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Functional Challenges of Tail Autotomy: Locomotor Responses to a Rapid Change in Mass and Loss of Tail Function

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Functional Challenges of Tail Autotomy: Locomotor Responses to a Rapid Change in Mass and Loss of Tail Function

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by

Kevin Jagnandan

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DEDICATION

This dissertation is dedicated to my family and friends for their unbridled love, support, and tolerance of my academic pursuits. In loving memory of my biggest supporter and grandfather, Robert Jagnandan.

ABSTRACT OF THE DISSERTATION

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Kevin Jagnandan

Doctor of Philosophy, Graduate Program in Evolution, Ecology and Organismal Biology
University of California, Riverside, December 2016
Dr. Timothy Higham, Chairperson

The ability to move effectively is critical in the survival of most animals, as locomotion plays a pivotal in prey capture, predator evasion, and other movements associated with acquiring resources and mates. However, terrestrial locomotion often requires compensation from the musculoskeletal system to overcome environmental or physiological demands. In this dissertation, I explore the demands associated with rapidly occurring changes in mass by addressing this phenomenon as a locomotor perturbation. In the leopard gecko, *Eublepharis macularius*, the tail accounts for 25% of the animal's mass, which is shed almost instantaneously via autotomy in response to a perceived threat. Autotomy thus provides an effective natural model for studying the impacts of rapid mass change on locomotor mechanics and neuromuscular function. Investigations of morphology, 3D kinematics, and ground-reaction forces (GRFs) before and after autotomy in this species revealed a significant anterior shift in the animal's center of mass (CoM) after sacrificing the tail, resulting in a shift to a more sprawled posture to preserve stability while adjusting to the change. Experimentally restricting natural undulatory movements of the tail revealed that this postural shift occurs when the tail is compromised in any way, not necessarily because of the altered mass. The tail is also suggested to be biomechanically linked to step length by rotation of the pelvic girdle and retraction of the femur, illuminating an additional source of the locomotor consequences of tail autotomy. Finally, electromyography (EMG) analyses were used to elucidate how motor control of the locomotor muscles are modulated in response to tail autotomy, revealing differential impacts in the forelimb and hind limb muscles that coincide with their locomotor functions. These results highlight the utility of tail autotomy as a system for studying the mechanical consequences of rapid mass change, the functional role of the tail, and the neuromuscular control of locomotion in response to altered demands. By understanding the functional consequences and adaptations of the locomotor system in response to tail autotomy, an evolutionary framework can be used to determine how selective pressures have shaped these responses.

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LIST OF ABBREVIATIONS

CF	caudofemoralis
CNS	central nervous system
CoM	center of mass
EMA	effective mechanical advantage
EMG	electromyography
F_m	muscle force
GAST	gastrocnemius
GRF	ground-reaction force
LG	lateral gastrocnemius
M_j	joint moment
M_m	muscle moment
PIT	puboischiotibialis
r	muscle moment arm
R	GRF moment arm
RIA	rectified integrated area
SVL	snout-vent length

INTRODUCTION

An animal’s ability to move effectively is critical for survival, as locomotion plays a pivotal role in catching prey, escaping predators, foraging, mating, and migrating. Naturally, animals encounter demands that can have diverse effects on the locomotor system, affecting their ability to perform these ecologically relevant tasks (Irschick and Garland, 2001). Locomotor perturbations stem from a number of sources (Figure 1), many of which result in a sudden shift in an animal’s center of mass (CoM) and destabilizing locomotion that alters the mechanical demands and constraints on the locomotor system. Perturbations are most frequently addressed in the context of terrestrial animals negotiating complex terrain in their natural environments. For example, behavioral and neuromechanical responses to environmental perturbations have been demonstrated using obstacles (Birn-Jeffery and Daley, 2012; Olberding et al., 2012), a drop in substrate height (Daley and Biewener, 2006; Daley et al., 2006), a decrease in surface roughness (i.e. a slip) (Clark and Higham, 2011), a change in surface compliance (Ferris et al., 1999), and unexpected lateral forces (Jindrich and Full, 2002) (Figure 1). Recovery from these perturbations generally occurs within a single stride (Daley and Biewener, 2006; Jindrich and Full, 2002), suggesting that feedforward and intrinsic mechanics contribute to maintaining dynamic stability. Internal factors, such as changes in body mass, can exert unavoidable physical demands that perturb locomotion (Garland, 1984; Higham and Irschick, 2013; Taylor, 1982) (Figure 1). How rapid changes in mass impact locomotor function is poorly understood, however.

Body size and mass play a significant role in shaping the evolution of terrestrial animals, as the scaling of structural elements with mass have been shown to have profound implications for how animals of different sizes move (Biewener, 1990; Biewener, 2005; Heglund et al., 1974; McMahon, 1975). At the individual level, organisms must also manage the demands imposed by changes in mass that occur throughout their lifetimes. Intra-individual body mass changes are ubiquitous and occur in response to a range of behavioral and physiological factors. These changes can also occur over a range of temporal scales (Figure 1). Mass change is typically a gradual process occurring over a period of months (e.g. pregnancy) or years (e.g. ontogeny). However, mass can also be gained rapidly, such as when an infant attaches to a parent, or lost quickly, such as during parturition or autotomy (voluntary loss of an appendage). In these examples, the time an individual has to compensate for the change in mass is significantly reduced compared to gradual shifts. Rapid mass changes thus require rapid compensation in order to correct for shifts in the individual’s CoM, relative muscle mass, and loading on the musculoskeletal system.

In this dissertation, I address rapid changes in mass as a locomotor perturbation and investigate its impact on locomotor biomechanics. In Chapter I, I review mechanisms by which mass is gained or lost rapidly in terrestrial vertebrates, and I integrate the existing information regarding the relationships between mass changes and locomotion across diverse taxa. I then use this to generate a framework for future studies on the skeletal, mechanical, neural, and muscular responses to rapid mass change. In the subsequent chapters, I utilize the voluntary shedding of the tail (autotomy) by leopard geckos (*Eublepharis macularius*) as a model system for naturally manipulating the mass of an animal to study rapid mass change in a series of experiments. In Chapter II, I determine specifically how much the CoM is shifted throughout the loss and regeneration of the tail, and how locomotor kinematics and kinetics are altered with these shifts. In Chapter III, I explore the role of the tail in locomotion and how the loss of tail function by autotomy impacts body and limb kinematics. In Chapter IV, I investigate how limb muscles are adapted to changes in loading during locomotion by quantifying shifts in *in vivo* muscle activity before and after autotomy. By understanding the functional consequences and adaptations to rapid changes in mass, an evolutionary framework can be used to determine how selective pressures have shaped these responses. In addition, understanding how the neuromuscular system rapidly adapts to altered demand will provide key insight into the plasticity of complex control systems.

CHAPTER I – How rapid changes in mass impact terrestrial locomotion: Muscle function and mechanics of a natural perturbation

Summary

Animals rarely move through their natural habitat without experiencing an environmental perturbation, such as an obstacle or drop in height. Rarely examined are rapid perturbations due to mass changes, which can have significant impacts on animal locomotion due to associated changes in relative muscle mass, altered body shape, shifts in the position of the center of mass (CoM), and changes in the overall load on the animal. I discuss locomotor perturbations, with an emphasis on the duration associated with them. I then address how commonly studied modes of rapid mass changes (e.g. parturition, autotomy, feeding) impact locomotion by examining the effects of natural and artificial changes in mass on locomotor performance, kinematics, kinetics, and muscle function in terrestrial animals. This information is utilized to generate a framework for future studies.

Rapid mass change as a locomotor perturbation

Aside from terrain disturbances, rapid perturbations also occur from external and internal alterations of mass (Figure 1). External mass changes involve an added load to an animal, such as carrying offspring or prey. Losing mass usually requires an internal physiological mechanism, such as when a lizard autotomizes its tail (Arnold, 1984). A commonality between environmental perturbations and rapid changes in mass is the sudden onset of the event. Slipping on a surface following a habitat transition and suddenly carrying or losing a load all occur with seconds or milliseconds. However, a key difference is the duration over which the perturbation is sustained. Although occurring suddenly, mass changes can persist and must be accompanied by longer term shifts in neural control and mechanics. This is in contrast to sudden environmental perturbations, which although potentially catastrophic, typically go away as quickly as they appear. For these cases, the ability to reflexively recover is paramount, but long-term control shifts are not likely necessary.

Perturbation experiments are useful for determining the control strategies that animals employ to maintain dynamic stability. Generally, perturbation experiments aim to rapidly displace an animal's CoM to observe the animal's response as it attempts to return to a normal locomotor cycle (Jindrich and Full, 2002). This may require some sensory input from the perturbation (e.g. mechanoreceptors sensing a change in substrate height) leading to a shift in neural signaling and a reactive motor response that alters limb kinematics or muscle use to stabilize the animal (Figure 2). Mechanisms for intrinsic mechanical stability may also play a role, allowing for perturbation recovery that occurs more quickly than explained by short latency reflexes. Mass changes that are concentrated in one region of the body can significantly alter the distribution of mass within an individual in a similar manner, and can thus prove useful for understanding how animals recover from different perturbations that occur naturally. Alternatively, sensory input before encountering the perturbation (e.g. visual cues) may lead to a proactive motor response to prevent a destabilizing motion (Figure 2). In mass change perturbations, it is unclear if control is primarily proactive or reactive. Can animals sense an upcoming change in mass and make the necessary adjustments in preparation for the event? Rapidly changing mass presents a notable perturbation that warrants neuromechanical compensation (or preparation) by the locomotor system.

Mechanical demands and consequences of rapid changes in mass

The shape and size of an animal largely determine animal movement due to the intricate relationship between locomotor mechanics and posture (Biewener, 1989b; Biewener, 1990; Biewener, 2005). During terrestrial locomotion, an animal exerts a force onto the ground with its limbs, generating a ground-reaction force (GRF), the largest component of which is vertical to support the body mass. The GRF generates moments (or torques) at the limb joints, which must be balanced by muscle force at those joints to support the body and sustain locomotion (Biewener, 2003). These relationships are demonstrated in the following equations (Eqn 1, Eqn 2):

$$\mathrm{GRF} \times R = F_m \times r \tag{1}$$

$$M_j = M_m \tag{2}$$

The joint moment (M_j) is equal to the product of the GRF and R (the moment arm of the GRF, or the length between the joint and line of force acting on that joint). The muscle moment (M_m) that balances the joint moment is equal to the product of F_m (the muscle force) and r (the muscle moment arm). Since GRF is proportional to body mass, it is not surprising that peak GRFs increase in direct proportion to body mass (Farley et al., 1993). Increasing GRFs result in a greater M_j, thus requiring more force from the limb muscles to support a greater mass (assuming a fixed muscle moment arm) (Biewener, 1989b; Biewener, 2005).

However, increasing the magnitude of muscle force generates greater stresses on the bones and tendons (Biewener, 1990), which can lead to higher risks of injury. An alternative for supporting increased mass is to shorten the moment arm of the GRF to reduce the muscle force requirement (assuming a fixed muscle moment arm). Adopting a more erect posture reduces M_j produced by the GRF, lowering the demand on the locomotor muscles for supporting the joint and avoiding potentially damaging stresses (Biewener, 1989b; Biewener, 1990; Biewener, 2005). This shift in posture increases the animal's effective mechanical advantage (EMA), defined as the ratio of the muscle moment arm to the GRF moment arm (Biewener, 1989b) (Eqn 3).

$$\mathrm{EMA} = r/R = \int \mathrm{GRF} / \int F_m \tag{3}$$

EMA generally increases with body size, as larger animals are observed to have more upright postures, reducing the magnitude of muscle force required to support the animal's mass (Biewener, 2005). However, this posture is hypothesized to limit maneuverability and acceleration compared to animals that support smaller masses while assuming more sprawled stances (low EMA) (Biewener, 1983; Biewener, 1989a; Biewener, 1989b).

The relationships between mass, EMA, muscle force, and locomotor ability are generally considered in the evolution of terrestrial locomotion. However, these principles are also applicable to mass changes that occur at the individual level. When mass is added to an individual, vertical GRFs must increase, consequently increasing the GRF moment and M_m at the joints if no postural shifts occur (Figure 3). Thus, an individual gaining mass can be problematic in terms of potential injury due to damaging stresses when muscle force increases. In natural systems, mass can increase substantially when animals carry loads in the form of prey, young, or materials. Whether these animals change their posture to reduce muscle force and consequent injury risk is a topic to be explored.

Can what is known about gaining mass be used to understand how animals respond to losing mass? When GRFs and joint moments decrease with the reduced mass, does EMA decrease as well (Figure 3)? This would generate a more crouched posture, which is suggested to increase the mechanical advantage for horizontal GRF production, allowing for greater maneuverability (Walter, 2003). Alternatively, if no postural shift occurs, muscle force would be reduced, making locomotion more efficient. Tail autotomy, parturition, and oviposition all involve a rapid reduction in mass as the tail, eggs, or young are released, although postural effects are undocumented. However, the physiological costs associated with recovering from gravidity is likely a factor in an animal's locomotor response (Olsson et al., 2000).

Locomotor stability may also be compromised by changes in mass. External loads added to turtles (Marvin and Lutterschmidt, 1997; Wren et al., 1998), geckos (Irschick et al., 2003), horses (Hoyt et al., 2000; Wickler et al., 2001), and other mammals (Baudinette and Biewener, 1998; Young et al., 2007) demonstrate similar locomotor impacts across these taxa. Generally, terrestrial quadrupeds exhibit reductions in stride length, stride frequency, and/or duty factor when mass is added, suggesting that an increased demand for support may be necessary for maintaining stability during locomotion with an added load. Less is known about how losing mass affects stability, although one can predict that rapidly shedding a large mass may have a dramatic impact. Perhaps most significant to locomotor stability is any rapid change in mass that alters an animal’s CoM (Figure 3). Many animals carry their young or prey in their mouth, adding significant mass to the cranial end of the body and shifting the CoM in that direction. A loss of caudal mass, such as what occurs in tail autotomy, should have a similar effect. These CoM shifts alter the distribution of mass and change the contributions of each set of limbs for support and propulsion (Arnold, 1984; Lee, 2011; Lee et al., 2004; Walter and Carrier, 2011). A large CoM shift could also generate a toppling moment that would alter locomotor forces and kinematics.

