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The Effects of Caudal Autotomy on the Kinematics of *Anolis Carolinensis* Running on an Arboreal-Like Substrate

by

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for eight credits.

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ABSTRACT

Caudal autotomy is a well-researched predation-escape mechanism used by *Anolis* lizards. This ‘self-sacrifice’ involves a neuromuscular event in which the tail is severed along an intravertebral fracture plane proximal to the point of a predator’s grip. The phenomenon of autotomy has persisted in anoles for millions of years due to its benefit of increasing the probability of survival. However, autotomy also incurs in the lizard a metabolic deficit, behavioral modifications, and locomotor consequences. Past research has documented post-autotomy changes in sprint speed, step length, and time spent pausing. I hypothesize that these gross locomotor effects can be explained by changes in limb kinematics. Using high-speed video cameras and MATLAB I recorded and compared the characteristic motions of the front and hind legs pre- and post-autotomy in running *Anolis carolinensis* (N=4), a trunk-crown anole with morphology representative of a broad range of anole ecomorphs. Post-autotomy, the lizards showed significant increases in average velocity, forelimb step frequency, and hindlimb step frequency ($p=0.025$; $p=0.02$; $p=0.035$). Kinematic variables of the limb did not significantly change after tail loss, so the gross locomotor changes observed are better explained as behavioral than biomechanical.

INTRODUCTION

The depth and breadth of knowledge of anole morphology, ecology, evolution, and behavior are nearly unrivaled by any other vertebrate taxon (Losos 2011). Containing approximately 361 species, the genus *Anolis* has been traced back roughly 40 million years (Losos 2011).

Anoles span a broad range of territories, from the northern half of South America, throughout Central America and Mexico, across the Caribbean and Pacific islands, and in the southern United States from North Carolina to eastern Texas. They are known for their phenotypic plasticity, and thus often vary in appearance depending on territory. However, though the genus is phenotypically plastic, it also contains much genetic variation and has a rich evolutionary history.

Anole evolution is a textbook example of adaptive radiation, a process by which different members of a clade diverge to adapt to the environment in different ways. Interspecific interactions drive divergence and the members of the clade radiate away from one another genetically, phenotypically, and behaviorally (Losos 2011). Anoles living in the same habitat—dubbed sympatric anoles—partition their resources by changing their resource use in one of three ways: prey size, structure of habitat, and thermal habitat (Losos 2011). This sympatric competition helps to structure the ecomorph composition of anole communities.

Ecomorphs are variants of an organism that have made physical adaptations to specific surroundings. The morphological and behavioral similarities between ecomorphs are not always indicative of phyletic relation, because they are independently derived adaptations that better suit the animal to its habitat. Leading research in anole ecomorphological evolution states that there are six extant ecomorphs, all of which can be found on the Greater Antilles, as well as elsewhere in the Caribbean: trunk-ground, trunk-crown, trunk, crown-giant, grass-bush, and twig (figure 1).

Each of these ecomorphs has earned its name based on the microhabitat in which it is found, and each has specific, advantageous physical attributes. The purportedly most abundant ecomorph, trunk-ground anoles are dark colored with a muscular build, and are commonly found posed with their heads down so that they may survey the ground for prey (Losos 2011). Trunk-crown anoles, dwelling on the upper trunks and branches of trees, move over a broad range of surface diameters. They typically have a slender body and a long tail, and are capable of changing the color of their skin from green to dark brown. Trunk anoles are best known for their short tails, flattened body, and laterally situated limbs. Crown-giants are aptly named for their massiveness, the largest species of which, *A. luteogularis*, often grows to half a meter in length. They accordingly have larger home ranges and diets including some small vertebrates. Grass-bush anoles unsurprisingly make their homes on narrow stems of vegetation, frequently moving in short hops rather than running. They have slender bodies and a long tail. Conversely, twig anoles have short tails paired with a slender body, and have been suggested to provide weak evidence for the theory that anole tails can be prehensile.

Anoles are a fruitful genus to study as a whole, but due to their adaptive radiation away from a common ancestor it is difficult to choose one model species to learn about the locomotion of all anoles. Evidence suggests that solitary anole species approximate the ancestral anole, the purported geographically isolated common ancestor of all extant anoles (Losos 2011). Current isolated species suggest that this ancestor was a trunk-crown anole, meaning it most likely limited its habitat to tree trunks and branches. Therefore, to study anole locomotion, the logical choice in research species is one that represents well the ancestral anole.

Members of the species *Anolis carolinensis* reside in the southern United States and make their home everywhere from leaf litter to high, narrow branches. In the presence of sympatric species, as they exist in Cuba, *A. carolinensis* are only found on trees from eye-level up. *A. carolinensis* is a trunk-crown anole, similar to the ancestral anole, but in the United States has undergone an ecological release in the absence of competing anole species. This ecological release is an additional asset to *A. carolinensis* as a research species because it has presumably allowed them to alter their locomotion in ways that make it representative of other ecomorphs, which have more limited microhabitats, in addition to trunk-crown anoles. These coincidental factors render *A. carolinensis* a reasonable subject of

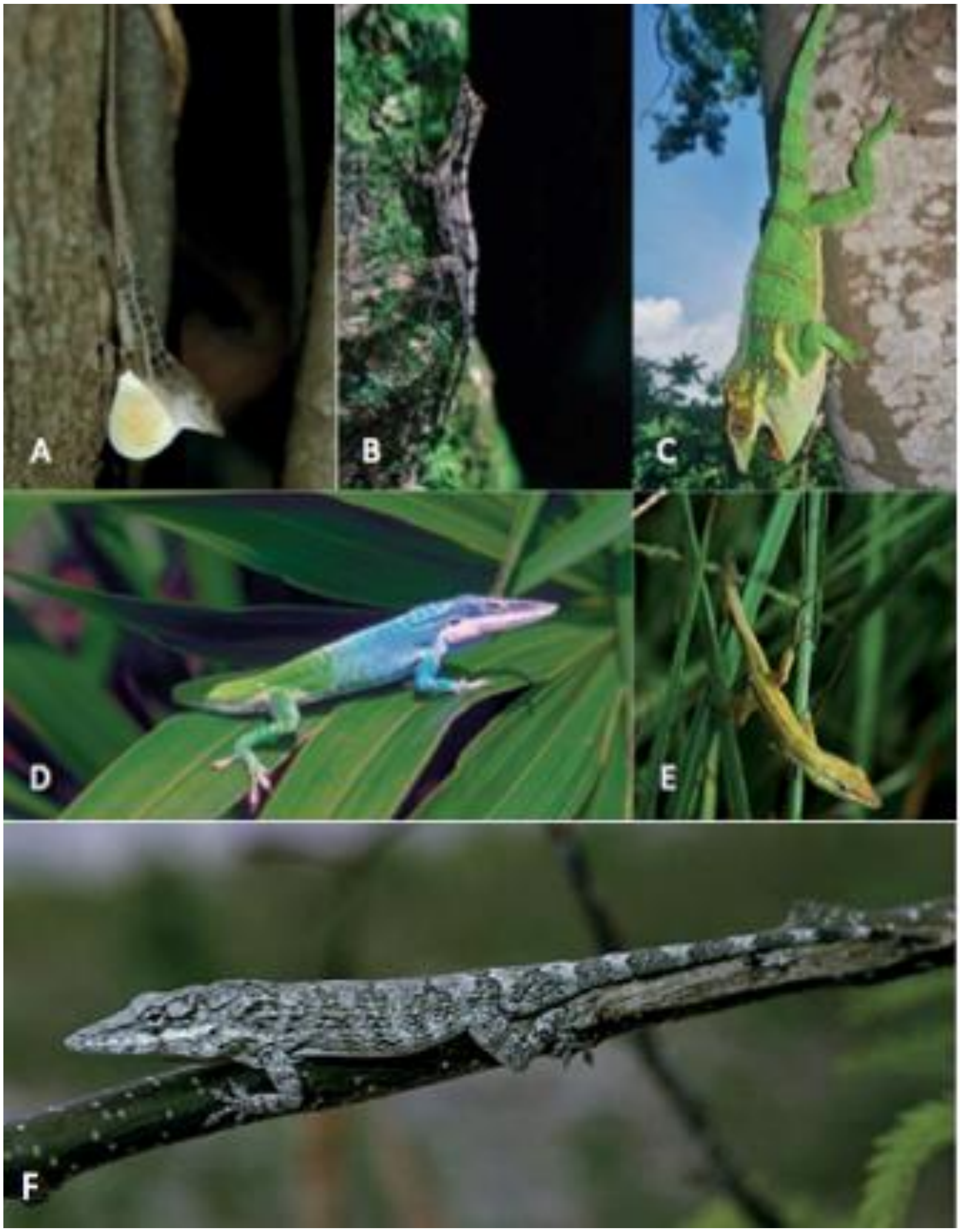

Figure 1-Anole Ecomorphs: Anole species belonging to each of the six anole ecomorphs, a) trunk-ground (*A. lineatopus*), b) trunk (*A. loysianus*), c) crown-giant (*A. smallwoodi*), d) trunk-crown (*A. allisoni*), e) grass-bush (*A. pulchellus*), and f) twig (*A. angusticeps*). Adapted from Losos, 2011.

study in characterizing anole locomotion.

