*, 100: 1489-1498.
- Cogger, H. G. and R. G. Zweifel. 1998. Encyclopedia of Reptiles and Amphibians, 2<sup>nd</sup> edition. Academic Press, San Diego.
- Dyhre-Poulsen, P. and A.M. Laursen. 1983. Programmed electromyographic activity and negative incremental muscle stiffness in monkeys jumping downward. *Journal of Physiology*, 1984: 121-136.
- Emerson, S.B. 1988. Testing for historical patterns of change: A case study with frog pectoral girdles. *Paleobiology*, 14: 174-186.
- Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, 70: 967-971.
- Essner, R.L., Suffian, D.J., Bishop, P.J., and S.M. Reilly. 2010. Landing in basal frogs: evidence of saltational patterns in the evolution of anuran locomotion. *Naturwissenschaften*, 97: 935-939.
- Gillis, G.B. and A.A. Biewener. 2000. Hindlimb extensor muscle function during jumping and swimming in the toad (*Bufo Marinus*). *Journal of Experimental Biology*, 203: 3547-3563.
- Gillis, G.B., Akella, T., and R. Gunaratne. 2010. Do toads have a jump on how far they hop? Pre-landing activity timing and intensity in forelimb muscles of hopping *Bufo marinus*. *Biology Letters*, 6: 486-489.

- Greenwood, R. and A. Hopkins. 1975. Muscle responses during sudden falls in man. *Journal of Physiology*, 254: 507-518.
- Hedrick, T. L. 2008. Software techniques for two-and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics*, 3: 1-6.
- Jones, M. and D.G.D. Watt. 1971a. Observations on the control of stepping and hopping movements in man. *Journal of Physiology*, 219: 709-727.
- Jones, M. and D.G.D. Watt. 1971b. Muscular control of landing from unexpected falls in man. *Journal of Physiology*, 219: 729-737.
- Lacquaniti, F. and C. Maioli. 1989. The role of preparation in tuning anticipatory and reflex responses during catching. *Journal of Neuroscience*, 9: 149-159.
- Marsh, R.L. 1994. Jumping ability of anuran amphibians. *Advances in Veterinary Science and Comparative Medicine*, 38B: 51-111.
- Mazyn, L.I.N., Montagne, G., Savelsbergh, G.J.P., and M. Lenoir. 2006. Reorganization of catching coordination under varying temporal constraints. *Motor Control*, 10: 143-159.
- McNitt-Gray, J.L. 1991. Kinematics and impulse characteristics of drop landings from three heights. *International Journal of Sport Biomechanics*, 7: 201-224.
- Nauwelaerts, S. and P. Aerts. 2005. Take-off and landing forces in jumping frogs. *The Journal of Experimental Biology*, 209: 66-77.
- Olson, J.M. and R.L. Marsh. 1998. Activation patterns and length changes in hindlimb muscles of the bullfrog *Rana catesbeiana* during jumping. *J Exp Biol* 201:2763-2777.
- Peters, S.E., Kamel, L.T., and D.P. Bashor. 1996. Hopping and swimming in the leopard frog. *Rana pipiens*: I. Step cycles and kinematics. *Journal of Morphology*, 230: 1-16.
- Prochazka, A., Schofield, P., Westerman, R.A., and S.P. Ziccone. 1977. Reflexes in cat ankle muscles after landing from falls. *Journal of Physiology*, 272: 705-719.



Purves, D., Augustine, GJ, Fitzpatrick, D., Katz, L.C., LaMantia, A., McNamara, J.O. and S.M. Williams. 2001. Neuroscience: 2<sup>nd</sup> edition. Sinauer Associates, Sunderland, MA.

Santello, M. 2005. Review of motor control mechanisms underlying impact absorption from falls. *Gait and Posture*, 21: 85-94.

Santello, M. and M.J.N. McDonagh. 1998. The control of timing and amplitude of EMG activity in landing movements in humans. *Experimental Physiology*, 83: 857-874.

Talbot, J.A. and D.L. Morgan. 1998. The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *Journal of Muscle Research and Cell Motility*, 19: 237-245.

Wells, K.D. 2007. The ecology and behavior of amphibians. University of Chicago Press, Chicago.

Yeow, C.H., Lee, P.V.S. and J.C.H. Goh. 2010. Sagittal knee joint kinematics and energetics in response to different landing heights and techniques. *The Knee*, 17: 127-131.

Zug, G.R. 1995. Anuran locomotion: fatigue and jumping performance. *Herpetologica*, 41: 188-194.