The effects of mass changes on locomotor performance are well documented. Sprint speed and endurance usually increase when mass is lost (Bauwens and Thoen, 1981; Cooper et al., 1990; Finkler et al., 2003; Qualls and Shine, 1998; Wapstra and O'Reilly, 2001), due to reduced force requirements for support and propulsion (Scales and Butler, 2007). In contrast, performance decreases in recently fed animals (Huey et al., 1984; Martin, 1996) and animals carrying loads (Hoyt et al., 2000; Kuo et al., 2011; Wickler et al., 2001). Greater variation is observed when mass is lost via tail autotomy, however, which is likely due to the functional role of the tail in different species (Anderson et al., 2012; Ballinger et al., 1979; Brown et al., 1995; Chapple and Swain, 2002; Cooper Jr et al., 2009; Cooper and Smith, 2009; Cooper et al., 2004; Cromie and Chapple, 2012; Daniels, 1983; Daniels, 1985; Ding et al., 2012; Downes and Shine, 2001; Formanowicz et al., 1990; Goodman, 2006; Huey et al., 1990; Kelehear and Webb, 2006; Lin and Ji, 2005; Lin et al., 2006; Martin and Avery, 1998; McConnachie and Whiting, 2003; McElroy and Bergmann, 2013; Medger et al., 2008; Pond, 1978; Punzo, 1982; Shine, 2003).

Utilizing mass change to understand neuromechanics

Considering the importance of muscles in driving locomotion, supporting body mass, and maintaining stability (Marsh, 1999), surprisingly little data is available on how the limb muscles adapt to changes in loading. This is despite the fact that the mechanical actions of muscles depend on the loads resisting motion. Are locomotor muscles capable of rapidly changing their function in the face of mechanical disturbances, such as rapid mass change? Are species that commonly undergo large and rapid changes in mass better at altering their neural control in the face of changing mass? If not, how long does it take to alter neural control after a perturbation, and what mechanisms might underlie differences in this time period?

Unique patterns of muscle function are predicted to emerge when animals experience a rapid change in mass, including changes in operating lengths, force generation, and the relative timing of activation among different limb muscles. In order to make predictions about shifts in muscle function with changes in mass, I draw from the literature on rapid perturbations. Recent work has focused on neuromuscular strategies for running over uneven terrain. One possibility is that the nervous system is not involved in the rapid response to a perturbation, but rather the dynamically stable mass-spring behavior of the locomotor system may result in passive leg adjustments (Daley, 2008). In other words, the pattern of locomotion in terrestrial vertebrates might be robust enough to adjust without higher control. However, many animals likely employ a reactive strategy, in which muscle activation is altered following the reception of sensory information from the perturbation (Figure 3). In humans, a slip perturbation leads to a rapid shift in neuromuscular control in order to recover balance. For example, Marigold and Patla (2002) found that, in humans, the mean muscle onset latencies ranged from 146-199 ms, indicating that polysynaptic reflexes were utilized to recover balance during a slip.

In running birds, the neuromuscular strategies in association with both drop perturbations and obstacles have been examined. The lateral gastrocnemius (LG) of guinea fowl exhibits an 80% increase in electromyography (EMG) intensity during perturbed obstacle strides in comparison to level locomotion (Daley and Biewener, 2011). This is in contrast to the perturbations involving a sudden drop, in which no significant change in LG EMG activity was observed (Daley et al., 2009). Given that the transmission delay for the stretch reflex of the gastrocnemius is about 6 ms, this suggests that a monosynaptic stretch reflex is involved. This alteration in control highlights the ability of the sensorimotor system to adapt to changing conditions. The same is expected for a sudden change in body mass. Unlike environmental perturbations, in which vision might play a role in providing sensory input prior to the perturbation, changes in mass will convey internal proprioceptive information. It is likely that articular mechanoreceptors are important for determining the magnitude of mass change. For example, a sudden drop in body mass after losing the tail will alter the amount of knee and ankle extension (likely more than elbow and wrist extension, due to the position of the tail), assuming that muscle activation remains the same. This altered joint position will be reported to the central nervous system (CNS) via joint mechanoreceptors (Riemann and Lephart, 2002). The animal can then reduce the level of neuromuscular activation, returning the joint angular excursion to normal after a period of time. Whether this occurs in animals that exhibit caudal autotomy is not known, but will form a prediction for future studies.

Much of what is known about neuromuscular changes in relation to changes in mass stems from artificial load studies. As load is added to animals, a common strategy is to increase duty factor and decrease stride length and frequency, suggesting a shift in motor unit recruitment from propulsive to supportive muscles, perhaps to enhance stability. Ellerby and Marsh (2006) measured muscle energy use in guinea fowl (*Numida meleagris*) carrying external loads, which increased under all experimental loading conditions, but differed between individual muscles. In trunk-loaded birds, most increases in energy use were restricted to stance-phase muscles, while distally-loaded birds exhibited increases in both stance- and swing-phase muscles. Trunk-loads also altered the contractile patterns of hind limb extensor muscles (indicated by enhanced stretch activation early in stance), as well as the intensity of muscle activation, demonstrated by an increase in the intensity of EMG signals from the muscles (McGowan et al., 2006). These findings provide further evidence for the increased demand on the muscles responsible for support and stability when mass is added, and may explain the kinematic changes observed in other species.

Aside from these data, studies that quantify *in vivo* muscle function in response to added or decreased loads are rare. Additionally, no data exists on how the locomotor muscles respond to natural changes in mass, such as those that occur with gravidity and autotomy. Some of the most significant demands ever placed on the neuromuscular system of non-human animals might be during a rapid gain or loss of mass, thus reflecting a critical gap in our understanding of how muscles can respond to change. This essentially addresses how plastic muscle function can be over short periods of time, in contrast to the large body of research examining atrophy and hypertrophy of muscles over longer durations (Edgerton et al., 2002).

Natural versus artificial experiments: gaining evolutionary insight

Much of what is known about perturbations involves artificial situations. For example, Clark and Higham (2011) examined how guinea fowl respond to a sudden decrease in the friction of the substrate. Although informative, this reflects an unnatural situation. I propose that examining natural perturbations, such as tail autotomy, will not only provide sensorimotor and mechanical insight into locomotor stability, but will also provide information regarding the evolution and ecology of perturbation recovery. However, a cost associated with natural experiments involving sudden changes in mass is that one cannot rule out concomitant physiological changes.

Evolution has shaped the ability of animals to deal with natural perturbations. We have yet to tap into this information to understand the evolution of neuromuscular and mechanical responses to destabilizing events. By examining terrestrial vertebrates that exhibit an array of responses to natural perturbations, we can tease apart the strategies that may enhance fitness. By doing so, we can identify phylogenetic constraints and perhaps multiple solutions to a similar problem. This may also help identify novel strategies that can then be adopted in the construction of stable robots that deal with carrying and releasing items. Lizards may be ideal for this endeavor, as tail autotomy is widespread and varies considerably among different lineages. The shape and size of the tail also varies dramatically, providing a great opportunity to examine the role of mass reduction in stability. The likely variation in tail function across lizards will also translate into unequal impacts on the animal following tail loss. Thus, multiple strategies for dealing with this natural perturbation are very likely, but understudied.

Conclusions

Gaining and losing mass quickly has dramatic impacts on animal locomotion, muscle function, and injury. However, very little is known about the role of rapid mass change in dynamic non-human locomotion, and how the ability to cope with these changes has evolved. I propose that tail autotomy in lizards will be an exceptional system for examining the role of rapid mass loss, given that it is a naturally occurring perturbation and is ultimately overcome through regeneration. The mechanical neuromuscular alterations (both short and long-term) following autotomy will illuminate a broader understanding of plasticity in physiological and biomechanical systems, and will provide a foundation for evolutionary studies.

CHAPTER II – Tail autotomy and subsequent regeneration alter the mechanics of locomotion in lizards

Summary

Animals undergo significant changes in mass due to a variety of causes. Autotomy, the voluntary shedding of an appendage in response to a predator stimulus, provides an effective model for measuring the effects of rapid mass change on locomotor behavior and the responses to more gradual mass gain, particularly in lizards capable of both autotomizing and regenerating their tail. Although the general effects of autotomy on locomotor performance are commonly explored, I investigated changes in locomotor mechanics associated with tail loss and long-term regeneration for the first time by measuring morphology, 3D kinematics, and ground-reaction forces (GRFs) in the leopard gecko, *Eublepharis macularius*. Tail autotomy resulted in a 13% anterior shift in the center of mass (CoM), which only partially recovered after full regeneration of the tail. Although no changes in body or forelimb kinematics were evident, decreases in hind limb joint angles signify a more sprawled posture following autotomy. Changes in hind limb GRFs resulted in an increase in weight-specific propulsive force, without a corresponding change in locomotor speed. Hind limb kinematics and GRFs following autotomy recovered to pre-autotomy values as the tail regenerated. These results suggest an active locomotor response to tail loss that demonstrates the causal relationships between morphological, kinematic, and force variation.

Introduction

Many animals encounter episodes of substantial changes in mass throughout their lifetime as a result of famine, obesity, illness, pregnancy, and even limb/tail loss. Losing and/or gaining mass in these ways can result in a variety of physiological effects, including changes in locomotor behavior. For example, organisms of several taxa, including salamanders (Finkler et al., 2003), lizards (Shine, 1980; Shine, 2003), and birds (Lee et al., 1996), suffer a reduction in locomotor performance when gravid. However, a major source of variation in locomotor strategies likely relates to differences in the length of time associated with mass loss or gain. An assessment of the effects of rapid mass loss on locomotion is necessary to more fully understand how animals overcome the particular functional challenges imposed by such eventualities.

Autotomy is a predator-escape strategy documented for a broad range of taxa (Fleming et al., 2007), including crabs (Bennett, 1973), spiders (Amaya et al., 2001; Parry, 1957), echinoderms (Emson and Wilkie, 1980), salamanders (Wake and Dresner, 1967), lizards (Arnold, 1984; Bateman and Fleming, 2009; Congdon et al., 1974; Cooper and Vitt, 1985; Daniels, 1983), and even some rodents (Layne, 1972; Sumner and Collins, 1918). Most commonly examined in lizards, this voluntary shedding of an appendage generally occurs in response to a predatory stimulus. Although this is an effective mechanism for increasing the likelihood of surviving an encounter with a predator (Dial and Fitzpatrick, 1984; Vitt et al., 1977), tail autotomy in lizards can have significant effects on the animal subsequent to the predator-prey interaction. The tails of many lizard species can play a significant role in energy storage (Avery, 1970; Bustard, 1967; Congdon et al., 1974), sexual display and reproduction (Smyth, 1974; Vitt et al., 1977). Additionally, the tail can be critical for locomotion and balance (Daniels, 1983; Etheridge, 1967), and thus its loss may markedly affect locomotor behavior and performance (Bateman and Fleming, 2009; Daniels, 1983; McElroy and Bergmann, 2013; Vitt et al., 1977).

It is clear from recent work that the lizard tail is important in a number of locomotor behaviors. Jusufi et al. (2008) showed that the tail can function as an emergency fifth leg and be employed to recover from slips while ascending a vertical surface, and that autotomy increases the likelihood of falling. Gillis et al. (2009) demonstrated a decrease in in-air stability during jumping after autotomy. Effects of tail loss on running speed have been found to be highly variable among lizard species (McElroy and Bergmann, 2013) and are thus hypothesized to be dependent upon the role of the tail in locomotion (Vitt et al., 1977). For example, a decrease in sprint speed following autotomy has been reported for six-lined racerunners (*Cnemidophorus sexlineatus*) (Ballinger et al., 1979), desert fringe-toed lizards (*Uma notata*) (Punzo, 1982), and greater earless lizards (*Cophosaurus texanus*) (Punzo, 1982), suggesting that locomotor performance is enhanced by the presence of the tail. Some lizards, however, exhibit no difference in sprint speed following autotomy (velvet geckos [*Oedura lesueurii*] (Kelehear and Webb, 2006)), whereas others can run more than twice as fast without the tail (marbled geckos [*Christinus marmoratus*] (Daniels, 1983)), suggesting that the tail does not assist in locomotion in these animals, but may actually impede faster running due to its relatively great weight or because it generates friction while being dragged along the ground, or both.

Although such studies have revealed notable relationships between morphology (tailed vs. tailless) and performance (sprint speed, jumping, and stability), the biomechanical changes in locomotion associated with tail autotomy are less well understood (Higham et al., 2013; McElroy and Bergmann, 2013). Knowledge of how force production and limb joint mechanics during locomotion change with the loss of mass and shift in center of mass (CoM) associated with tail autotomy is critical for being able to link the radical change in morphology with measured performance differences. Furthermore, studies considering the time course of locomotor changes associated with regenerating the tail following autotomy are rare (Chapple and Swain, 2002; Downes and Shine, 2001).

I investigated how locomotor mechanics are affected by the rapid loss and gradual recovery of mass associated with tail autotomy and regeneration in the leopard gecko, *Eublepharis macularius*. The tail of this desert-dwelling species is one of the largest (relative to body size) among geckos, accounting for approximately one-third the length of the body and one-fourth of the animal's mass (Higham and Russell, 2010; Higham and Russell, 2012). Since losing a relatively large tail is likely to elicit a greater change in locomotion (McElroy and Bergmann, 2013), this species is well-suited for the examination of the effects of autotomy on locomotor mechanics. Additionally, leopard geckos are an established study system for autotomy, and the relative ease of inducing autotomy in this species has been noted (Delorme et al., 2012; Higham and Russell, 2010; Higham and Russell, 2012; Lynn et al., 2013; McLean and Vickaryous, 2011).

To examine the effects of autotomy and regeneration on locomotor mechanics, I addressed three questions. (1) How do tail loss and regeneration affect the lizard's CoM? I hypothesize that the removal of substantial caudal mass as a result of autotomy will shift the animal's CoM anteriorly, and regeneration of the tail will return the CoM to its original position. (2) How do removal of the tail and the associated shift in CoM affect locomotor mechanics while running on a level surface? I hypothesize that a large tail weighs down the posterior end of the trunk, and that removal of that mass will result in the rising of the posterior end. Therefore, I anticipate an increase in hip height following autotomy, resulting in passive changes in forelimb and hind limb joint kinematics. Additionally, propulsive hind limb ground-reaction forces (GRFs) are expected to decrease proportionally with the loss of mass. (3) As the tail is regenerated, do joint kinematics and propulsive hind limb GRFs change continually and ultimately return to the original state? After a shift in kinematics and GRFs immediately following autotomy, I hypothesize that these metrics may either return to the "pre-autotomy" state over time, or the lizard may adopt some manifestation of the "post-autotomy" mode of locomotion as a permanent solution.