The subject of anole locomotion has captured ample attention, much of it focused on the effects of limb length variation on locomotor capabilities such as sprint speed and surefootedness (Losos 2011). These two variables are at odds with one another, and limb length variations are an example of the plasticity of anole adaptation. For example, long-legged lizards run two times faster than short-legged lizards on large diameter branches, and as fast as short-legged lizards on small diameter branches. So why did short legs evolve at all? The answer is that short-legged lizards trip and stumble less often than long-legged lizards, which affords them better escape capabilities. The existence of short-legged lizards is a testament to the importance of surefootedness as a key adaptation (Losos 2011). Behavior is closely tied to locomotion. Long-legged lizards, such as grass-bush anoles, jump often because their legs do not allow them to run quickly on blades of grass. Short-legged lizards rely on stealth, rather than speed, to flatten themselves to a branch and slink, undetected, away from a predator (Losos 2011). Many phenotypic characteristics are shown to be adaptive by their repeated, independent development across clades (Losos 2011). These characteristics can include specific aspects of locomotion, such as the use of the tail. In running and jumping, the tail is thought to be used as a counterbalance, as well as being integral in maneuvering in the air during jumps (Losos 2011).

A. carolinensis is one of many lizards possessing the ability to autotomize, or self-amputate, their tails. Autotomy is a well-documented phenomenon in an array of species. Certain spiders, mice, and lizards are all known to exhibit autotomy. This behavior is employed as a self-defense mechanism in times of perceived danger, with the intent being to release the body part by which the predator is holding the organism so that it may escape. Different parts of the body are autotomized in different creatures; spiders can sacrifice a leg, African Spiny mice release patches of skin, octopi leave behind an arm, and lizards lose their tails. (Punzo 1997; Seifert *et al.* 2012; Budelman 1998; Arnold 1984). Besides releasing the lizard from its predator's grip, caudal, or tail, autotomy is additionally beneficial in that the autotomized tail writhes for up to half an hour after it has been detached (Cromie and Chapple 2012). This distracts the predator and affords the lizard time to flee. Caudal autotomy is an ancestral trait in squamates, possessed by 13 of the approximately 20 families of lizards (Fleming *et al.* 2013); its persistence is a strong testament to its usefulness as a predation escape mechanism. Autotomy is thought to be a discrete neuro-mechanical event instigated by the lizard, rather than solely the result of the mechanical force between the predator and the prey, though this force is speculated to be necessary (Highman *et al.* 2013). Detailed biochemical events of autotomy are as yet unrevealed, but it is known that the break occurs along a fracture plane immediately proximal to the predator's grip (Highman *et al.* 2013; Fleming *et al.* 2013; Bustard 1968).

There are several factors that affect a lizard’s likelihood to autotomize. In his 1984 comprehensive literature review of lizard caudal autotomy, E.N. Arnold found evidence indicating that tree-and rock-climbing species do not rely as heavily on their tails for locomotion as ground-dwelling lizards and therefore may undergo caudal autotomy more frequently. However, other groups have found that autotomy causes significant locomotor deficits for climbing species in arboreal habitats (Brown *et al.* 1995; Fleming *et al.* 2013). Young lizards, being smaller, more vulnerable to attack, and less able to fight off a predator than larger adults, might gain a greater benefit from autotomy and thus be more likely to autotomize (Arnold 1984, Highman *et al.* 2013). The effectiveness of other predation escape efforts also contributes to the inclination to autotomize. Many species of lizards rely on crypsis, the act of standing or lying perfectly still and blending in to avoid detection. In an open environment such as the desert, which provides no foliage in which to hide, crypsis is not sufficient to avoid capture and caudal autotomy is more heavily relied upon as a predation response than in protected environments (Naya *et al.* 2007). Accordingly Bateman and Fleming, in their 2009 study of two lizard populations in environments of differing predation risk, found that autotomy was more easily achieved in species that experience high predation pressure than by those in low predation risk environments. The balance between the costs and benefits of caudal autotomy governs the factors associated with the behavior. Many of the negative consequences of caudal autotomy, though costly, are temporary because lizards are capable of regenerating their tails within a few months (Cromie and Chapple 2012).

Caudal autotomy is advantageous but metabolically unfavorable. Since the tail is used in lipid storage, autotomy is an energetic sacrifice, as demonstrated by lizards returning to the site of autotomy and eating their previously lost tail to recover lost energy (Sanggaard *et al.* 2012). In an effort to lose as little energy as possible during autotomy, the lizard can fracture its tail at different planes along its length. It typically detaches along a fracture plane immediately proximal to the grip of the predator, conserving all except what must absolutely be lost in order to escape (Bustard 1968). Lost tails grow back with a cartilaginous tube rather than individual vertebrae, so though regenerated tails can still serve as lipid storage units, fine movements may be lost (Fleming *et al.* 2013). Regenerated tails also exhibit reduced readiness to autotomize because they lack the intravertebral fracture planes of original tails (Fleming *et al.* 2013). However, the remainder of the original tail can readily autotomize multiple times since autotomy occurs with economy to reduce waste of tail above the predator's grip (Fleming *et al.* 2013).

Aside from energetic costs, lizard caudal autotomy has additional consequences in social status, risk-taking behavior, and locomotion. Many of these costs can be offset by the reward of escaping capture and by regeneration (Fleming *et al.* 2013). Autotomy manifests socially because long tails are more desirable than shorter counterparts. During fights, male lizards try to knock off their opponents' tails to reduce social standing, because long tails are related to dominance (Bateman and Fleming 2009). However, sometimes a regenerated tail is shorter and more club-like than the original, which can be, ironically, a more effective weapon during fighting (Bateman and Fleming 2009). Other mechanisms of escape, such as running and climbing, might be negatively affected by autotomy, thus further discouraging the lizard from taking risks. These locomotor effects of autotomy have long been observed and quantified, but certain aspects of the effects are less well understood.

Research into post-autotomy locomotion has focused largely on the gross locomotor effects of tail loss, such as speed and duration of sustained fleeing. Tails can drag against the substrate, slowing the animal, position the animal’s center of mass posteriorly, providing its hindlimbs with additional propulsive power, and can counterbalance the body during undulation in locomotion (McElroy and Bergmann 2013). Loss of the tail can therefore lead to many different resulting locomotor effects, whether beneficial or deleterious. For example, Brown *et al.* (1995) found that, on arboreal substrates, animals that had undergone autotomy were slower and ran shorter distances than lizards with their original tails. Similarly, Martin and Avery (1998) studied lizard movement on a flat surface with good traction and also found that tailless lizards ran more slowly and for shorter distances during bursts of locomotion than tailed individuals. The same study found that the average stride length of the animals decreased after tail loss and the time spent pausing between running bursts increased. The increased pausing time is a potential behavioral modification adapted to preserve energy in the wake of losing the substantial energy available in the tail. Martin and Avery (1998) also noted that a positive relationship between stride length and speed remained after autotomy. Cromie and Chapple (2012) studied autotomy in two skink species and also reported that speed was reduced after tail loss. They made note that, at their maximum spring speed, skinks' bodies move sigmoidally and autotomy reduces much of the lateral movement necessary for this behavior, altering their gait.

At times, altering the substrate or temperature affects the post-autotomy behavior. For example, despite finding significant locomotor deficits on an arboreal substrate after autotomy, Brown *et al.* (1995) found that, on a flat surface, the lizards ran faster and farther post-autotomy. Skinks are found to perform better on all parameters at their optimal temperature (Cromie and Chapple 2012). Reviews have suggested that, overall, the tail is more likely to enhance locomotor performance (McElroy and Bergmann 2013), and thus losing it during an autotomy event is likely to have an adverse effect.