Materials and Methods

Study organisms

Seven adult female *E. macularius* (mass=39.4±1.2 g; SVL=121.9±2.2 mm) with original tails were obtained from commercial suppliers and housed individually in terraria (50.8×25.9×32.0 cm) maintained at 28-33°C. Geckos were fed a diet of live crickets *ad libitum* until satiation throughout the duration of the experiment. Prior to locomotor trials, small dots of white nail polish were applied to the following points on the animals for visualization in high-speed videos: dorsal midpoint of the body along the midline of the back, center of the pectoral/pelvic girdles along the midline, shoulder/hip, elbow/knee, wrist/ankle, and the metapodial-phalangeal joint of the fourth toe. Joints were all marked on the left forelimbs and hind limbs. Following pre-autotomy measurements/trials, the base of the tail was gently pinched to initiate autotomy at the proximal-most fracture plane. Animals were sacrificed at the conclusion of the experiments using 0.05 mL of sodium pentobarbital (390 mg ml^{-1}). All sacrificed animals and autotomized tails were utilized for morphological analyses.

Measuring tail morphology and CoM

Body mass (including tail mass), SVL, tail length (distance from the vent to the tip of the tail), and tail diameter (obtained from the widest point along the tail) were measured before and after the tail was autotomized, and bi-weekly for 22 weeks as the tail regenerated. Measurements of body mass, tail length, and tail diameter ceased showing significant changes after 18 weeks, suggesting that tail regeneration was complete by week 22. Fully regenerated tails were removed with a scalpel at the point of autotomy after sacrifice in order to compare the mass and volume of original and regenerated tails. Volumes of the tails were measured by liquid displacement in a graduated cylinder (Vitt et al., 1977).

After sacrificing and freezing the geckos and tails until rigid, CoM measurements were obtained via a thread suspension method (Alexander, 1983). The location of the CoM was determined for each individual with its fully regenerated tail (still attached), no tail (after removal of the regenerated tail), and original tail (reattached using 2-3 drops of super glue).

Experimental set-up

Stride kinematics and GRF data were obtained from each gecko as it ran on a level flat trackway (1.0 × 0.15 m) covered with cork to prevent slipping. The temperature of the experimental room was maintained at approximately 30°C when filming. Following the pre-autotomy trials, autotomy was induced and each animal was allotted 20-30 minutes to rest in a cloth bag with restricted movement. This ensured that the post-autotomy trial was representative of the animal's first strides without a tail, while also minimizing potential effects of fatigue or stress associated with the removal of the tail (Langkilde and Shine, 2006). Strides were then recorded at two-week intervals until week 14, and four-week intervals thereafter, until regeneration ceased at 22 weeks, yielding a total of 11 trials.

Stride kinematics

Lizards were filmed at 250 frames s^{-1} with a shutter speed of 1/2000 s using two Photron APX-RS cameras (Photron USA, San Diego, CA, USA). The cameras were oriented to provide a direct lateral view and an oblique dorsal view (45°) and synchronized with an external trigger. A pre-measured calibration object constructed of LEGO™ blocks was used to produce 3D coordinates for digitizing. Three forelimb and three hind limb strides were recorded for each combination of individual and trial as the animal moved along the trackway at a constant speed (we did not attempt to elicit maximal performance). I digitized the points marked along the body and limbs using DLT DV 5 custom software (Hedrick, 2008) for MATLAB (version R2012a, The MathWorks, Natick, MA, USA) in order to obtain x, y and z coordinates to describe antero-posterior, medio-lateral, and dorso-ventral movements, respectively. These coordinates were then used to calculate body speed (distance traveled by the point at the center of pectoral girdle throughout the stride divided by the duration of the stride), stride length, duty factor, hip height, shoulder height, body pitch, and joint angles (humerus/femur depression, humerus/femur retraction, and elbow, knee, and ankle angles) throughout each stride. Extensive details of how these calculations are made are available elsewhere (Foster and Higham, 2012).

Hind limb locomotor forces

Hind limb GRFs were quantified using a custom-made force platform, which consisted of a Nano17 6-axis force/torque sensor (ATI Industrial Automation, Apex, NC, USA) and a carbon-fiber top plate (DragonPlate, Elbridge, NY, USA). This was covered in cork to prevent a change in substrate for the moving animal, and embedded in the center of the trackway with its surface (0.10 x 0.05 m) flush with the latter. This allowed ample room for the gecko to move over the force plate with only one hind limb making contact. Only runs in which the gecko stepped completely onto the force platform with one hind limb (without contact from any other limbs) were used in this study. Three runs were obtained per individual for each trial. Fore-aft, medial-lateral, and vertical GRFs were sampled at 5000 Hz using AcqKnowledge 4.0.0 software (BIOPAC Systems, Inc., University of Cambridge, England). Peak vertical and peak propulsive GRFs were calculated from the accelerative phase of each hind limb footfall. Relative (weight-specific) GRFs were obtained by correcting for the weight of each individual.

Statistical analyses

To compare original and fully-regenerated tail morphology, paired *t*-tests were used to analyze differences in mass and volume, and a repeated measures ANOVA was used to compare changes in tail length and diameter throughout autotomy and regeneration. Measurements at each trial (pre-autotomy, post-autotomy, 2 weeks post-autotomy, etc.) were used as the repeated measures. The latter method was also used to compare shifts in CoM position between lizards with original, autotomized, and regenerated tails.

Averages of the kinematic and GRF variables of the three runs for each individual per trial were used for all statistical analyses. The effects of speed on kinematics and force variables were removed by regressing all of the variables individually against body speed. The residuals of all variables that had a significant relationship ($\alpha \leq 0.10$) with speed were used for subsequent statistical analyses while all other variables remained in their original form. A repeated measures ANOVA was again used to compare each variable throughout autotomy and regeneration, and *post hoc* tests with Bonferroni corrections were used for pairwise comparisons of the pre-autotomy trial with each successive trial. Assumptions for normality and equal variances were not violated for any of the variables measured. All statistical analyses were performed using SYSTAT 13.00.05.

Results

Body mass, tail length, and tail diameter ceased showing significant changes after 18 weeks (*post hoc* comparison of weeks 18 and 20: body mass, t=-0.842, d.f.=6, P=0.432; tail length, t=-2.303, d.f.=6, P=0.061; tail diameter, t=-1.396, d.f.=6, P=0.212), justifying the cessation of the study after 22 weeks. The snout-vent length (SVL) and body mass (without the tail) of each individual did not differ significantly at the start and end of the study (paired t-tests: SVL, t=0.473, d.f.=6, P=0.653; body mass, t=-2.238, d.f.=6, P=0.067), indicating that there was no growth of the animals aside from the regeneration of the tail. In addition to the absence of vertebrae, regenerated tails differed in size and shape from the originals (Figure 4). Paired t-tests indicated that regenerated tails were significantly smaller in both mass (original, 22% body mass; regenerated, 15% body mass) and volume (original, 7.8×10^3 mm^3; regenerated, 5.8×10^3 mm^3) than the originals (mass, t=6.457, d.f.=6, P=0.001; volume, t=3.742, d.f.=6, P=0.010). Regenerated tails grew to only 61% of the original length (repeated measures ANOVA, $F_{2.5}$=585.764, *P*<0.001), but attained 131% of the original maximum diameter (repeated measures ANOVA, $F_{2,5}$=394.159, *P*<0.001). These changes in tail morphology resulted in a significant anterior shift in location of the CoM from a mean of 65.9±1.6% SVL with the original tail, to 52.7±1.6% SVL with no tail, and finally a posterior shift to 60.5±1.3% SVL with a fully regenerated tail (repeated measures ANOVA, $F_{2,5}$=10.174, *P*=0.017) (Figure 5). A *post hoc* comparison indicated a significant difference in the CoM location with the original and the fully regenerated tail (*t*=3.306, d.f.=6, *P*=0.016), suggesting that the CoM was not restored to its original position.

In running trials, geckos ran at speeds ranging from 0.23-2.4 SVL s^{-1}, and autotomy and regeneration did not have a significant effect on speed (repeated measures ANOVA, $F_{2,5}$=2.877, *P*=0.147). Maximum hip height, maximum shoulder height, pitch of the body, stride length, stance time, and duty factors of the fore- and hind limbs were not significantly impacted by autotomy and regeneration (Table 1). However, although forelimb joint kinematics were unaffected, changes in hind limb joint angles were prominent (Figure 6). Immediately following autotomy, significant decreases in maximum angles of femur depression (*t*=6.534, d.f.=6, *P*=0.001), femur retraction (*t*=5.872, d.f.=6, *P*=0.001), and knee flexion (*t*=3.807, d.f.=6, *P*=0.009) were observed, which then notably increased throughout tail regeneration. *Post hoc* tests suggest that the extent of femoral depression and retraction recovered to the “pre-autotomy state” two weeks following autotomy (depression, *t*=2.078, d.f.=6, *P*=0.083; retraction, *t*=2.294, d.f.=6, *P*=0.062), whereas knee angle showed a more gradual increase as regeneration proceeded, returning to the original value at 10 weeks following autotomy (*t*=1.080, d.f.=6, *P*=0.322).

Peak vertical GRF significantly decreased following autotomy (*post hoc* test, *t*=6.739, d.f.=6, *P*=0.001) and gradually increased until returning to the pre-autotomy state after 12 weeks of regeneration (*post hoc* test, *t*=0.445, d.f.=6, *P*=0.672) (Figure 7A). After correcting for the changes in mass associated with autotomy and regeneration of the tail, weight-specific peak vertical GRF showed no significant change over the 11 trials (repeated measures ANOVA, $F_{2,5}$=4.419, *P*=0.078) (Figure 7B). However, *post hoc* tests still suggest a significant decrease in vertical GRF after autotomy, irrespective of the loss of mass (*t*=3.207, d.f.=6, *P*=0.018). Relative peak propulsive GRF significantly increased after autotomy (*post hoc* test, *t*=-4.473, d.f.=6, *P*=0.004) and returned to the pre-autotomy state at week eight (*post hoc* test, *t*=-1.782, d.f.=6, *P*=0.125) (Figure 7C).

Discussion

Tail autotomy is a remarkable strategy for escaping predatory attacks. Although survival is likely enhanced, I present several biomechanical ramifications of the associated alteration of both body mass and relative location of the CoM. For this I used *E. macularius*, which has the ability to autotomize and relatively rapidly regenerate a bulky tail (Lynn et al., 2013). Previous work relating to the effects of autotomy have focused on differences in gross morphology, anatomy, histology, and development between original and regenerated tails. Although it is frequently noted that the structure of the regenerated tail differs from that of the original (Delorme et al., 2012; Goss, 1969; McLean and Vickaryous, 2011), disparities in size and shape are only briefly mentioned (Lynn et al., 2013; Whimster, 1978), and have not previously been quantified. Additionally, such studies have generally been conducted on juvenile animals, and although ontogenetic differences in tail regeneration are unknown, growth and maturity have been suggested to influence regeneration rates in geckos (Congdon et al., 1974; Lynn et al., 2013; Vitt et al., 1977). Here, I show that in mature adult female leopard geckos, fully regenerated tails are smaller in both mass and volume than the original tails and take on a new shape, being shorter and wider than the original (Figure 4). Interestingly, the regenerated tails of juveniles of the same species are not smaller in mass (Lynn et al., 2013), suggesting that tail regeneration is not afforded such a high priority in adults, and that the costs of regenerating a larger tail may outweigh the benefits. I hypothesize that the tail does not return to its original shape because regenerating a replica of the original might not be critical for locomotion in *E. macularius.* In *Aspidoscelis sexlineatus*, the tail is suggested to function as a counter-balance mechanism while running (Ballinger et al., 1979). In contrast, leopard gecko tails are hypothesized to be devoted primarily to fat storage (Lynn et al., 2013), for which tail shape may be less critical. Mixed results have been found when comparing the sizes of original and regenerated tails in other lizard species (Daniels, 1983; Medger et al., 2008; Vitt et al., 1977), which may also be linked to the function of the tail in locomotion.

The changes observed in CoM associated with autotomy and regeneration were not surprising. As expected, complete removal of the autotomizable portion of the tail resulted in a significant shift of the CoM anteriorly. Additionally, because the regenerated tail is shorter and weighs less than the original, the CoM never returned to its original position. Instead, the CoM location recovered to only about half of its initial antero-posterior displacement once the tail was fully regenerated. These findings provide empirical support for mathematical models of CoM (Gillis et al., 2013), which suggest that CoM changes are proportionally related to the size of the tail. The fact that the CoM never returned to its original location suggests that long-term compensation may occur in the underlying neuromuscular control of locomotion. Possibly, these lizards permanently "adapt" to this altered morphology, much like other animals can adjust to increases in body mass (Irschick et al., 2003; Kuo et al., 2011; Lind et al., 2010). However, long-term recordings of neuromuscular function are needed to determine whether this is the case.

Because the CoM is located in a different position relative to the propulsive system (the hind limbs) following autotomy, I expected a reduction in stability during locomotion. One approach animals can take to quickly compensate for such reduced stability is to deploy kinematic adjustments of the limbs and/or body (Gillis et al., 2013). My prediction that the gecko's body pitch would be affected by the anterior shift in CoM was not upheld, suggesting that the animal is able to immediately adjust to the shift in mass. This is supported by the decreases in femoral depression, retraction, and knee angles (Figure 6) observed immediately following autotomy, all of which are indicative of the animal adopting a more sprawled posture (Fuller et al., 2011). Since the hind limbs are perceived to be the primary generators of locomotor thrust (Chen et al., 2006; Russell and Bels, 2001), it is likely that a locomotor response to autotomy and the loss of mass would occur here. Thus, the changes in hind limb kinematics are not surprising. I hypothesize that this sprawled posture may be a means of enhancing stability and balance by lowering the CoM (Foster and Higham, 2012; Rewcastle, 1981) as the animal adjusts to the change in mass distribution during its first steps after losing its tail. Interestingly, each of these hind limb kinematic parameters begins to return to the pre-autotomy state by the next trial (two weeks after autotomy), and are statistically indistinguishable from values obtained with the original tail by week 10. The differential return to pre-autotomy conditions observed among femoral depression, retraction, and knee angles is likely related to the non-uniform changes in tail form as regeneration proceeds. The initial stages of tail regeneration are represented mostly by increases in tail length, whereas tail diameter does not begin to significantly increase until week 6-8 (Figure 4D). Thus, it is likely that increase in tail mass throughout regeneration is not linear, resulting in a non-linear response in hind limb kinematics. Additionally, frequent observations of hind limb kinematics over the days, or even hours/minutes following autotomy would likely reveal the amount of time or number of strides necessary for the animal to begin “recovering”.