As the majority of research on post-autotomy locomotion has focused on flat, horizontal substrates and has emphasized broader performance attributes such as speed, distance traveled, and behavior, little is known about more natural locomotor substrates and the more detailed nuances of a lizard's stride after tail loss. Losos and Irschick (1996) found a positive relationship between hind limb length and acceleration while sprinting; shorter legs lead to decreased acceleration. Perhaps autotomy affects the effective length of the hind limbs by altering the way the lizard uses them. Given that the tail is implicit in an animal's balance, it is reasonable to hypothesize that lack of stability after tail removal may lead to compensatory changes in stride mechanics, and ultimately, decreased stride length (Martin and Avery 1998). Yet, there have been few attempts to address these more detailed potential consequences of caudal autotomy for locomotion. The aspects of caudal autotomy that have received much scientific attention focus on gross locomotion and behavior. I propose that these topics are downstream results brought about by specific kinematic changes of the limbs themselves that have yet to be characterized. The scientific community has devoted years of time and attention to the 'what' of caudal autotomy and largely ignored the 'why'.

My work here aims to characterize the motions in a lizard's stride both before and after tail loss to gain understanding of the missing kinematic connection between autotomy and resulting gross locomotor changes. In particular, I hypothesize that the demands of increased balance after tail loss lead to the animal taking shorter strides at the same frequency as before tail loss, thus resulting in the decreased speeds often found as a locomotor cost of autotomy. These shorter strides will be characterized by smaller angular excursions at specific limb joints (e.g., shoulder, elbow and knee). My work will not only reveal whether shorter strides are characteristic of post-autotomy locomotion in *A.*

carolinensis, but, if they are, it will also detail the specific limb joints most accountable for the shorter strides.

MATERIALS AND METHODS

Animals

This study used four Green Anoles (*Anolis carolinensis)* with a mean mass of 4.13g, ranging from 3.50g to 4.54g, and a mean snout-vent length (SVL), measured from the tip of the nose to the opening of the cloaca, of 57mm, ranging from 54mm to 58mm. The animals were obtained from Petco Animal Supplies in Holyoke, Massachusetts and Springfield, Massachusetts and housed in a temperature-controlled room at 26° Celsius with a 12:12 light:dark cycle beginning at 7am, but with individual heat lamps on constantly. The animals were misted with water once a day and fed 6 mealworms and excess small crickets.

Arena

The lizards ran up a wooden dowel of 2.5cm diameter, placed at 30° to the horizontal in a carpeted octagonal arena of 1.4 by 0.46 meters (figure 2). Around the dowel was a tightly wrapped surgical towel of negligible thickness to provide traction, as the surface of the dowel alone was not conducive to anole locomotion (pers. obs.). The arena was lit by two 500-Watt Lowel TOTA lamps, standing on opposite sides of the tank.

Figure 2-Experimental Arena: The arena containing the experimental environment.

The animal was filmed running up the dowel using two Fastec Imaging high-speed digital video cameras at right angles to one another, both capturing the left side of the animal. Video was taken at a frame rate of 506 frames per second (fps) for one animal and 500 fps for three animals and with a resolution of 1280 pixels x 1024 pixels.

Experiments and Data Collection

Each lizard was housed in an individual container. Each animal's left side was marked using LiquidPaper® (Newell Rubbermaid, Oak Brook, IL) or BIC Wite-Out® (BIC Corporation, Shelton, CT, USA) with small dots approximately 2mm in diameter on the shoulder, elbow, wrist, hip, knee, and ankle and three dots in a cross on the animal's back. One of the animals was marked with one dot on its back rather than three, and so this animal did not contribute data to certain parameters assessed for the other three lizards.

To initiate a locomotor trial, fully tailed animals housed in small plastic containers (12.5 x 14cm) were first allowed to sit under high-wattage lights to warm up for several minutes before being placed at the low end of the dowel. Running was stimulated by gently prodding a lizard's tail or hindlimbs, and only runs in which the lizard remained atop the dowel, rather than running on the side or bottom of the dowel, were considered. One stride was captured per run, and five to eight runs were captured per lizard, though only five runs from each lizard were analyzed to maintain statistical consistency. The stride chosen was an intermediate step, neither the first nor the last, to ensure that the data most typical of running were gathered. If a lizard gave a several-step run, data were collected from non-consecutive, intermediate steps. This decision was based on the assumption of consistency of steps. Between each run the animal was returned to the plastic container for 1-3 minutes.

Autotomy was induced directly following locomotor data collection by pinching a point on the tail with rat tooth forceps such that approximately 75% of the tail by length would be autotomized. The autotomized tails were weighed, and, for the night following tail loss, animals were held in their individual tanks and were not provided branches for climbing to practice locomoting without a tail. Tailless locomotor data were collected approximately 20 hours after the autotomy event in the same manner as tailed data. These experiments were approved by the Mount Holyoke Institutional Animal Care and Use Committee.

Data Analysis

MATLAB software was used to digitize each step collected in three dimensions. The marked points on the lizard were selected in one video while a blue line traced the same point on the lizard in the other video (figure 3), as dictated by unique calibration of a 64-point cube in the arena.

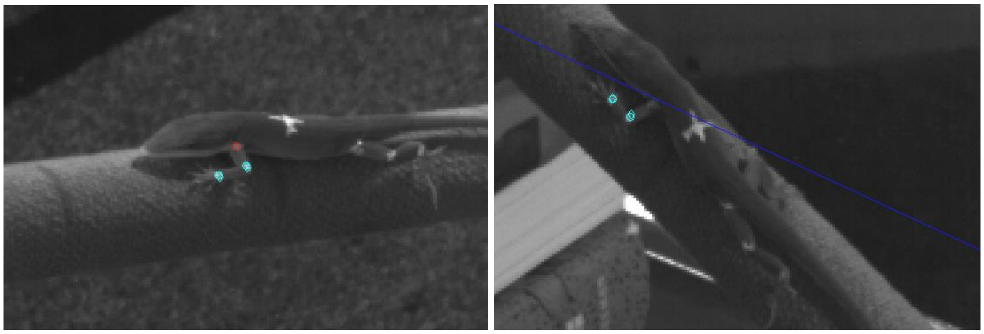

Figure 3-MATLAB Three-Dimensional Digitizing: One point is selected manually in one video and MATLAB traces the corresponding point in the second video. The blue points on the wrist and elbow indicate that these points are completed. The shoulder point is in progress.

MATLAB and 3D digitizing served to transform videos into data. Four animals were assessed five times in each tail condition: pre-autotomy and post-autotomy. A repeated-measures analysis of variance was conducted to assess whether there were differences in ten kinematic variables after autotomy. The independent variables used were trial, having five levels for trials 1, 2, 3, 4, and 5, and tail status, having two levels, with a tail and without a tail. The dependent variables assessed were average velocity, forelimb step length, hindlimb step length, forelimb step frequency, hindlimb step frequency, maximum elbow angle, minimum elbow angle, maximum knee angle, minimum knee angle, and maximum humeral protraction. Figure 4 depicts knee and elbow angle. Protraction is a measure of the angle in the x-y plane between an axis along the humerus and the lizard's body (figure 5). 'Excursion' signifies the difference between the maximum and minimum of a variable. Five of the ten variables assessed, average velocity, forelimb step length, hindlimb step length, forelimb step frequency, and hindlimb step frequency, chronicle the gross locomotor effects of autotomy. The remaining five variables give information about more detailed limb joint kinematics.

Figure 4-Knee Angle and Elbow Angle Diagram: Knee angle (hindlimb) and elbow angle (forelimb) of *Anolis carolinensis* (Kamath 2014).

Figure 5-Protraction Diagram: Protraction is a measure of the angle between the axis along the lizard’s body and the axis through the humerus (Deppe 2011).

RESULTS

Table 1 provides animal mass, snout-vent length (SVL), percent of tail removed, and recovery, or the time between autotomy and the post-autotomy data collection.

Gross Locomotor Effects

The lizards ran significantly faster after losing their tails, *F*(1,3)=17.32, *p*=0.025 (figure 6). Velocity averaged 0.42 m/s before autotomy and 0.60 m/s after autotomy. There was no significant change in the step-length of the forelimb, which averaged approximately 5.5 cm regardless of tail condition, *F*(1,3)=0.70, *p*=0.46 (figure 7). Hindlimb step-length was shorter, on average, after tail autotomy, but also did not change significantly, *F*(1,3)=8.069, *p*=0.07 (figure 8) . Step frequency increased significantly after tail loss for both the forelimb, *F*(1,3)=21.26, *p*=0.02 (figure 9), and the hindlimb, *F*(1,3)=13.46, *p*=0.035 (figure 10).

Limb Joint Kinematics

When a lizard runs there are cyclical changes in the angles of the various limb joints with each step (figure 11). My work focuses on the knee and elbow, in the middle of the hindlimbs and forelimbs respectively. Figure 11 shows that the knee maxima and the elbow minima occur roughly simultaneously, as do the knee minima and the elbow maxima. The lizards showed a significantly reduced maximum elbow angle after autotomy, $F(1,3)=12.73$, $p<0.05$ (figure 12). There was a marginally significant difference between the maximum knee angle achieved by the lizards before and after autotomy, $F(1,3)=9.23$, $p=0.056$ (figure 13). Neither minimum elbow angle (figure 14) nor minimum knee angle (figure 15) changed significantly after tail loss, $F(1,3)=0.03$, $p=0.875$; $F(1,3)=3.36$, $p=0.164$. Three of the four lizards were assessed for protraction and retraction of the humerus. Humeral protraction couples with changes in joint angle to produce a step, and figure 16 shows how humeral movements coincide with elbow angle during a step. Maximum protraction of the humerus was not found to change significantly after autotomy, $F(1,2)=0.97$, $p=0.428$ (figure 17)

All variables showed no significant change with trial with the exception of maximum elbow angle, $F(4,12)=18.817$, $p<0.05$ (figure 18). The significant effect of trial on maximum elbow angle shows a pattern indicating that the change in elbow angle from trial to trial was likely not meaningful.

Table 1-Profile of Research Subjects: Profile of the four *Anolis carolinensis* used in this study.

Lizard	**Mass (g)**	**SVL (mm)**	**Tail Removed (%)**	**Recovery (hrs)**
1	3.5	54	75.2	20.4
2	4.25	58	81.7	20.4
3	4.24	58	76.4	21.5
4	4.54	58	70.7	20.2

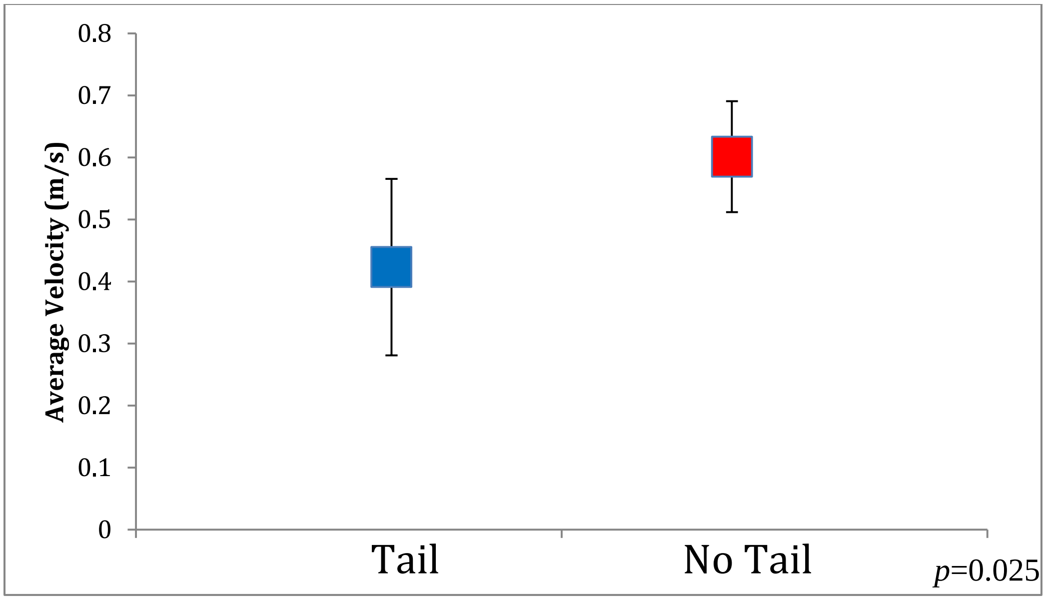

Figure 6-Average Velocity: Average velocity before and after autotomy (N = 4 lizards, 5 steps per lizard). Lizards ran significantly faster after losing 75% of their tails. Error bars represent standard deviations.

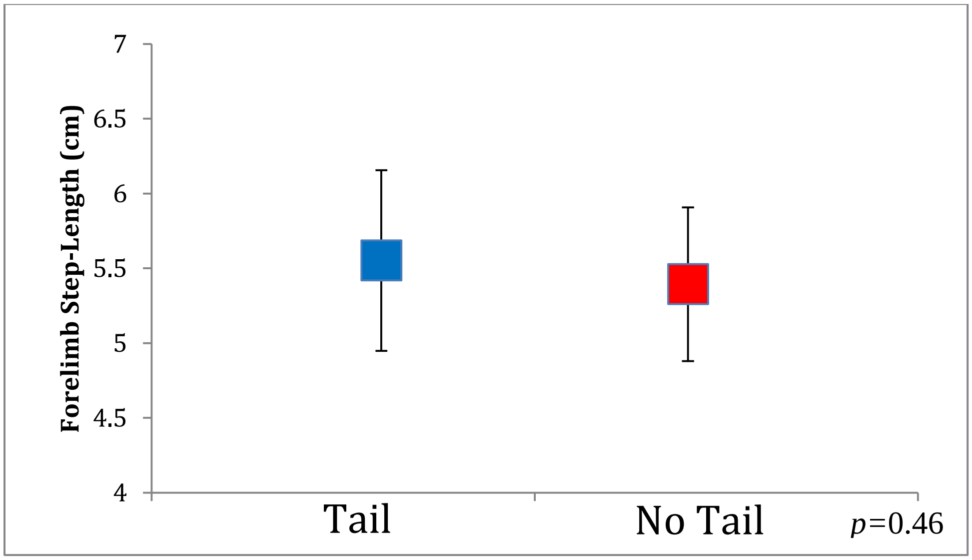

Figure 7-Forelimb Step-Length: Average forelimb step-length before and after autotomy (N = 4 lizards, 5 steps per lizard). There was no effect of autotomy on forelimb step length. Error bars represent standard deviations.

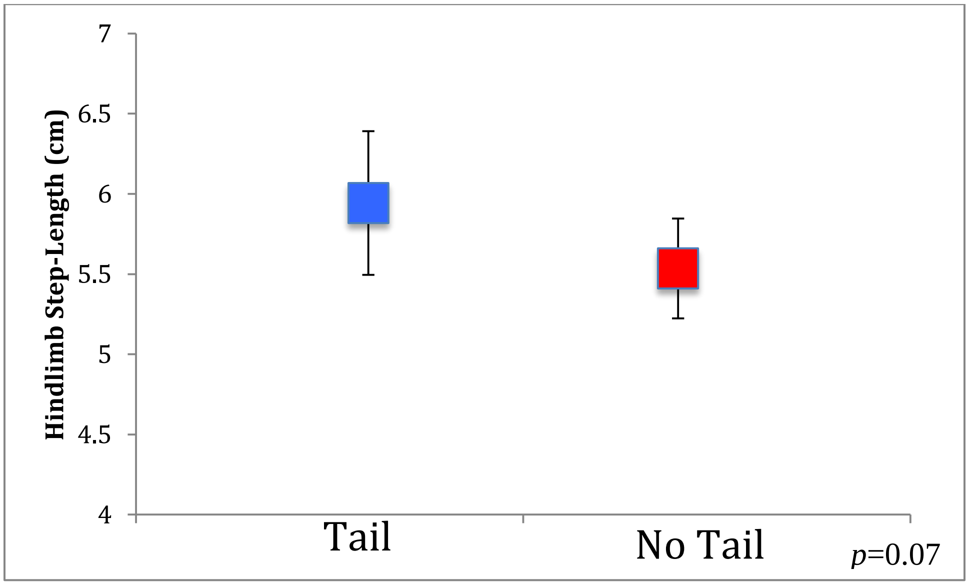

Figure 8-Hindlimb Step-Length: Average hindlimb step-length before and after autotomy (N=4 lizards, 5 steps per lizard). The decrease in hindlimb step-length after autotomy is marginally significant. Error bars represent standard deviation.