Additionally, the changes in CoM location observed are thought to have significant effects on hind limb GRF production. As expected, peak vertical GRF decreased after the tail was autotomized because less force is needed to lift the reduced mass of the animal against the force of gravity, and it gradually increased as the tail regenerated (Figure 7A). Although this relationship vanished when peak vertical GRF was corrected for weight, the hind limbs were still found to be producing significantly less weight-specific vertical force after autotomy (Figure 7B). It is therefore possible that the geckos may be overcompensating for the loss of caudal mass immediately after losing the tail by generating less vertical force in the hind limbs, which may be directly related to the more sprawled posture of the animal and the resulting change in the lever arms of the muscles responsible for body support. Additionally, a long heavy tail, like that of the leopard gecko, is proposed to position the CoM posteriorly so that the hind limbs can apply a greater propulsive force to the substrate during locomotion (Snyder, 1949). Thus, by shifting the CoM anteriorly, tail autotomy should decrease hind limb propulsive force, which would in turn reduce sprint speed (Ballinger et al., 1979; Punzo, 1982). However, I did not find any changes in speed following autotomy, and perhaps more interestingly, my findings show that weight-specific hind limb propulsive GRFs follow the opposite of the expected trend (Figure 7C). Because the hind limbs are actually producing more propulsive force without an increase in speed, I hypothesize that during the first 4-6 weeks following autotomy, the forelimbs may be playing a more significant role in braking to counteract the effect of increased force from the hind limbs. Data on the forelimb GRFs are needed to support this hypothesis, however.

Tail autotomy is a valuable way of assessing the impacts of shifting the location of the CoM, especially given that it is a naturally occurring event. In addition, in order to alter the location of the CoM in other vertebrates, such as mammals, mass would need to be added to the body. For example, Lee et al. (2004) manipulated the distribution of mass on the mechanics of level trotting in dogs. An anterior shift in CoM (as occurred in my study) was achieved by adding 10% of body mass at the pectoral girdle. This alteration did not impact kinematics, and the mean fore-aft force was unchanged from that of unloaded trotting (Lee et al., 2004). It is difficult to equate this increase in mass to the decrease in mass observed here, especially since tail autotomy in leopard geckos results in more than a 10% reduction in body mass. This may explain why I found differences in kinematics between the conditions. Adding mass likely results in a change in the energetic cost of locomotion and the location of the CoM, which would make it difficult to tease apart the differential impacts of these variables. Tail autotomy reduces overall body mass, and may not impact the energetic cost of locomotion, highlighting the utility of this system. Finally, tail autotomy permits the assessment of GRFs over a period of time, as was accomplished in my study, something that is rarely (if ever) done in studies that manipulate CoM location by adding mass.

Morphological variation has been proposed to drive kinematic variation, which then drives force variation in terrestrial running (McElroy and Reilly, 2009). My findings provide support for this, and form a baseline for future work in determining the effects of rapid mass change on locomotor mechanics. Only one other study has incorporated kinematic data into performance measures associated with autotomy, but just two kinematic variables (body curvature and stride length) were recorded (Medger et al., 2008). Neither of these variables was significantly affected by tail autotomy. Furthermore, no other steady locomotion force data related to the changes in mass associated with autotomy and regeneration are available. Such observations will provide insight into how lizards may actuate movement during post-autotomic predator-prey interactions. Whether these permanently modified mechanics and motion result in a decrease in escape ability is something to be addressed in future work. It is possible that locomotion following autotomy has been under selective pressure for those lizards exhibiting the ability to autotomize their tail. An interesting parallel study would be to remove the tails of non-autotomizing species to determine whether there is a lack of compensation due to the lack of a post-autotomic period for selection to act on.

Rapid mass change is a ubiquitous phenomenon across vertebrates and invertebrates, and can occur for a number of reasons. Although force data describing the effects of mass change related to gravidity are available (Scales and Butler, 2007), there are likely a number of other physiological variables associated with gravidity that can contribute to locomotion. Whether autotomy also results in physiological changes that could impact locomotion is unclear, but autotomy is likely a good model system for studying the effects of rapid mass loss. Autotomy and regeneration studies conducted on lizards allow for precise manipulations of mass change and mass distribution without detrimental effects to the study organisms.

CHAPTER III – Both the mass and motion of the tail are critical for gecko locomotion: an integrative study combining tail restriction and autotomy

Summary

Tails are an intricate component of the locomotor system for many vertebrates. In lizards capable of voluntarily shedding the tail via autotomy, these locomotor functions can be lost or impaired. Leopard geckos (*Eublepharis macularius*) are a model system for tail autotomy in which a large, fat-storing tail is laterally undulated during steady locomotion. However, autotomy results in the loss of tail function and a substantial loss in body mass, leading to a shift in the center of mass (CoM). It is unclear which of these losses are driving locomotor changes that occur with autotomy. To elucidate the function of tail undulations, I investigated changes in body and limb kinematics of lizards before and after disabling the tail, both artificially by restricting tail undulations and naturally by removal of the tail via autotomy. Restricting tail undulations resulted in kinematic adjustments similar to those that occur following tail autotomy, primarily characterized by more flexed hind limb joints and a more sprawled posture. These data suggest that effects of autotomy on locomotion in geckos may be linked to the loss of tail movements rather than the loss of mass. In addition, I provide empirical support for the link between lateral tail undulations and step length through the rotation of the pelvic girdle and retraction of the femur. Disabling undulations by restriction and autotomy of the tail limits pelvic rotation, which reduces femur retraction and decreases step length. These

findings demonstrate a functional role for tail undulations in geckos, which likely applies to other terrestrial vertebrates.

Introduction

A defining feature of chordates is the post-anal tail, which has evolved many key functions across taxa (Hickman, 1979). These include courtship (Dakin et al., 2016), signaling (Barbour and Clark, 2012; Cooper, 1998), the maintenance of fat stores (Lemelin and Schmitt, 2004; Young et al., 2007), and defense/combat (Arbour, 2009; Arnold, 1984). Tails also have functional roles in animal locomotion, most notably when used directly for propulsion, as in countless swimming animals (Fish, 1984; Lauder, 2000) and when used to power pentapedal locomotion in kangaroos (O'Connor et al., 2014). Perhaps less obvious is the tail's role in maintaining balance and enhancing maneuverability or stability (Ballinger, 1973; Carrier et al., 2001; Hsieh, 2016; Walter and Carrier, 2002). Although prehensile tails serve as an extra limb to reduce the risk of falling in arboreal environments (German, 1982; Lemelin, 1995), several taxa utilize non-prehensile tails for a similar advantage. Mice have been documented undulating the tail for balance when crossing a narrow perch (Buck et al., 1925). Primates with long tails utilize sweeping movements of the tail when navigating narrow supports to alter the momentum of their body (Larson and Stern, 2006). And cats utilize tail adjustments to realign their hips over a perch to avoid falling (Walker et al., 1998). Even on broad level terrain, tails can adjust the balance of the body to counteract pitching effects of leg movements (Alexander and Vernon, 1975), and tails have been shown to be useful for initiating turns and maneuvering (Wilson et al., 2013).

Lizards are ideal for studying tail function because all of the functions described above are represented within their tremendous diversity. The tail can be dragged behind the lizard, pushed against the substrate during climbing, raised, curled, used as a prehensile "fifth limb", used for counter-rotation during jumping, or undulated as they walk, run, and/or climb (Arnold, 1984; Higham and Anderson, 2013; Higham et al., 2001; Vitt et al., 1977). Despite the importance of the tail in various forms of locomotion (Ballinger et al., 1979; Hsieh, 2016; Jusufi et al., 2008; Punzo, 1982; Snyder, 1949; Snyder, 1962), most lizard species voluntarily shed the tail (autotomy) as a predator-escape strategy (Arnold, 1984; Bateman and Fleming, 2009). How tail autotomy impacts locomotion has thus become a topic of much interest in recent years (Gillis and Higham, 2016; Higham et al., 2013; Hsieh, 2016; McElroy and Bergmann, 2013). Performance effects are variable across species, likely due to differences in the role of the tail in locomotion (Vitt et al., 1977). Species for which locomotor performance is improved after autotomy generally have large fatty tails that impede faster running (Daniels, 1983), while locomotion is impaired by tail loss in species that depend on the tail for balance, stability, and/or maneuverability (Ballinger et al., 1979; Punzo, 1982).

In some species, autotomy does not influence performance, but significant changes in locomotor mechanics occur. Changes in locomotor kinematics and hind limb ground-reaction forces (GRFs) were recently investigated in the leopard gecko, *Eublepharis macularius* (Jagnandan et al., 2014), a padless desert-dwelling species and an established model system for tail autotomy and regeneration (Delorme et al., 2012; Higham and Russell, 2012; Lynn et al., 2013; McLean and Vickaryous, 2011; Russell et al., 2015). Geckos lower their center of mass (CoM) by taking a more sprawled posture after autotomy, a change that was attributed to a reduction in stability due to the significant loss of caudal mass (*E. macularius* has one of the largest tails relative to body size) (Jagnandan et al., 2014). However, it is unclear if stability is impaired by the change in mass or the loss of tail function. The tail of *E. macularius* serves a primary role in the storage of fats (Lynn et al., 2013), but unlike many other large-tailed reptiles, the tail is not dragged behind the animal as it walks. Instead, the tail is lifted off the ground and swings laterally. Undulations of the vertebral column generate a standing wave in the trunk that transforms into a traveling wave moving caudally along the tail as the lizard walks (Hamley, 1990).

The function of lateral undulations of the tail during locomotion remains unclear, although several hypotheses have been presented. Tail movements in arboreal mammals are suggested to aid in balance and stability when traversing narrow perches (Buck et al., 1925; Larson and Stern, 2006; Walker et al., 1998). Recent data on green anoles demonstrate that mediolateral tail movements are most prominent on the narrowest perches and compensate for instabilities imposed by a small perch diameter (Hsieh, 2016). Undulating the tail during otherwise steady locomotion may also be a useful mechanism for rapidly responding to unexpected perturbations by imparting angular momentum on the body and resisting the destabilizing motion (Larson and Stern, 2006; Walker et al., 1998). The tail is also suggested to play a role in force generation by the caudofemoralis, the muscle that retracts the femur (Irschick and Jayne, 1999; Nelson and Jayne, 2001; Reilly, 1994; Russell and Bauer, 1992; Snyder, 1952; Snyder, 1954; Snyder, 1962). Undulating the tail could alternately lengthen the caudofemoralis muscles attached to each hind limb as the tail is swung from side to side. Lengthening the muscle to a more optimal length would lead to greater actin-myosin overlap within the muscle sarcomere, which would thus enhance the force generated for propulsion by the caudofemoralis. Tail undulations could also contribute to rotation at the pelvic girdle due to inertial effects. A large undulating tail could provide the momentum necessary for rotating the pelvic girdle, which could influence both the length of a hind limb step as well as the angle at which the femur can retract to drive propulsion.

For both axial and appendicular structures that move during locomotion, function can be revealed by either removing all or some of the structure (Higham et al., 2005; Jagnandan et al., 2014), by adding to the structure (Carrier et al., 2001; Wickler et al., 2004), or by restricting motion of the structure (Ashby and Delp, 2006; Carr and Gentile, 1994). Although the voluntary loss of the tail has been studied, little is known about the differential role of mass *versus* motion of the tail during locomotion. I examined how the tail is used in leopard geckos walking on level terrain and determine how these tail movements change with speed. I then disabled normal tail movements, both artificially by restricting tail undulations with a graphite rod and naturally by autotomizing the tail in the same individuals. We hypothesized that restricting the motions of the tail will cause changes in locomotion that are comparable to those that occur following tail autotomy (Jagnandan et al., 2014). Thus, we predicted that autotomy-induced changes in locomotion result from the loss of tail undulations, not a loss of mass. I specifically investigated changes in limb joint angles to augment balance or stability, as well as changes in the rotation of the pelvic girdle when an undulating tail is compromised.

Materials and Methods

Study organisms

Ten adult *E. macularius* (mass, 36.3±1.9 g; SVL, 104.6±2.1 mm) with original tails intact were obtained from commercial suppliers and housed in terraria (50.8×25.9×2.0 cm) maintained at 28-33°C. Geckos were fed a diet of live crickets *ad libitum*, but fasted the day before the experiment until trials were complete. Prior to experimental trials, white nail polish was applied to the following points on the animals to visualize body and joint movements in high-speed videos: dorsal midpoint of the body, center of the pectoral/pelvic girdles, shoulder/hip, elbow/knee, wrist/ankle, and the metapodial-phalangeal joint of the middle toe. Joints were marked on the right forelimbs and hind limbs. Five points were also evenly distributed from the base of the tail to the tail tip to track the tail movements.

Experimental set-up

Stride kinematics were obtained from each lizard as it ran on a level trackway (1.0×0.13 m) with sandpaper substrate to prevent slipping. A mirror mounted at 45° above the trackway provided a dorsal view for the trials. The temperature of the experimental room was maintained at ~30°C. Lizards were recorded moving along the trackway under three tail treatments: original, restricted, and autotomized. After recording trials with a lizard's original tail intact, a lightweight (<1.0 g) hollow graphite rod was attached along the entire length of the tail using non-toxic glue. The rod restricted undulations of the tail, while still permitting the lizard to lift its tail off the ground to prevent friction drag while walking. Locomotor trials were then repeated with the restricted tail. Following these trials, the rod was gently removed from the tail, and the base of the tail was gently pinched to initiate autotomy at the proximal-most fracture plane. Trials were then repeated for lizards with autotomized tails. Between trials for each treatment, each individual was allotted 20-30 minutes to rest in order to minimize potential effects of fatigue or stress associated with the restriction and removal of the tail (Langkilde and Shine, 2006). However, I limited the amount of walking between trials to avoid any short-term adjustments.

Stride kinematics

Locomotor movements were captured at 250 frames s^{-1} with a shutter speed of 1/2000 s using two Photron APX-RS cameras (Photron USA, San Diego, CA, USA), which provided a lateral and dorsal view synchronized with an external trigger. A pre-measured calibration object constructed of LEGO™ blocks was used to generate 3D coordinates for digitizing. Three to five forelimb and hind limb strides were recorded for each combination of individual and tail treatment. I digitized the points marked on the animals using DLT DV5 custom software (Hedrick, 2008) for MATLAB (version R2012a, The MathWorks, Natick, MA, USA) to obtain x, y, and z coordinates to describe antero-posterior, medio-lateral, and dorso-ventral movements, respectively. These coordinates were then used to calculate speed, stride length, stance time, duty factor, and joint angles for the right fore- and hind limb throughout each stride. Details of these calculations are available elsewhere (Foster and Higham, 2012; Jagnandan et al., 2014). Tail coordinates were used to calculate the height of the tail off the ground and lateral displacement of the tail (measured as the lateral displacement of the tail tip relative to the pelvic girdle) throughout each stride.

Statistical analyses

Averages of each kinematic variable for each individual per tail treatment were used for all statistical analyses. For the tail variables (tail height and lateral displacement), a regression analysis was used to examine the relationship between tail movements and walking speed. The effects of speed on fore- and hind limb joint kinematics were removed by regressing the variables against body speed. Residuals of the variables that had a significant relationship ($\alpha \leq 0.10$) with speed were used for subsequent statistical analyses, while all other data were analyzed in their original form. A repeated-measures ANOVA was used to compare each variable between original, restricted, and autotomized tail treatments, and *post hoc* tests with Bonferroni corrections were used for pair-wise comparisons among the treatments. Assumptions for normality and equal variances were not violated for any of the variables measured. All statistical analyses were performed using SYSTAT 13.00.05.

Results

In running trials, geckos ran at speeds ranging from 0.59 to 3.36 SVL s^{-1}, which was not significantly affected by restricting or autotomizing the tail (repeated measures ANOVA, $F_{2,8}=4.075$, $P=0.060$). Lateral displacement of the tip of the tail relative to the pelvic girdle exhibited a significant negative relationship with speed ($F_{1,8}=5.870$, $P=0.042$, $R^2=0.423$) (Figure 8), although no relationship was observed between the height of the tail and speed ($F_{1,8}=0.100$, $P=0.759$, $R^2=0.012$). Restricting the tail reduced the lateral displacement of the tail as intended ($t=3.112$, d.f.=9, $P=0.012$) and did not affect the tail height off the ground ($t=0.734$. d.f.=9, $P=0.482$).

Stride lengths, stance times, and duty factors of the fore- and hind limbs were not significantly impacted by restricting or autotomizing the tail (Table 2). Forelimb joint kinematics were also unaffected. However, step length (the distance traveled during the stance phase of the hind limb) was significantly reduced by restricting ($t=3.509$, d.f.=9, $P=0.007$) and autotomizing ($t=3.447$, d.f.=9, $P=0.007$) the tail. Both restricting and autotomizing the tail also significantly decreased the maximum angles of femur depression (restriction, $t=6.225$, d.f.=9, $P<0.000$; autotomy, $t=7.869$, d.f.=9, $P<0.000$), femur retraction (restriction, *t*=2.94, d.f.=9, *P*=0.016; autotomy, *t*=3.305, d.f.=9, *P*=0.009), knee flexion (restriction, *t*=4.541, d.f.=9, *P*=0.001; autotomy, *t*=4.627, d.f.=9, *P*=0.001), and ankle flexion (restriction, *t*=3.997, d.f.=9, *P*=0.003; autotomy, *t*=4.157, d.f.=9, *P*=0.002) in the hind limbs (Figure 9). The angular excursion at each of these hind limb joints was also significantly reduced after tail restriction (femur depression, *t*=3.069, d.f.=9, *P*=0.013; femur retraction, *t*=3.527, d.f.=9, *P*=0.006; knee, *t*=2.939, d.f.=9, *P*=0.017; ankle, *t*=3.577, d.f.=9, *P*=0.006) and after tail autotomy (femur depression, *t*=5.090, d.f.=9, *P*=0.001; femur retraction, *t*=3.115, d.f.=9, *P*=0.012; knee, *t*=5.661, d.f.=9, *P*<0.000; ankle, *t*=5.825, d.f.=9, *P*<0.000). No significant differences were observed between the restricted and autotomized tail treatment groups (step length, *t*=0.859, d.f.=9, *P*=0.412; maximum femur depression angle, *t*=1.089, d.f.=9, *P*=0.305; maximum femur retraction angle, *t*=0.051, d.f.=9, *P*=0.960; maximum knee angle, *t*=1.229, d.f.=9, *P*=0.250; maximum ankle angle, *t*=1.176, d.f.=9, *P*=0.270; angular excursion of femur depression, *t*=2.510, d.f.=9, *P*=0.063; angular excursion of femur retraction, *t*=-1.157, d.f.=9, *P*=0.277; angular excursion of the knee, *t*=2.123, d.f.=9, *P*=0.063; angular excursion of the ankle, *t*=1.917, d.f.=9, *P*=0.087).

Pelvic girdle rotation decreased significantly when the tail was compromised, as indicated by a lower angular excursion in lizards with restricted (*t*=2.287, d.f.=9, *P*=0.048) and autotomized (*t*=3.129, d.f.=9, *P*=0.012) tails when compared to lizards with original tails intact (Figure 10). No significant differences in pelvic girdle rotation were observed between the restricted and autotomized treatments (*t*=-0.247, d.f.=9, *P*=0.810).

Discussion

Tail autotomy in lizards can result in both a significant loss of mass and a loss function that results from tail motion. Analysis of locomotor kinematics of *E. macularius* under experimental conditions in which the tail was compromised revealed the function of tail motions when walking and their relationship to pelvic rotation and step length. Specifically, I observed a more sprawled posture when lateral undulations of the tail were restricted and when the tail was completely autotomized, suggesting that geckos must compensate for not only the loss of caudal mass, but also for the loss of tail motion after an autotomy event. Additionally, restricting tail undulations reduced the rotation of the pelvic girdle, retraction of the femur, and step length, thereby providing evidence for a significant role of the tail in gecko locomotion. These results, elaborated below, reveal key functions of tails during locomotion that are likely applicable to any terrestrial vertebrate that relies on tail motion to move effectively.

Despite having a large fatty tail that accounts for one-fourth of the animal's body mass, the tail of *E. macularius* is slightly raised and laterally undulated instead of being dragged on the ground while walking. As the base of the tail moves laterally, the femora are alternately retracted to generate propulsion. The base of the tail is flexed towards the protracted hind limb during each cycle of hind limb movement, and the remainder of the tail follows this basal movement in an undulatory manner. Interestingly, I found that lateral displacement of the tip of the tail exhibits a negative relationship with the speed at which the gecko walks (Figure 8), suggesting that the tail swings less at higher speeds.

This more rigid posture of the tail streamlines the body of the lizard, and is suggested to be appropriate when lizards are moving forward quickly (Carrier et al., 2001). In these experiments, lizards were restricted to a narrow trackway to promote running in a straight line. However, if the lizard had to quickly navigate obstacles that required higher maneuverability, arching or undulating the tail would likely be beneficial in reducing the rotational inertia of the body.

After losing its tail, *E. macularius* adopts a more sprawled posture during locomotion, as previously indicated by decreases in femur depression, femur retraction, knee angle and ankle angle (Jagnandan et al., 2014). This locomotor response to autotomy is hypothesized to augment stability and balance that may be impaired due to the altered mass distribution and/or the loss of tail as a stabilizing appendage. Restricting the tail allowed us to tease apart the locomotor effects of autotomy due to losing mass versus losing tail function. By effectively modifying the tail into a stiff rod, the gecko was permitted to lift the tail off the ground to prevent friction, but prevented from swinging and laterally undulating the tail as it walked. This modification produced the same locomotor response as autotomizing the tail. Both removing and restricting the tail impact the location of the CoM, with removal shifting the CoM forward (Gillis et al., 2013; Jagnandan et al., 2014) and restriction limiting the CoM to the midline (Hsieh, 2016; Snyder, 1962). Forelimb kinematics were unaffected by restriction and autotomy, but maximum joint angles and angular excursions in the hind limbs decreased (Figure 9). These results suggest that tail undulations have a functional role in locomotion on level terrain, a role that is lost after autotomy and requires compensation by altering hind limb kinematics. In fact, it is likely that the impacts of autotomy on locomotion are a result of losing potentially beneficial tail movements, and not necessarily related to the loss of mass.

The function of tail undulations during steady locomotion is more clearly elucidated by the observed changes in pelvic rotation and its downstream effects on femur retraction and step length. Both restricting and autotomizing the tail reduced the degree of rotation of the pelvic girdle throughout the stride (Figure 10). I hypothesize that swinging the heavy tail laterally provides momentum for rotation at the pelvic girdle. Lizards generally exhibit greater pelvic rotation in order to facilitate a more sprawled posture compared to most other terrestrial quadrupeds (Reilly and Delancey, 1997a; Reilly and Delancey, 1997b; Reilly et al., 2005). Thus, a reduction in pelvic rotation should be expected to generate a more upright posture. This in stark contrast to what is observed after tail autotomy, in which lizards become more sprawled to maintain stability. Decreased pelvic rotation after autotomy thus results in a reduced step length during steady locomotion to maintain the sprawled posture. Additionally, pelvic rotation influences the angle at which the femur can protract and retract (Snyder, 1952; Snyder, 1954; Snyder, 1962). The reduction in the angle of femur retraction observed in lizards with restricted and autotomized tails (Table 2) coincides with the reduction in pelvic rotation. These data provide empirical support for the proposed link between lateral tail undulations and step length by rotation of the pelvic girdle and retraction of the femur (Peterson, 1984).

Autotomy is therefore likely to impact lizards that have a functional tail that provides momentum for rotating the pelvic girdle.

Our findings demonstrate that the tail serves a functional role in locomotion by undulating and rotating the pelvic girdle, thus contributing to femur retraction and step length. To further reveal the locomotor function of tail undulations in terrestrial lizards, I propose a series of future experiments that will elucidate how the tail is used and how animals compensate for the lost appendage. First, the effects of tail loss on dynamic stability and maneuverability should be tested by examining if/how lizards utilize the tail to navigate obstacles, drops, and turns. Experiments that record the timing and intensity of muscle activation in the tail will reveal whether these movements are passively or actively controlled. Passive control may suggest that undulating the tail is simply a means of dissipating energy from the laterally undulating body during locomotion, while active control would suggest a role in balance or stability. Electromyography (EMG) experiments would also be insightful when testing how the tail undulations affect the activation of the caudofemoralis and its role in retracting the femur (Irschick and Jayne, 1999; Reilly, 1994). Finally, I hope to explore the evolution of tail function by using these methods to explore the diversity of tail morphologies and their related locomotor functions across lizard taxa.

Tail autotomy in lizards provides an effective and natural system for understanding tail function. Hypothesized functions of tails commonly arise from studies on tail autotomy and locomotion. A negative impact of tail loss on performance suggests that the tail serves a role in balance, stability, maneuverability, or propulsion (Ballinger et al., 1979; Gillis et al., 2009; Punzo, 1982). Other attempts at assessing tail use in locomotion involve invasive surgeries with irreparable effects on the study animals (Buck et al., 1925; Snyder, 1949; Walker et al., 1998). However, tail autotomy allows for a removal of the tail in a natural manner with minimal physiological effects (Langkilde and Shine, 2006) in order to study its function.

CHAPTER IV – Modulation of the neuromuscular control of locomotion in autotomized geckos

Summary

Animal locomotion is driven by the underlying musculature of the axial and appendicular skeleton. Naturally, these muscles must be able to rapidly respond to environmental and physiological demands for locomotion to be effective. However, little is known about how muscles respond to morphological shifts, such as those that occur with tail autotomy in lizards. In leopard geckos (*Eublepharis macularius*), tail loss results in a 25% decrease in caudal mass and significant kinematic alterations to retain stability. To elucidate how motor control of the locomotor muscles are modulated with these shifts, I used electromyography (EMG) to quantify patterns of *in vivo* muscle activity in fore- and hind limb muscles before and after autotomy. This assessment revealed differential impacts of autotomy on the locomotor muscles. Forelimb muscles (biceps and triceps) exhibited no changes in motor recruitment, consistent with unaltered kinematics after autotomy. Amplitude of activity of propulsive muscles of the hind limbs (caudofemoralis and gastrocnemius) was significantly reduced and coincided with decreases in the propulsive phases of femur retraction and ankle extension, respectively. The puboischiotibialis did not exhibit these changes, despite significant reductions in femur depression and knee angle, suggesting that reduction in mass and vertical ground-reaction force by autotomy allows for the maintenance of a more sprawled and stable posture without increasing motor recruitment of the support muscles. These results highlight the utility of tail

autotomy as a system for studying the neuromuscular control of locomotion and emphasize the importance of examining muscle function in relation to locomotor demands.

Introduction

Locomotion is fundamental to most aspects of animal survival, including behaviors associated with prey capture and predator evasion. The ability to successfully accomplish these tasks often requires mobile animals to effectively alter locomotor strategies as they encounter environmental and physiological demands (Irschick and Garland, 2001). Although many of these changes can be visibly observed through analyses of external behavior and locomotor kinematics (Russell and Bels, 2001), it is the underlying muscles of the body and limbs that actuate these movements by driving propulsion, initiating braking, and maintaining balance and stability (Roberts et al., 1997; Schilling et al., 2009).

Studies of the modulation of neuromuscular control in response to locomotor challenges illuminate how animals navigate heterogeneous environments. Changes in surface grade, for example, alter the impact of gravity on the propulsive mechanisms (Daley and Biewener, 2003; Irschick and Jayne, 1998), thus requiring an adjustment in the magnitude of muscle activity of hip extensors and femur retractors to function effectively (Carlson-Kuhta et al., 1998; Gillis and Biewener, 2002; Higham and Jayne, 2004; Pierotti et al., 1989; Schilling et al., 2009). Shifts in the timing of muscle activity can also occur with changes in structural demands (Foster and Higham, 2014) or sudden environmental perturbations (Daley and Biewener, 2011; Daley et al., 2009; Marigold and Patla, 2002).

Although the response of locomotor muscle activity and recruitment are well-described in regards to environmental demands, little is known about how muscles respond to morphological changes. How muscle function can rapidly adapt to alterations in body form, such as changes in mass, is a relatively unexplored, but insightful area of study that can further elucidate the functional versatility of musculoskeletal systems (Gillis and Higham, 2016; Gillis and Biewener, 2003). Muscles play a pivotal role in the movement and support of body mass (Marsh, 1999), yet few data exists on how limb muscles adapt to changes in loading. The neuromuscular changes that occur with mass have been briefly addressed in animals carrying artificial loads, in which there is evidence of increased demand and recruitment of muscles for support and stability with the added mass (Ellerby and Marsh, 2006; McGowan et al., 2006). Although useful for understanding the adaptability of muscle, artificial loading experiments represent unnatural conditions that may limit ecological relevance.