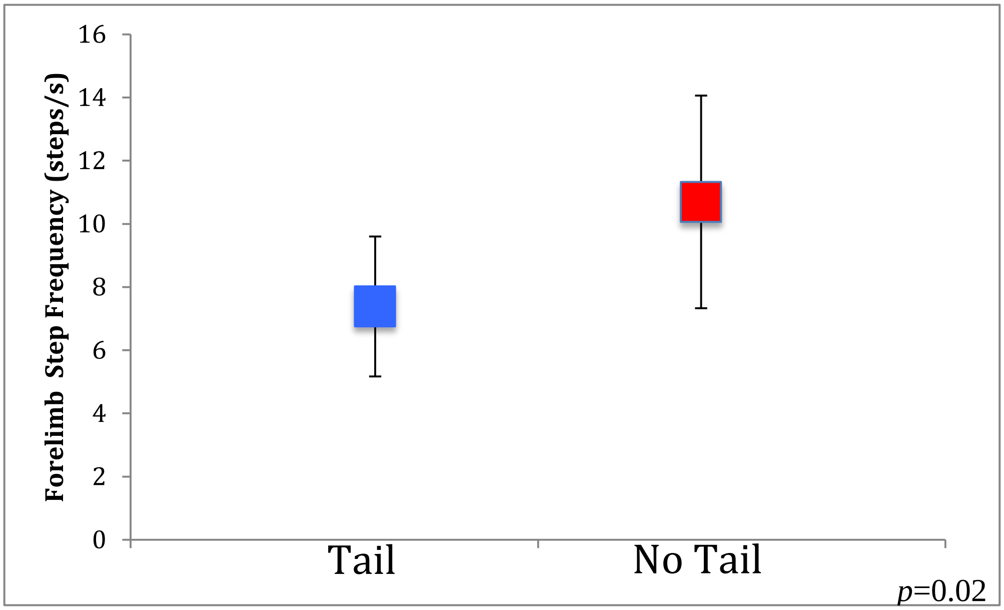

Figure 9-Forelimb Step Frequency: Average forelimb step frequency before and after autotomy (N=4 lizards, 5 steps per lizard). Lizards stepped significantly more frequently with their forelimbs after autotomy than before. Error bars represent standard deviation.

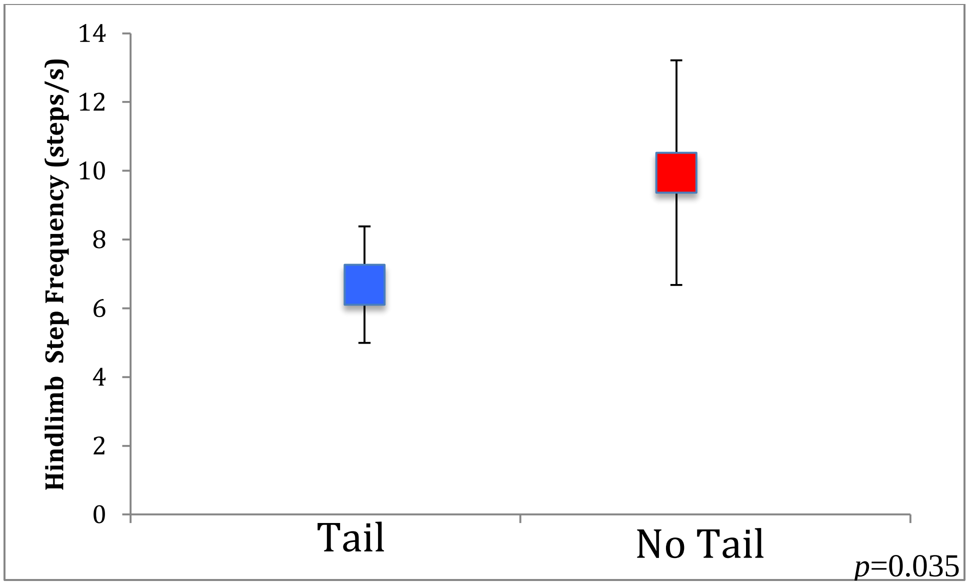

Figure 10-Hindlimb Step Frequency: Average hindlimb step frequency before and after autotomy (N=4 lizards, 5 steps per lizard). Lizards stepped significantly more frequently with their hindlimbs after autotomy than before. Error bars represent standard deviation.

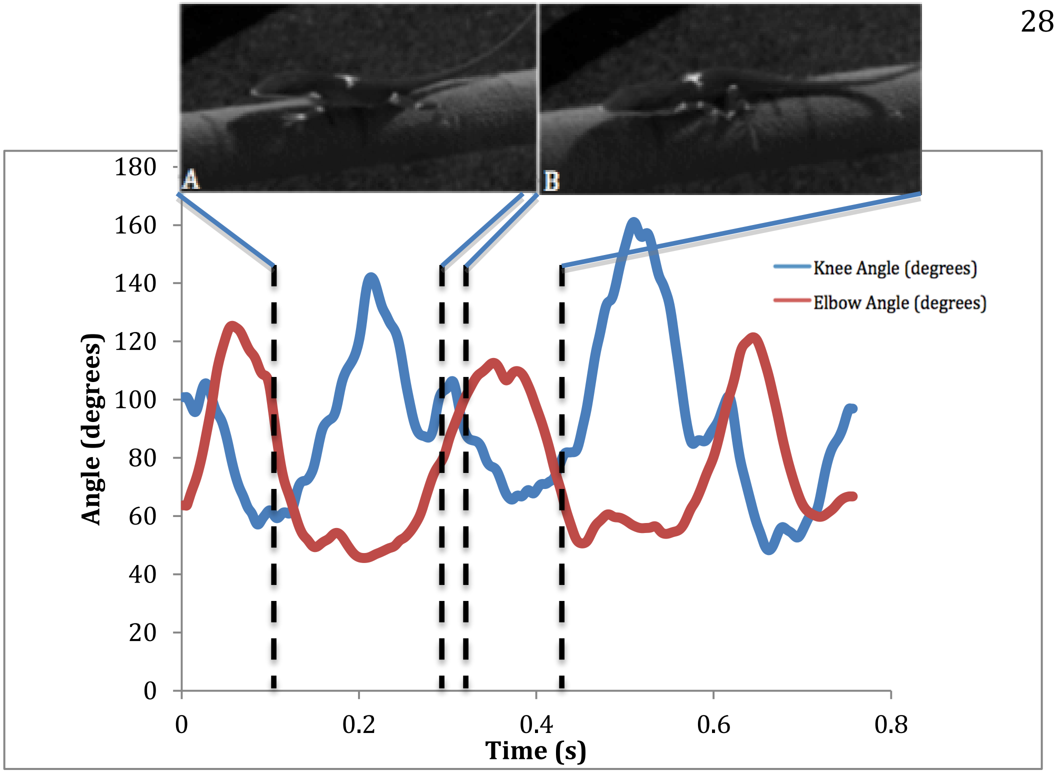

Figure 11-Knee and Elbow Angles of Several Steps: One lizard taking three steps with the forelimb and two steps with the hindlimb. Image A is the body position at a small elbow angle and a large knee angle, while image B is the body position at a large elbow angle and a small knee angle.

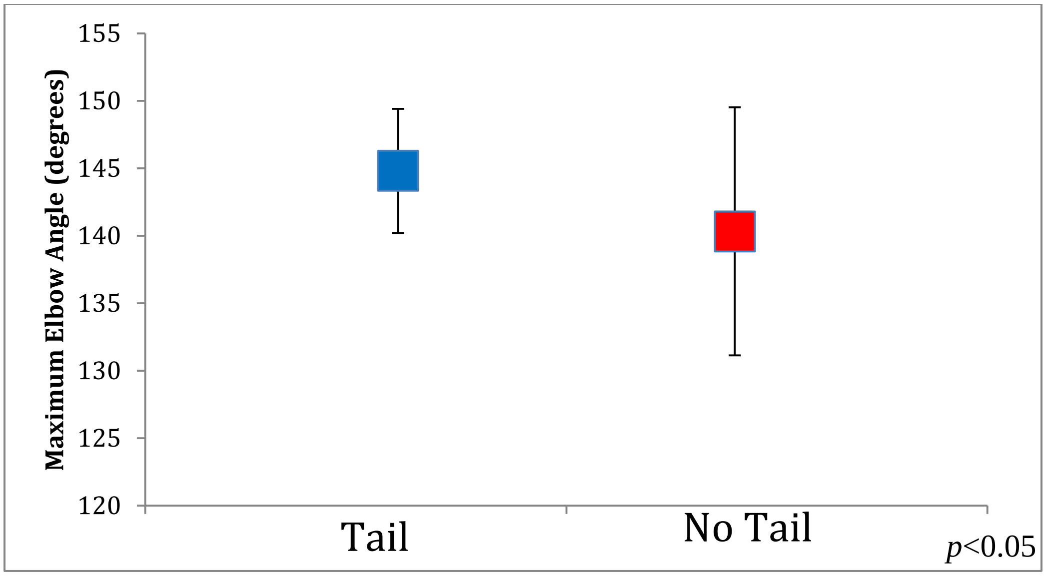

Figure 12-Maximum Elbow Angle: Average maximum elbow angle before and after autotomy (N=4 lizards, 5 steps per lizard). Lizards achieved a significantly smaller maximum elbow angle after losing 75% of their tails. Error bars represent standard deviation.