Naturally, animals can gain or lose mass rapidly, but no data exists on how the locomotor muscles respond to these morphological shifts. Tail autotomy in lizards presents a fascinating system in which mass is lost rapidly from the caudal end as the tail is voluntarily shed from the rest of the body (Arnold, 1984). Unsurprisingly, this extreme behavioral response to perceived threats can have a substantial impact on the animal subsequent to the encounter (Bateman and Fleming, 2009; Higham et al., 2013). The tail can play important roles in jumping (Gillis et al., 2009; Gillis et al., 2013; Kuo et al., 2012), vertical climbing (Jusufi et al., 2008), and locomotor stability (Hsieh, 2016), each of which is negatively impacted following an autotomy event. Effects of autotomy on locomotor performance (e.g. sprint speed) are variable and dependent on role of the tail in steady locomotion (McElroy and Bergmann, 2013; Vitt et al., 1977)

Autotomy in the leopard gecko (*Eublepharis macularius*) is particularly interesting with respect to mass change because the tail of this species represents approximately 25% of the animal's mass, yet is readily autotomized when stimulated (Higham and Russell, 2010; Higham and Russell, 2012; Jagnandan et al., 2014). Recent data on the changes in locomotor mechanics associated with tail autotomy in this species revealed that a significant anterior shift in the center of mass (CoM) occurs following removal of the tail (Jagnandan et al., 2014). In response, geckos alter joint kinematics and assume a more sprawled posture during locomotion to lower their CoM and counteract destabilizing effects. The response of the locomotor muscles to the change in the distribution of mass and their roles in controlling the kinematic changes have not been previously examined. Using electromyography (EMG) to quantify activation patterns of the locomotor muscles before and after autotomy would reveal how neuromuscular control is altered by the loss of the tail.

Given the rapid loss of considerable mass, shift in the CoM, and postural changes observed after autotomy in *E. macularius*, I examined the modulation of locomotor muscle activity using EMG in this species to determine if and how the limb muscles are recruited differently after tail loss. I hypothesized that geckos must quickly alter motor output and activation of the limb muscles to compensate for the altered mass and CoM displacement. Specifically, I predicted that shifting the CoM anteriorly transfers the load distribution closer to the forelimbs, thus requiring greater recruitment and activation of forelimb muscles to support the increased load. Additionally, I expected a reduction in activity in the propulsive muscles of the hind limb due to a lower requirement of the muscles to move the animal forward (i.e. less mass to move). Finally, I predicted that geckos will exhibit greater motor output to hind limb muscles associated with lowering the CoM closer to the substrate and maintaining a more sprawled position throughout a stride.

Materials and Methods

Study organisms

Ten adult *E. macularius* (mass, 30.7±1.7 g; SVL, 110.0±1.5 mm) with original tails were obtained from commercial suppliers and housed individually in terraria (50.8×25.9×32.0 cm) maintained at 28-33°C. Geckos were fed a diet of live crickets *ad libitum*, but fasted for 24 hours prior to the surgery and through the end of the experimental trials.

Surgery and experimental set-up

Using previous kinematic data of *E. macularius* (Fuller et al., 2011; Jagnandan et al., 2014) and literature on the anatomy and function of the appendicular musculature of this species (Zaaf et al., 1999), five muscles were selected for EMG implantation (Figure 11). The biceps and triceps were implanted to record *in vivo* muscle activity associated with flexion and extension of the elbow, respectively, to assess the effects of the altered loading on the anterior limbs. In the hind limb, muscles hypothesized to drive the kinematic changes previously observed with autotomy were selected. These include the caudofemoralis (CF) (linked to retraction of the femur), the puboischiotibialis (PIT) (linked to depression of the femur and flexion of the knee), and the gastrocnemius (GAST) (linked to extension of the ankle).

Implantation of the EMG electrodes into each of these muscles followed an established surgical procedure (Higham and Jayne, 2004). Lizards were anaesthetized with an intramuscular injection of ketamine (100 mg kg^{-1}) prior to surgery. Fine-wire bipolar EMG electrodes were constructed following Jayne (1988) using 0.051 mm diameter polycoated stainless-steel wire (California Fine Wire Co., Grover Beach, CA, USA). Wires were inserted through the tip of a 26-gauge hypodermic needle used to implant the electrode through the skin and into the mid-belly of each muscle. EMG wires were then individually sutured to the skin, just proximal to the site of implantation and on the dorsal surface of the lizard's back, using 5-0 coated vicryl suture (Ethicon, Inc., Somerville, NJ, USA). This prevented the wires from pulling at the muscles and reduced the likelihood of accidental removal of the electrodes. Plastic model cement glue was then used to glue all of the electrodes into a single cable, which provided sufficient length for unrestricted movement of the lizard. Following the surgery, small dots of white nail polish were used to mark the center of the pectoral/pelvic girdles, shoulder/hip, elbow/knee, wrist/ankle, and the metapodial-phalangeal joint of the middle toe of the right fore- and hind limbs for concurrent kinematic analyses. Lizards were then placed individually into clean terraria and allowed to recover from anesthesia for at least 12 hours.

Following full recovery from surgery and anesthesia, locomotor trials were observed for each lizard as it ran on a level trackway (1.0×0.13 m) with sandpaper substrate to prevent slipping. A mirror mounted at 45° above the trackway provided a dorsal view for trials. Room temperature was maintained at approximately 30°C. Lizards were recorded before and after autotomy, by which the base of the tail was gently pinched to initiate autotomy at the proximal-most fracture plane. Lizards were allotted 20-30 minutes of rest with limited movement to minimize potential locomotor effects of fatigue or stress associated with tail autotomy (Langkilde and Shine, 2006). Data were obtained from a minimum of five forelimb and hind limb strides for each lizard per tail treatment, in which lizards moved steadily through the field of view. Stride kinematics for each individual were also obtained prior to surgery to ensure that behavior was not significantly altered by the surgery or presence of EMG electrodes.

Animals were sacrificed at the conclusion of the experiments using an overdose intraperitoneal injection of sodium pentobarbital (300 mg kg^{-1}). Dissections were performed on euthanized lizards to verify electrode placement in the forelimb and hind limb muscles.

Electromyography analysis

EMG signals were amplified 10,000 times using GRASS QP511 quad amplifiers (Natus Neurology Inc., Warwick, RI, USA) with a 60 Hz notch filter and low- and high-bandpass filters of 0.1 Hz and 3000 Hz, respectively. Signals were recorded at 5000 sample s^{-1} using a BIOPAC MP150 data acquisition system with the UIM100C module and AcqKnowledge 4.0.0 software (BIOPAC Systems, Inc., Goleta, CA, USA). An external trigger was used to synchronize EMG and video data.

EMG signals were bandpass filtered (2500 Hz and 70 Hz high- and low-bandpass filtered, respectively) and rectified. Signal noise was then subtracted from the rectified EMG signals. These signals were used to calculate two amplitude variables: the magnitude of peak burst amplitude and the total rectified integrated area (RIA), which reflects the relative proportion of the muscle that was active during the period of time for which it was calculated. These variables were expressed relative to the maximum amplitude ever observed for that muscle per individual in order to allow for comparisons between treatments and individuals. In addition to these amplitude variables, several timing variables were calculated, including onset and offset for each burst of muscle activity, burst duration, and the timing of peak burst amplitude. All timing variables were standardized by stride duration and expressed relative to footfall. To approximate the shape of the EMG burst, the time at which half of the total burst RIA was achieved was calculated and expressed relative to burst duration (Roberts et al., 2007). Extensive details of how each of these variables were calculated are available elsewhere (Foster and Higham, 2014).

Stride kinematics

High-speed video of locomotor movements were recorded simultaneously with the EMG signals using two Edgertronic SC1 cameras (Sanstreak Corp., San Jose, CA) at 250 frames s^{-1} with a shutter speed of 1/2000 s. A pre-measured calibration object constructed of LEGO^{TM} blocks were used to generate x, y, and z coordinates for digitizing. Points marked on the animals were digitized using DLT DV5 custom software (Hedrick, 2008) for MATLAB (version R2012a, The MathWorks, Natick, MA, USA). These 3D points were used to calculate body speed and joint angles for the fore- and hind limb throughout each stride. Details of these calculations are available elsewhere (Foster and Higham, 2012; Jagnandan et al., 2014).

To link muscle function to joint kinematics, EMG signals and kinematic variables from strides of different lengths were averaged by dividing values from stance and swing phases into 40 and 20 equal-duration bins, respectively. This is consistent with a duty factor of roughly 70%, as previously observed in *E. macularius* (Jagnandan et al., 2014).

The resulting trace for each muscle was then compared to the binned kinematic data to observe the timing of muscle activity relative to angular changes in the limb joints.

Statistical analyses

Averages of each EMG and kinematic variable for each individual before and after autotomy were used for all statistical analyses. The effects of speed on fore- and hind limb muscle activity and joint kinematics were removed by regressing the variables against body speed. Residuals of the variables that expressed a significant relationship ($\alpha \leq 0.10$) with speed were used for subsequent statistical analyses, while all other data were analyzed in their original form. To test for significant differences between mean values of pre- and post-autotomy treatments, paired *t*-tests were used for pairwise comparisons. Assumptions for normality and equal variances were not violated for any of the variables measured. All statistical analyses were performed using SYSTAT 13.00.05.

Results

Details of the changes in limb kinematics that occur with tail autotomy have been published in extensive detail elsewhere (Jagnandan et al., 2014). Geckos in this study exhibited the same changes in kinematics after tail autotomy, and no differences were observed before and after implantation of EMG electrodes. Briefly, geckos exhibited no differences in joint angles during flexion and extension of the forelimbs (Figure 12A-B), while significant decreases in femur retraction (Figure 13A-B), ankle angle (Figure 14A-

B), femur depression (Figure 15A-B), and knee angle (Figure 15C-D), were observed after the tail was removed.

Timing of muscle activity was consistent with hypothesized functions of the muscles in relation to movement at the joints (Zaaf et al., 1999) (Figures 12-15). The biceps exhibited two bursts of activity during the stride (Figure 12C-D). The first began just before footfall and persisted through the first half of stance, which is consistent with flexion of the elbow that occurs in the beginning of the stance phase. This was followed by a second smaller burst just before the swing phase, coinciding with elbow flexion as the foot is lifted off the ground. Similarly, the triceps also had multiple bursts that correspond with extension of the elbow in the second half of stance and again in the second half of swing before footfall (Figure 12E-F).

The CF was characterized by a single prolonged burst of activity beginning at or just before footfall that persisted throughout the entire stance phase (Figure 13C-D). Bursts were generally highest in amplitude near the beginning of the burst, as indicated by a relatively early time of half burst RIA. CF activity occurred as the femur was retracted throughout stance (Figure 13). The GAST remained active throughout much of stance as well, but was also characterized by a high-amplitude burst near the end of swing phase (Figure 14C-D). The ankle was extended by the GAST in the second half of stance and again near the end of swing (Figure 14A-B). Activity of the PIT consisted of a prolonged burst throughout stance that coincides with a gradual depression of the femur, and a higher amplitude burst at the beginning of swing as the knee is flexed when it is moved forward (Figure 15).

Statistical differences in the effects of the removal of the tail via autotomy are summarized in Tables 3-5. Amplitude and timing variables of the forelimb muscles were not significantly affected by the anteriorly shifted CoM due to autotomy. In contrast, the CF incurred a significant reduction in maximum amplitude and stance RIA when the tail was removed. A significant decrease in amplitude variables was also evident in the first burst of activity (during stance) in the GAST. Interestingly, timing variables and the shape of the bursts were not significantly altered in these hind limb muscles. Despite significant reductions in femur depression and knee angle, no statistical differences in amplitude, timing or shape of the activity bursts of the PIT were observed.

Discussion

Tail autotomy in lizards can result in a significant loss of mass and an anterior shift in CoM, resulting in modulation of limb kinematics to compensate for the changes that occur (Jagnandan et al., 2014). My assessment of *in vivo* muscle activity in *E. macularius* revealed differential impacts of autotomy on locomotor muscles throughout the fore- and hind limbs. Specifically, I observed no shifts in muscle activity in the forelimbs and a significant reduction in motor recruitment in propulsive muscles of the hind limbs. Muscles that regulate locomotor posture, however, did not change in amplitude or timing of activation.

Although the link between neuromuscular control and locomotor kinematics are well-supported (de Leon et al., 1994; Higham and Biewener, 2008; Reilly et al., 2005; Schilling et al., 2009), changes in muscle activity are not always coupled with changes in kinematics. When faced with external demands on locomotion, motor control may be altered to counteract the altered demand in order to preserve limb kinematics, or vice versa (Foster and Higham, 2014). Thus, although forelimb kinematics have been previously shown to be unaffected by tail autotomy, modulation of muscle activation in the forelimb muscles in response to a shift in load distribution via autotomy was still predicted to occur. In running dogs, for example, artificially shifting the CoM forward using added weights increased the contribution of the forelimbs to acceleration (Lee, 2011; Walter and Carrier, 2011). Interestingly, I found that motor control patterns of the biceps and triceps were not decoupled from forelimb kinematics in my study, as no changes in the amplitude or timing of the activation of these muscles were observed. These data suggest that, despite the anterior shift in CoM and theoretical increase in load on the forelimbs, biomechanical compensation for the tail autotomy takes place primarily in the hind limbs, the site that is closer to where the morphological change occurs.

The primary neuromuscular changes that occurred in the hind limbs were observed in the muscles that drive propulsion. The CF is a propulsive muscle of particular interest in regards to autotomy because of its critical role in retracting the femur (Irschick and Jayne, 1999; Nelson and Jayne, 2001; Reilly, 1994; Russell and Bauer, 1992; Snyder, 1952; Snyder, 1954; Snyder, 1962) and its point of origin at the proximal caudal vertebrae (Snyder, 1962; Zaaf et al., 1999). Tail autotomy occurs most proximally at a vertebral fracture plane that is distal to the origin of the CF, so the muscle remains intact after removal of the tail (Russell and Bauer, 1992). However, these results suggest that its functional role in locomotion may be altered. A notable decrease in the amplitude of activation of the CF is present throughout stance phase after autotomy (Figure 13C-D), which is consistent with the loss of mass that occurs as the animal requires less work from the muscles to move the body, as well as a reduction in retraction of the femur. However, EMG signals also reveal a higher amplitude of activation just after footfall in intact geckos, despite femur retraction occurring continuously throughout stance. This suggests a secondary function that may be related to movements of the tail during locomotion. During steady locomotion, the tail of *E. macularius* is laterally undulated, with the base of the tail flexed toward the protracted hind limb during each cycle of limb movement. I hypothesize that this high amplitude of muscle activity at footfall is functionally related to the swinging of the tail toward the hind limb that is beginning stance, since the early CF burst is absent in autotomized geckos.

Additionally, the GAST exhibits a reduced amplitude and RIA of muscle activity (Tables 3, 5) that is consistent with a reduction in ankle extension in the second half of stance (Figure 14). This again suggests that with the loss of mass via autotomy, less work is needed to propel the hind limb forward, and the activation of the propulsive muscle is modulated accordingly. Geckos also exhibit a small increase in ankle angle near the end of the stride as the ankle is extended just before footfall. A large burst of activity of the

GAST is present at the end of the stride that corresponds with this extension of the ankle, but this burst is not altered by autotomy. This suggests that only propulsion in the stance phase is impacted by autotomy, while muscle activity when the limb is off the ground is unaltered. Moreover, the high amplitude of this second burst suggests a functional role besides extension of the ankle. I hypothesize that the activity of the GAST just prior to footfall may be associated with braking and stability to stiffen the ankle joint as the foot is brought down to the ground, similar to pre-landing activation of limb muscles in hopping frogs (Ekstrom and Gillis, 2015; Gillis et al., 2010).

The PIT, however, is a hind limb muscle that exhibited no changes in motor pattern after the mass of the tail was removed, despite significant reductions in femur depression and knee angle. These kinematic changes are indicative of a more sprawled posture, which was proposed to preserve stability as the lizard adjusts to its lower mass and shifted CoM (Jagnandan et al., 2014). Sprawled postures lower an animal’s effective mechanical advantage (EMA), thereby increasing the demand on the locomotor muscles (assuming all else is constant) (Biewener, 1989a; Biewener, 1989b; Biewener, 1990; Biewener, 2005). Tail autotomy, however, reduces the gecko’s mass and vertical ground-reaction force (GRF) (Jagnandan et al., 2014), thus countering this demand on the support muscles and allowing geckos to support a more sprawled, stable posture without altering motor recruitment in those muscles.

Tail autotomy in lizards is an effective natural model for investigating the neuromuscular control of locomotion in response to altered demands. These findings demonstrate differential responses of limb muscles to the morphological shift that occurs with tail autotomy. Whether these changes in motor control persist or continuously change as the tail is regenerated is something to be addressed in future work using long-term EMG implants. Another interesting follow-up study would combine demands brought on by physiological mechanisms (autotomy) with demands of the environment (e.g. changes in surface grade) to further investigate the adaptability of neuromuscular control of the locomotor system. For example, does mass loss by autotomy help to compensate for the increased demands of locomotion up an incline? Or do the shifts in motor control make navigating demanding terrain more problematic? Answers to these questions will illuminate a greater understanding of muscle plasticity and have broad applications for biomechanical, physiological, and evolutionary research.

CONCLUSIONS

My utilization of tail autotomy in geckos has been valuable in illuminating the mechanical consequences of rapid mass change, revealing the functional role of the tail in locomotion, and elucidating the neuromuscular control of locomotion in response to functional demands. Specifically, these experimental studies brought forth four major conclusions. First, tail autotomy in geckos can result in a significant loss of mass and a resulting shift in the CoM, particularly in species with relatively large tails such as *E. macularius*. Second, this rapid morphological change necessitates compensation by the animal to maintain stability, which is accomplished by altering hind limb GRFs and joint kinematics to assume a more sprawled posture. Third, analysis of tail movements during locomotion demonstrates the role of tail undulations and suggests that the effects of autotomy may be linked to both the loss of functional tail movements and altered mass. Finally, EMG analyses reveal differential impacts of autotomy on the patterns of motor control of the locomotor muscles that coincide with the loss of mass and adoption of a more stable, sprawled posture.

With these results, there is ample room for future research that address unanswered questions related to rapid mass change, tail function, and the consequences of tail autotomy. Direct measures of dynamic stability, for example, will further elucidate how changes in mass impact terrestrial locomotion. Navigating obstacles, inclines and declines, and other heterogeneous environments alter demands on the locomotor system on their own. Revealing how altered mass and other consequences of autotomy impact locomotion on non-uniform or non-level terrain could tease apart the complexity of locomotor adaptations to concurrent physiological and environmental demands. Furthermore, comparative studies using similar methods would also reveal whether some species are better at compensating for changes in mass than others. Ecological and morphological factors may a play role in the strategies that animals take to overcome these functional demands. An evolutionary approach to determine the relationships between rapid mass changes, stability, mechanics, muscle function, and performance across lizard species is also useful for understanding where selection acts in regards to locomotor adaptability.

Broader applications of this research can also aid in exercise science and medical research. Artificial increases in mass and alterations of mass distribution are frequent occurrences during human exercise and athletic training. The mechanisms that lizards naturally utilize to counteract mass changes due to autotomy and regeneration can provide insight on the effectiveness and efficiency of altered locomotor mechanics and muscle function in humans. Additionally, these results may be applied to research on biologically-inspired robotics, where load-carrying and locomotor stability are often of primary concern.

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Table 1. Summary of kinematic variables before and after autotomy in the leopard gecko *Eublepharis macularius*

Variable	Pre-autotomy	Post-autotomy	22 weeks post-autotomy	*F*-ratio	*P*
Body pitch (deg)	3.72 ± 0.61	2.44 ± 0.77	2.78 ± 0.80	0.867	0.475
Forelimb					
stride length (SVL)	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00	0.682	0.547
stance time (s)	0.33 ± 0.04	0.28 ± 0.02	0.35 ± 0.02	3.344	0.120
duty factor	0.70 ± 0.03	0.70 ± 0.02	0.73 ± 0.02	0.613	0.578
shoulder height (SVL)	0.11 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	1.374	0.334
max humerus depression (deg)	29.02 ± 8.62	45.00 ± 15.63	67.28 ± 17.20	2.881	0.147
max humerus retraction (deg)	54.61 ± 5.98	63.90 ± 4.93	53.97 ± 3.51	4.475	0.077
max elbow angle (deg)	135.65 ± 3.01	133.04 ± 2.60	138.56 ± 3.17	1.667	0.279
Hind limb					
stride length (SVL)	0.73 ± 0.00	0.72 ± 0.00	0.72 ± 0.00	0.263	0.779
stance time (s)	0.43 ± 0.02	0.41 ± 0.02	0.43 ± 0.02	0.294	0.757
duty factor	0.75 ± 0.01	0.72 ± 0.01	0.73 ± 0.01	1.984	0.232
hip height (SVL)	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	3.044	0.137
max femur depression (deg)	65.77 ± 7.50	21.75 ± 4.96	38.36 ± 6.18	19.465	**0.004**
max femur retraction (deg)	59.73 ± 5.60	42.00 ± 3.88	61.23 ± 6.63	16.984	**0.006**
max knee angle (deg)	160.49 ± 3.57	140.78 ± 3.89	160.70 ± 4.40	7.449	**0.032**
max ankle angle (deg)	141.80 ± 3.57	129.92 ± 2.55	132.66 ± 4.81	12.244	**0.012**

Means + residuals (± s.e.m.) for each variable are given for pre-autotomy, post-autotomy and 22 weeks post-autotomy. Statistical significant (repeated measures ANOVA) of changes in each variable is also given. Significant results are indicated in bold type.

Table 2. Summary of kinematic variables in the leopard gecko *Eublepharis macularius* across tail treatments

Variable	Original	Restricted	Autotomized	*F*-ratio	*P*
Forelimb					
Stride length (SVL)	0.63 ± 0.15	0.60 ± 0.05	0.49 ± 0.06	2.986	0.108
Step length (SVL)	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	1.452	0.290
Stance time (s)	0.54 ± 0.03	0.55 ± 0.03	0.50 ± 0.02	0.640	0.552
Duty factor	0.70 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.765	0.497
Humerus depression (deg)					
Maximum	45.59 ± 8.31	34.29 ± 4.88	29.30 ± 2.91	0.898	0.445
Angular excursion	101.38 ± 15.88	72.67 ± 7.72	72.26 ± 4.30	1.818	0.223
Humerus retraction (deg)					
Maximum	54.78 ± 2.68	68.51 ± 2.45	73.02 ± 3.13	2.897	0.113
Angular excursion	44.41 ± 1.80	51.21 ± 2.57	59.23 ± 2.41	3.505	0.081
Elbow angle (deg)					
Maximum	151.04 ± 1.23	144.82 ± 1.91	142.56 ± 1.59	1.986	0.199
Angular excursion	92.49 ± 2.27	83.17 ± 2.64	93.76 ± 2.73	4.383	0.052
Wrist angle (deg)					
Maximum	165.31 ± 2.00	161.83 ± 1.43	164.83 ± 1.45	1.233	0.341
Angular excursion	71.93 ± 3.09	70.38 ± 2.51	73.33 ± 2.73	0.353	0.713

Means + residuals (± s.e.m.) for each variable are given for original, restricted, and autotomized tail treatments. Statistical significance (repeated measures ANOVA) of changes in each variable is also given. Significant results are indicated in bold type.

Table 2. (continued)

Variable	Original	Restricted	Autotomized	*F*-ratio	*P*
Hind limb					
Stride length (SVL)	0.62 ± 0.15	0.72 ± 0.05	0.70 ± 0.06	1.275	0.331
Step length (SVL)	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	5.836	**0.027**
Stance time (s)	0.64 ± 0.03	0.60 ± 0.03	0.56 ± 0.01	0.923	0.436
Duty factor	0.78 ± 0.01	0.78 ± 0.01	0.77 ± 0.01	0.578	0.583
Femur depression (deg)					
Maximum	49.44 ± 2.49	23.01 ± 1.44	20.79 ± 1.30	29.601	**< 0.000**
Angular excursion	52.42 ± 3.25	30.56 ± 1.63	23.29 ± 1.48	30.447	**< 0.000**
Femur retraction (deg)					
Maximum	55.00 ± 2.18	40.94 ± 1.65	39.82 ± 2.03	6.106	**0.025**
Angular excursion	82.57 ± 2.29	65.26 ± 2.03	69.50 ± 1.95	5.637	**0.030**
Knee angle (deg)					
Maximum	164.49 ± 0.93	154.84 ± 1.47	151.75 ± 1.30	1.674	**0.003**
Angular excursion	98.83 ± 1.39	88.08 ± 1.68	81.48 ± 2.40	14.282	**0.002**
Ankle angle (deg)					
Maximum	139.65 ± 2.03	129.69 ± 1.90	126.19 ± 1.97	9.85	**0.007**
Angular excursion	85.19 ± 2.38	70.41 ± 1.71	63.19 ± 2.61	16.589	**0.001**

Means + residuals (± s.e.m.) for each variable are given for original, restricted, and autotomized tail treatments. Statistical significance (repeated measures ANOVA) of changes in each variable is also given. Significant results are indicated in bold type.

Table 3. Stance RIA for each muscle before and after tail autotomy

Muscle	Pre-autotomy	Post-autotomy	*t*-statistic	*P*
Biceps	72.81 ± 4.98	60.53 ± 5.87	1.301	0.229
Triceps	60.45 ± 5.43	69.80 ± 6.57	-0.925	0.382
CF	74.85 ± 4.58	30.85 ± 9.58	6.450	**< 0.000**
GAST	72.22 ± 3.43	57.79 ± 3.68	8.281	**< 0.000**
PIT	53.43 ± 7.02	48.89 ± 8.93	0.180	0.861

Means + residuals (± s.e.m.) for stance RIA are given for pre-autotomy and post-autotomy. Values are expressed relative to the maximum amplitude observed for each muscle. Statistical significance (paired *t*-tests) of changes in each variable is also given. Significant results are indicated in bold type.

Table 4. Summary of EMG variables in forelimb muscles of the leopard gecko *Eublepharis macularius*

Muscle	Burst	Kinematics	Variable	Pre-autotomy	Post-autotomy	*t*-statistic	*P*
Biceps	1	Elbow flexion (stance)	Max burst amplitude	49.82 ± 8.63	38.44 ± 5.76	0.952	0.373
			Onset	-11.24 ± 1.96	-12.86 ± 1.81	0.628	0.550
			Burst duration	38.79 ± 2.62	42.93 ± 2.93	-1.245	0.253
			Time of max burst amplitude	4.62 ± 1.44	2.92 ± 4.75	0.162	0.875
			Time of half burst RIA	44.48 ± 2.70	39.11 ± 3.59	1.448	0.191
	2	Elbow flexion (swing)	Max burst amplitude	47.12 ± 8.71	28.93 ± 5.23	2.115	0.067
			Onset	56.67 ± 5.29	56.28 ± 7.88	0.197	0.849
			Burst duration	30.81 ± 2.21	28.78 ± 2.71	0.671	0.521
			Time of max burst amplitude	54.69 ± 2.96	61.94 ± 3.90	-1.590	0.150
			Time of half burst RIA	51.49 ± 3.35	54.94 ± 2.35	-1.121	0.260
Triceps	1	Elbow extension (stance)	Max burst amplitude	35.86 ± 8.15	23.62 ± 4.36	0.752	0.477
			Onset	12.65 ± 2.81	8.29 ± 1.70	1.127	0.297
			Burst duration	47.31 ± 3.88	51.34 ± 1.86	-0.800	0.450
			Time of max burst amplitude	30.35 ± 2.75	34.16 ± 3.28	-0.957	0.370
			Time of half burst RIA	56.74 ± 3.18	58.53 ± 0.96	-0.575	0.584
	2	Elbow extension (swing)	Max burst amplitude	6.90 ± 1.76	5.58 ± 1.12	-0.479	0.657
			Onset	70.26 ± 1.64	69.49 ± 1.21	0.141	0.895
			Burst duration	24.92 ± 1.64	19.71 ± 2.23	1.778	0.150
			Time of max burst amplitude	73.52 ± 4.03	70.60 ± 2.50	0.420	0.696
			Time of half burst RIA	55.91 ± 4.50	53.16 ± 2.94	0.417	0.698

Means + residuals (± s.e.m.) for each variable are given for pre-autotomy and post-autotomy. Amplitude variables (shaded) are expressed relative to the maximum amplitude observed for each muscle. Timing variables (not shaded) are standardized by stride duration and expressed relative to footfall. Statistical significance (paired *t*-tests) of changes in each variable is also given. Significant results are indicated in bold type.

Table 5. Summary of EMG variables in hind limb muscles of the leopard gecko *Eublepharis macularius*

Muscle	Burst	Kinematics	Variable	Pre-autotomy	Post-autotomy	*t*-statistic	*P*
CF	1	Femur retraction	Max burst amplitude	53.73 ± 9.04	21.83 ± 8.01	3.705	**0.014**
			Onset	-1.68 ± 2.26	-6.90 ± 1.32	1.475	0.200
			Burst duration	81.45 ± 2.00	81.52 ± 0.47	-0.468	0.660
			Time of max burst amplitude	14.01 ± 3.08	10.04 ± 3.26	0.668	0.534
			Time of half burst RIA	36.94 ± 4.12	42.50 ± 2.55	-1.146	0.304
GAST	1	Ankle extension (stance)	Max burst amplitude	38.49 ± 5.12	15.41 ± 0.69	4.747	**0.018**
			Onset	6.40 ± 2.30	7.66 ± 2.87	-1.257	0.298
			Burst duration	64.41 ± 4.26	61.70 ± 3.80	2.155	0.120
			Time of max burst amplitude	33.79 ± 5.49	36.78 ± 7.49	-0.503	0.649
			Time of half burst RIA	52.15 ± 3.57	45.82 ± 5.81	-0.117	0.915
	2	Ankle extension (swing)	Max burst amplitude	54.50 ± 5.90	56.04 ± 10.87	-1.453	0.384
			Onset	85.06 ± 2.31	76.75 ± 10.37	0.174	0.890
			Burst duration	13.23 ± 1.56	23.87 ± 9.62	-1.019	0.494
			Time of max burst amplitude	94.74 ± 1.28	95.63 ± 2.63	0.536	0.687
			Time of half burst RIA	65.99 ± 1.61	64.93 ± 10.28	-0.462	0.724

Means + residuals (± s.e.m.) for each variable are given for pre-autotomy and post-autotomy. Amplitude variables (shaded) are expressed relative to the maximum amplitude observed for each muscle. Timing variables (not shaded) are standardized by stride duration and expressed relative to footfall. Statistical significance (paired *t*-tests) of changes in each variable is also given. Significant results are indicated in bold type.