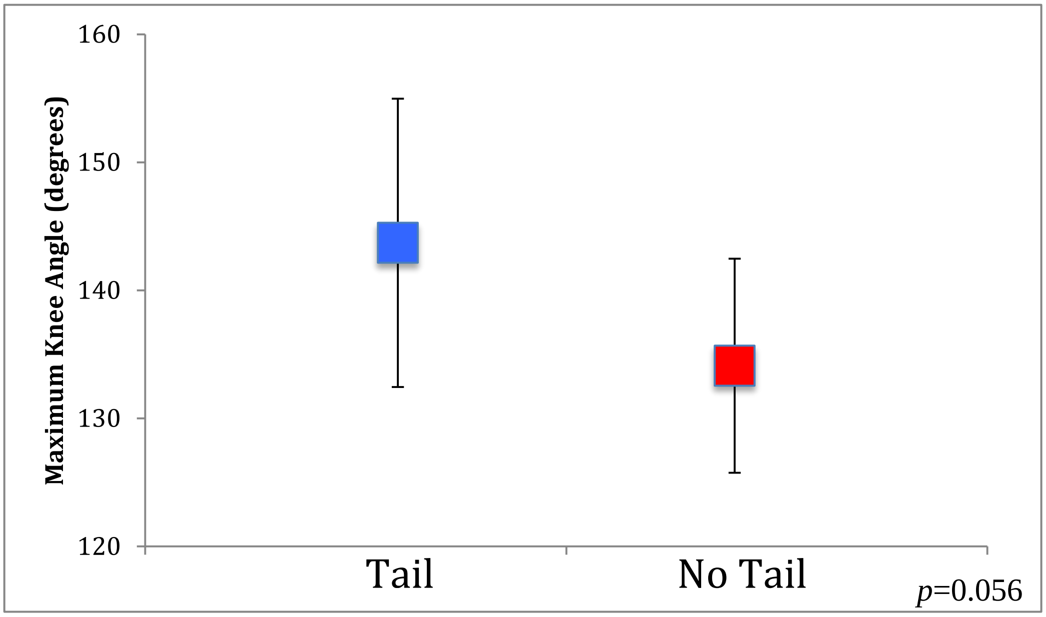

Figure 13-Maximum Knee Angle: Average maximum knee angle before and after autotomy (N=4 lizards, 5 steps per lizard). Error bars represent standard deviation.

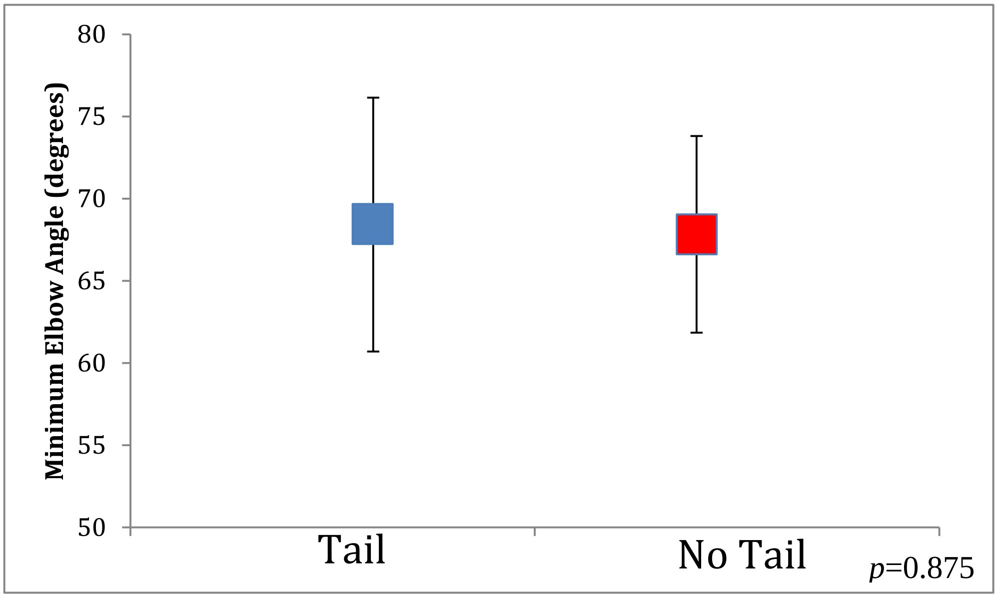

Figure 14-Minimum Elbow Angle: Average minimum elbow angle before and after autotomy (N=4 lizards, 5 steps per lizard). Error bars represent standard deviation.

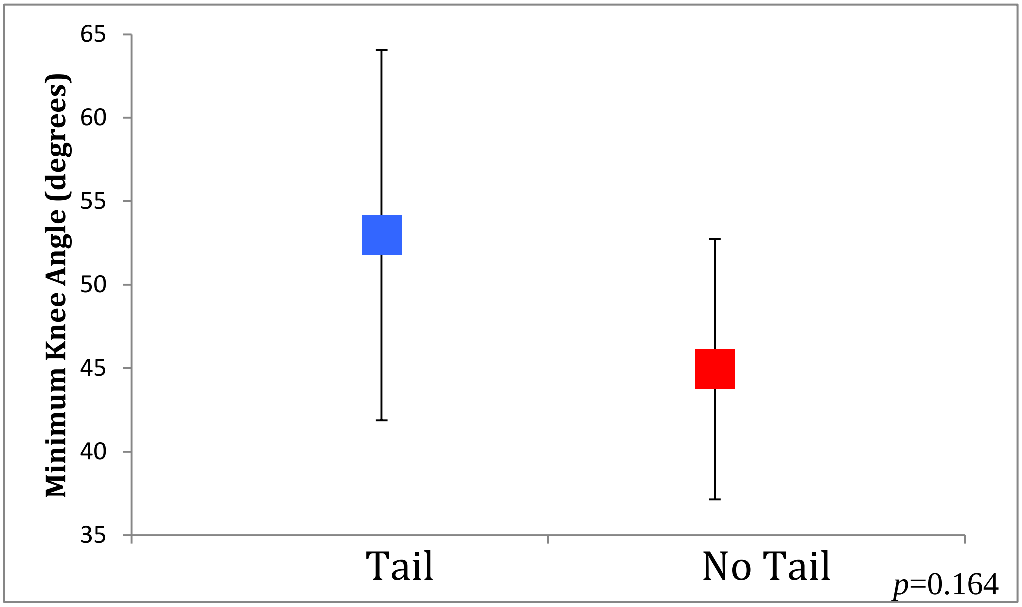

Figure 15-Minimum Knee Angle: Average minimum knee angle before and after autotomy (N=4 lizards, 5 steps per lizard). Error bars represent standard deviation.

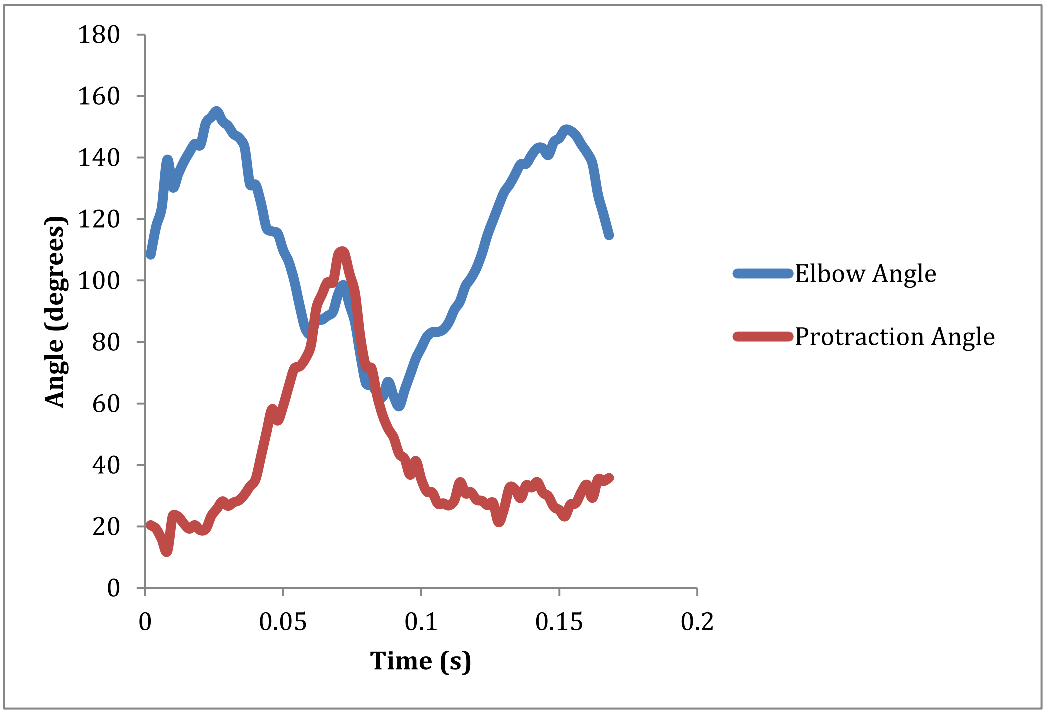

Figure 16-Forelimb Protraction and Elbow Angle: Protraction coinciding with elbow angle in one lizard taking between one and two steps.

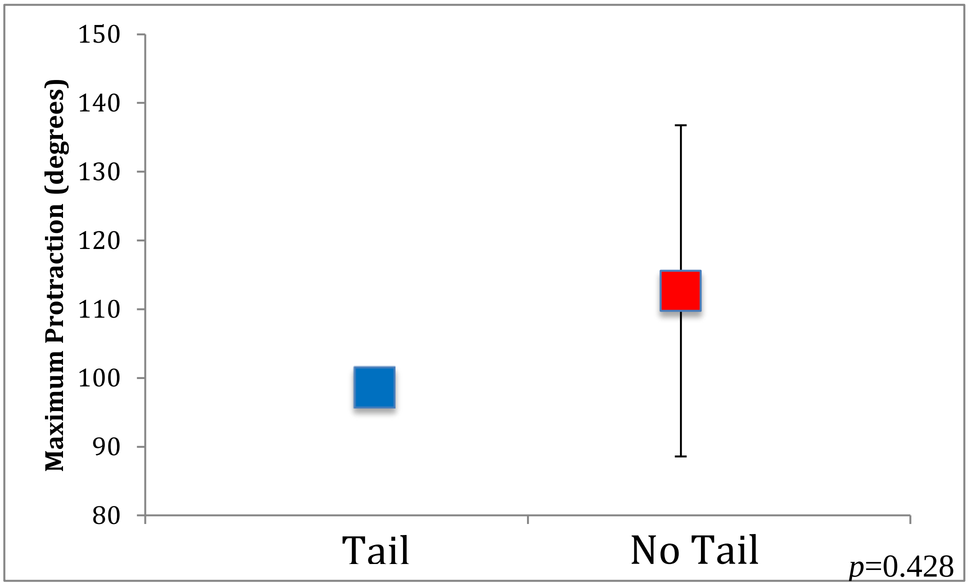

Figure 17-Maximum Protraction: Average maximum protraction before and after autotomy (N=3 lizards, 5 steps per lizard). Error bars represent standard deviation (pre-autotomy SD=1.323).

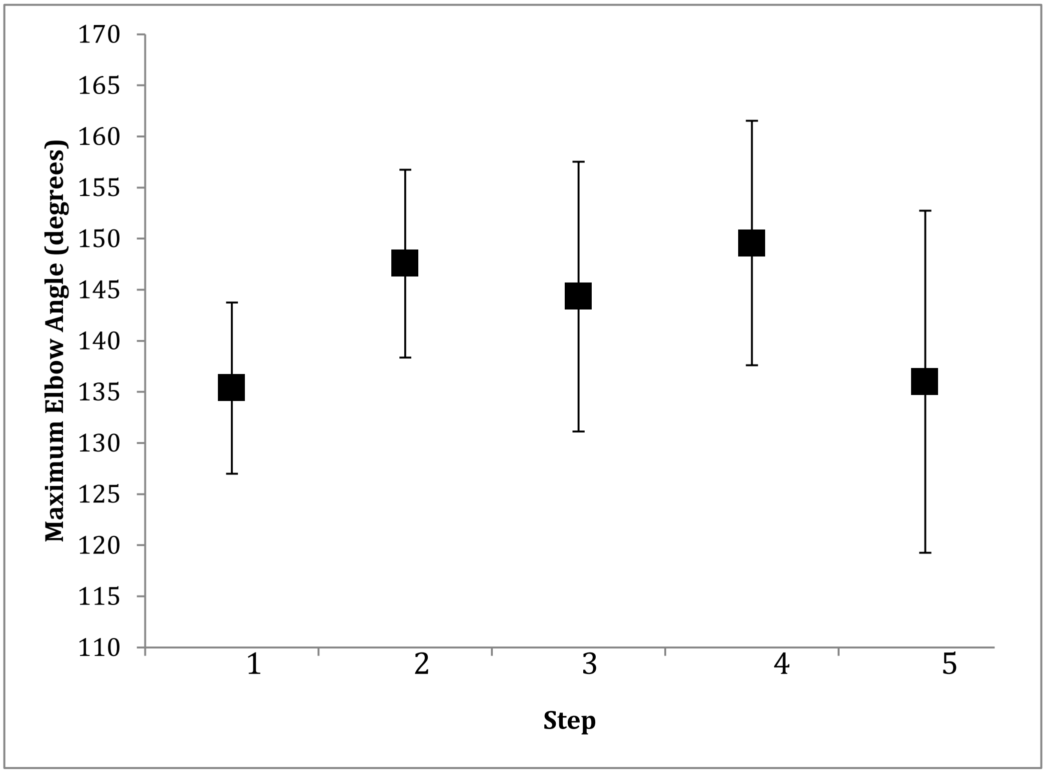

Figure 18-Effect of Trial on Maximum Elbow Angle: There was a significant difference in maximum elbow angle due to trial. The significant effect of trial on maximum elbow angle does not appear to have a meaningful pattern. Each step above, 1-5, represents the average maximum elbow angle across all four lizards.

DISCUSSION

Anolis carolinensis spend many locomoting hours in fine branch environments, and should therefore be studied on substrates closely imitating the natural, arboreal habitat (Losos 2011). Many factors of a natural habitat are dynamic and very difficult to reproduce, including the fluctuating temperature of the air, the wind direction and speed, the shifting compliance of the branch as the lizard runs toward or away from a tree trunk, the presence of twigs along the branch, friction of the wood, knots in the branch, and wetness.

Winnowing these confounding elements simplifies the animal’s locomotion and thus reveals the genuine kinematics of traversing a branch-like substrate. I constructed an arena in which the lizards ran along a support of moderate friction, with diameter and slope comparable to that of similar research, to recreate an arboreal environment (Losos and Irschick 1995; McElroy and Bergmann 2013; Brown *et al*. 1995). This was a larger diameter substrate than is likely to be found among fine branches of an arboreal environment, but smaller diameter dowels introduce the confounding elements for which I wished to control in order to study specificities of *A. carolinensis* running.

This study aimed to detect differences between the kinematics of *A. carolinensis* running before and after caudal autotomy. I characterized limb joint movements and hypothesized that changes in detailed limb movements might help explain previously reported gross locomotor effects of caudal autotomy in lizards. I predicted that past findings, such as decreased step length and slower sprint speed, were downstream effects of the lizard using different arm and leg motions (Martin and Avery 1998; Brown *et al*. 1995). I assessed these motions using excursions of the knee, elbow and humerus.

Limb Kinematics

My pre-autotomy running kinematics agreed with research done by Spezzano and Jayne (2004) on stride length, but diverged from their results on stride frequency. Their study evaluated the hindlimb kinematics of *Anolis sagrei*, a trunk-ground ecomorph, on substrates of varying diameter and incline. The diameter and incline most similar to those used in my experiment were a substrate of 45° incline and 3cm diameter. My results found a comparable average stride length (mean=5.9cm in my study versus approximately 6.5cm in theirs), but a smaller average stride frequency (mean=6.7 Hz in my study versus approximately 9Hz in theirs). The discrepancy in stride frequency may represent differences between the two species, or may be a result of the slightly different inclines (45° rather than 30°), though Spezzano and Jayne (2004) found no significant change in stride frequency with incline.

Spezzano and Jayne (2004) found higher speeds than results of this study under similar conditions. Again with diameter 3cm and 45° incline, Spezzano and Jayne (2004) commonly found speeds of greater than 100cm/s for *A. sagrei*, while

I found average velocity to be 42cm/s for *A. carolinensis*. This discrepancy again may be attributable to differences in the species and slight differences in experimental conditions.

Knee angle results also agree with past research on arboreal lizards, as shown in Higham and Jayne (2004). They studied the kinematics of the arboreal chameleon *Chameleo calyptratus* on different inclines as well as on a flat surface to compare these movements to those of *Dipsosaurus dorsalis*, a terrestrial generalist. Though data for *C. calyptratus* knee angle on an incline was not reported, on a treadmill the lizard had a knee angle maximum of ~150° and a knee angle minimum of ~65°. Its maxima and minima are comparable to knee angles found for *A. carolinensis* in this study (average maximum knee angle=156°, average minimum knee angle=53°).

While results for knee angle in this study are corroborated by past research with arboreal lizards, they differ from findings in the predominantly terrestrial iguana *D. dorsalis* (Jayne and Irschick 1998). This lizard typically runs faster than *A. carolinensis* is reported to run in this study, presumably because they are many times larger. In addition to this difference, *D. dorsalis* exhibited knee minima higher than *A. carolinensis* knee minima and knee maxima lower than *A. carolinensis* knee maxima (Jayne and Irschick 1998). This large knee excursion in *A. carolinensis* might be considered an arboreal adaptation, since it is comparable to the arboreal *C. calyptratus* but larger than the terrestrial *D. dorsalis*.

Effects of Autotomy

I rejected my hypothesis that *A. carolinensis* have different limb joint movements post-autotomy when results showed no significant change in the majority of kinematic variables tested. Average velocity, however, did increase significantly after autotomy. In his 1983 study of the gecko *Phyllodactylus marmoratus*, Daniels similarly found an increase in average running speed. In this gecko, the tail accounts for approximately 25% of the total body weight. This led Daniels to conclude that the tail drags during locomotion, impeding forward motion both because of its weight and the friction developed between it and the substrate. He concluded that losing the tail removes these impediments and allows lizards to run faster, stating that this new speed is a necessary compensation for losing autotomy as a predation escape behavior. In lizards suspected to have more actively functioning tails than *P. marmoratus*, such as the quadrupedal *Cophosaurus texanus* and the quadrupedal and electively bipedal *Uma notata*, autotomy is associated with a decrease in running speed (Punzo 1982). These two lizards have much less massive tails, with the *C. texanus* tail accounting for ~10% of the body weight, so any drag they impart may not contribute to speed during locomotion.

Recent research has illuminated the importance of the tail in arboreal locomotion in lizards (Gillis *et al*. 2009; Jusufi *et al*. 2008; McElroy and Bergmann 2013). Since *A. carolinensis* tails account for approximately 5% of their body weight, in light of the work of Punzo and Daniels, the tail would not provide sufficient drag to alter running speed; running speed would be predicted to decrease after autotomy. Given that I found the opposite, perhaps a behavioral explanation is needed. Caudal autotomy is well-documented as a costly, but ultimately beneficial, predation escape behavior. Its costs range from metabolic to social to locomotor, and it is associated with several shifts in behavior (Higham *et al.* 2013; McElroy and Bergmann 2013; Cromie and Chapple 2012). Tails have been found to govern risk-taking behavior; after autotomy, tailless lizards spend less time in open areas due to the inherent risk of capture. They prefer habitats with coverage that provide safety from predation, and restrict the size of their home range (Naya *et al.* 2007). After autotomy, one of the lizard's most effective mechanisms for escape is no longer available, so taking fewer risks and remaining well-protected might be a response to a reduced number of escape behaviors. In lieu of explanatory, associated changes in limb joint kinematics, I attribute the increase in average velocity observed in my experiments to a behavioral response. I speculate that the increase in average velocity documented in this research is a result of a vulnerable lizard in an exposed arena. Mechanically, the increase in average velocity is consistent with other findings.

Limbed animals have two means of increasing speed: increasing step-length and increasing step frequency. These two can be employed independently or in combination to produce increased gait speed. Upon finding an increase in average velocity of the lizards post-autotomy, I turned to step length and step frequency, expecting a correlated change in either or both. Step length showed no significant change after tail loss, indicating that the increase in average velocity was not attributable to a lengthening of the steps. In fact, hindlimb step length showed a marginally significant decrease after autotomy (p=0.07) from a pre-autotomy mean of 5.94 cm to a post-autotomy mean of 5.54 cm. If coupled with a constant step frequency, a decrease in step length would yield a decrease in average velocity. I predicted an increase in hindlimb step frequency large enough to overcome the marginally significant decrease in hindlimb step length and still result in an increase in average velocity.

Results indicated a significant increase in hindlimb step frequency from a pre-autotomy mean of 6.7 steps per second to a post-autotomy mean of 10.0 steps per second, a 33% increase in frequency. This increase in step frequency was also observed in the forelimb, which changed from a pre-autotomy mean of 7.4 steps per second to a post-autotomy mean of 10.7 steps per second. This finding is amenable with the marginally significant decrease in hindlimb step length. The hindlimbs showed a greater percent increased frequency than the forelimbs by 2%, which is logical considering that the hindlimbs must increase frequency by more than their decrease in step length to increase velocity.

The absence of significant changes in step length in the hindlimbs and forelimbs is consistent with the absence of significant changes in maximum knee angle, minimum knee angle, minimum elbow angle, and maximum humeral protraction. If the lizard does not change the length of its step, the angles through which it moves its limbs to produce a step are not expected to change. Maximum elbow angle was the only significantly changing limb joint variable ($p<0.05$) decreasing from 145° pre-autotomy to 140° degrees post-autotomy. This minute change in the degree of extension of the forelimb after tail loss has no ultimate effect on step length.

One possible explanation for the reduced maximum elbow angle is the increase in speed of the body as driven by increased hindlimb step frequency. In taking a post-autotomy step, the forelimb may begin the step with normal pre-autotomy positioning—minimum elbow angle—but, while protracting and increasing the elbow angle, the body may move faster than it does with a tail as a result of the increased hindlimb step frequency. This propulsion from the hind end speeds the body forward, forcing the forelimb to return to the ground to support the moving body before it has achieved as great a maximum elbow angle as it does with a tail.

Sample size was small for my study, although in the field of comparative biomechanics, time-intensive data collection dictates a commonly accepted sample size of four or five animals. Nevertheless, results from studies using such small sample sizes should be extrapolated to a larger population only with caution. The small sample size of this study unfortunately has implicit inaccuracy; individual lizards vary and any unrepresentative nuance in a subject's locomotion heavily influences the data reported here.

In choosing individual steps from videos of several-step runs, I chose only non-consecutive, intermediate steps. Choosing intermediate steps ensured that I avoid unrepresentative kinematics of initial and final steps. Choosing non-consecutive steps prevented me from cutting and analyzing the same step twice. Lizard three gave several runs of more than four steps. In these instances I took more than one step from a run under the assumption that two non-consecutive steps from the same run are as different as two steps from different runs. This assumption is technically and statistically not sound. I corrected this error in my methodology but, due to time constraints, chose to keep the data collected in that manner.

MATLAB technology is efficient, but three-dimensional digitizing leaves room for human error to alter data. Depending on the brightness and focus of the cameras, certain of the videos were very difficult to digitize with heightened accuracy. In these instances, digitization was approximated to the best of my ability, but may be less precise than is ideal. In addition, my data aggregation and manipulation, as well as calculations, were done in Microsoft Excel. They were carried out meticulously, but certain room for error and error propagation exist when working in several workbooks with the same formulaic skeleton.

Conclusion

In this study, four *Anolis carolinensis* were assessed for changes post-caudal autotomy in limb joint kinematics and gross locomotor characteristics. Lizards run faster post-autotomy, which is mechanically explained by an increase in step frequency by the forelimbs and hindlimbs. Step length did not change for the forelimbs or the hindlimbs. This result agrees with the fact that there was no change in maximum knee angle, minimum knee angle, and minimum elbow angle after tail loss. Maximum elbow angle significantly decreased post-autotomy, potentially as a result of increased body speed due to increased hindlimb step frequency. The forelimbs were not found to protract significantly differently after autotomy.

A. carolinensis, as a trunk-crown ecomorph having undergone an ecological release on mainland USA, is taken to be a suitable model species for locomotion of the Anole genus. It would be interesting to test the validity of this designation by repeating this study with alternative species of anole.

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