Table 5. (continued)

Muscle	Burst	Kinematics	Variable	Pre-autotomy	Post-autotomy	*t*-statistic	*P*
PIT	1	Femur depression	Max burst amplitude	35.29 ± 8.57	31.96 ± 12.27	0.652	0.550
			Onset	-5.03 ± 1.54	-2.13 ± 4.72	-0.491	0.649
			Burst duration	49.11 ± 4.08	50.55 ± 3.61	-1.079	0.341
			Time of max burst amplitude	21.53 ± 6.75	13.78 ± 11.11	0.331	0.757
			Time of half burst RIA	45.09 ± 5.47	34.71 ± 4.45	1.617	0.181
	2	Knee flexion	Max burst amplitude	39.05 ± 8.94	37.23 ± 5.70	-0.235	0.823
			Onset	60.59 ± 6.56	62.14 ± 3.46	-0.236	0.823
			Burst duration	33.98 ± 7.24	28.67 ± 2.95	0.198	0.851
			Time of max burst amplitude	72.12 ± 6.16	76.59 ± 3.04	-0.497	0.640
			Time of half burst RIA	51.02 ± 4.41	58.97 ± 7.04	-0.718	0.505

Means + residuals (± s.e.m.) for each variable are given for pre-autotomy and post-autotomy. Amplitude variables (shaded) are expressed relative to the maximum amplitude observed for each muscle. Timing variables (not shaded) are standardized by stride duration and expressed relative to footfall. Statistical significance (paired *t*-tests) of changes in each variable is also given. Significant results are indicated in bold type.

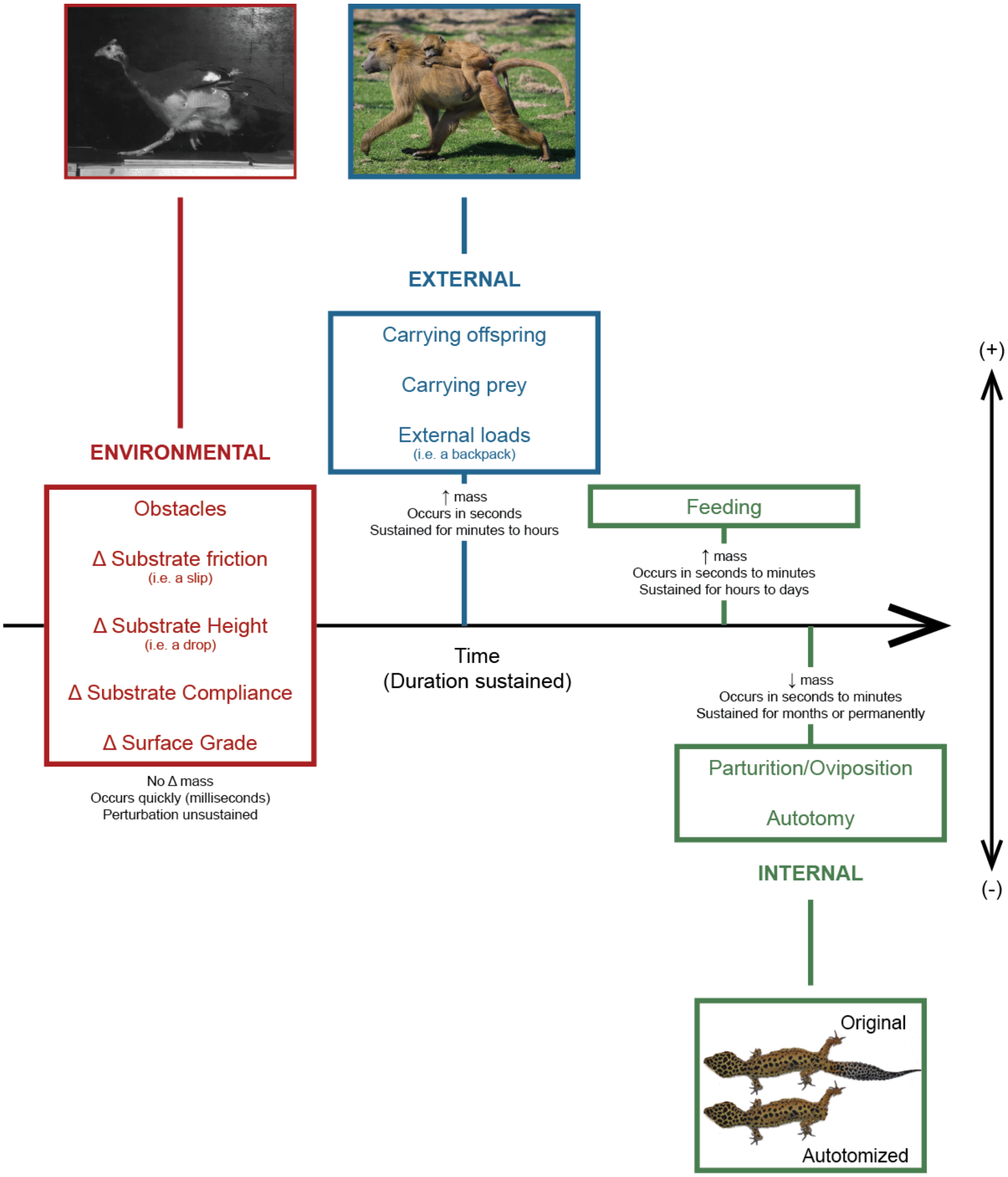
EXTERNAL
Carrying offspring
Carrying prey
External loads
(i.e. a backpack)
↑ mass
Occurs in seconds
Sustained for minutes to hours
ENVIRONMENTAL
Obstacles
Δ Substrate friction
(i.e. a slip)
Δ Substrate Height
(i.e. a drop)
Δ Substrate Compliance
Δ Surface Grade
No Δ mass
Occurs quickly (milliseconds)
Perturbation unsustained
Feeding
↑ mass
Occurs in seconds to minutes
Sustained for hours to days
Time
(Duration sustained)
↓ mass
Occurs in seconds to minutes
Sustained for months or permanently
Parturition/Oviposition
Autotomy
INTERNAL
Original
Autotomized
(+)
Δ Mass
(-)

Figure 1. **Proposed classification for locomotor perturbations.** (A) Environmental perturbations rapidly alter an animal's CoM without changing mass. These occur most rapidly, thus requiring a quick recovery, but are not sustained changes. Image: Guinea fowl experiencing a rapid change in surface friction. (B) External perturbations involve a load rapidly added onto an animal, which can be sustained for several hours. Image: Guinea baboon (*Papio papio*) carrying its young on its back. (C) Internal perturbations can involve a rapid gain or loss of mass. These changes also occur rapidly, but are sustained for an extensive amount of time. Image: Leopard gecko (*Eublepharis macularius*) before and after autotomy of the tail.

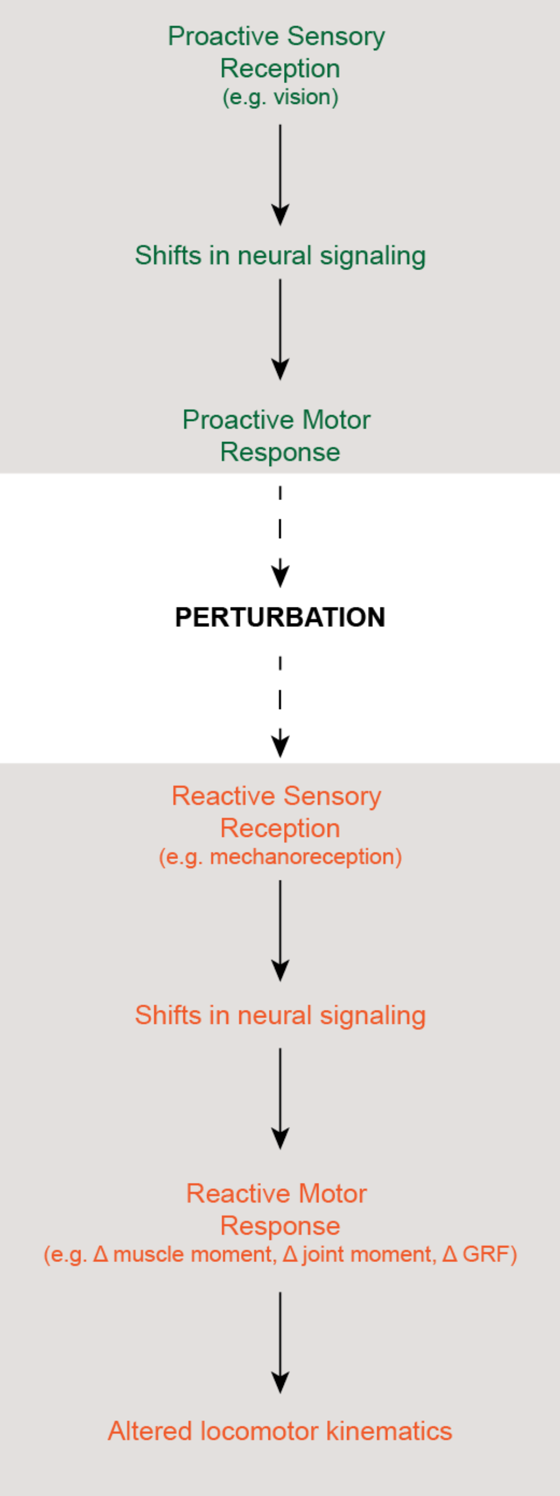
Proactive Sensory Reception
(e.g. vision)
Shifts in neural signaling
Proactive Motor Response
PERTURBATION
Reactive Sensory Reception
(e.g. mechanoreception)
Shifts in neural signaling
Reactive Motor Response
(e.g. Δ muscle moment, Δ joint moment, Δ GRF)
Altered locomotor kinematics

Figure 2. **Proactive and reactive control of perturbations.** Proactive control requires an initial sensory input before encountering the perturbation, such as a visual cue, which leads to a proactive motor response in preparation for the event. Reactive control occurs after experiencing the perturbation and instead involves a recovery phase consisting of sensing the perturbation and adjusting the locomotor system accordingly. Control strategies to maintain dynamic stability during a perturbation can be proactive, reactive, or both.

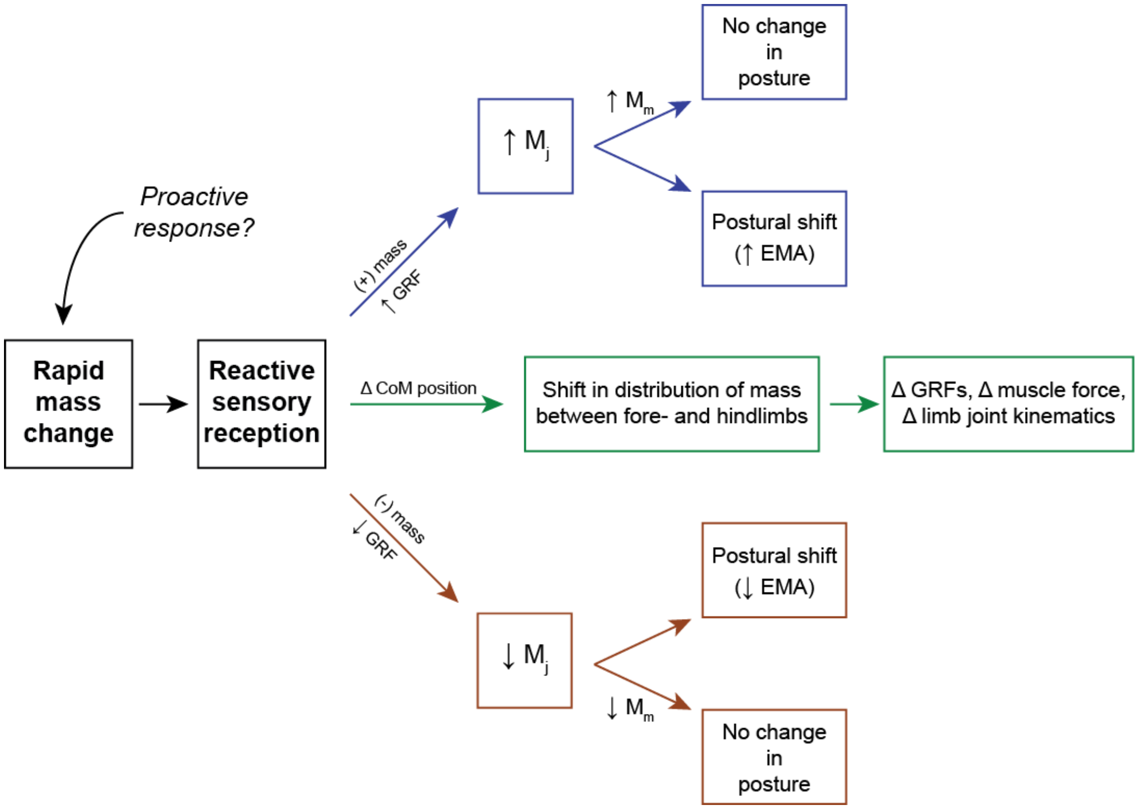

Figure 3. **Motor responses to rapid mass changes.** Increases in mass (blue) increase ground-reaction forces (GRFs), which increase the joint moments (M_j). To compensate for change, the limb muscles can generate more force, thereby increasing the muscle moment (M_m), or the animal can increase its limb effective mechanical advantage (EMA) and switch to a more erect posture. When mass is lost (orange), GRFs decrease, reducing M_j. The muscles can generate less force to lower M_m and make locomotion more efficient, or the animal can decrease its limb EMA and switch to more sprawled posture to increase maneuverability or stability. Mass changes that shift the center of mass (CoM) (green) alter the distribution of the mass between the limbs, which can lead to changes in GRFs, muscle force and kinematics in either or both sets of limbs, thus impacting posture and locomotor mechanics.

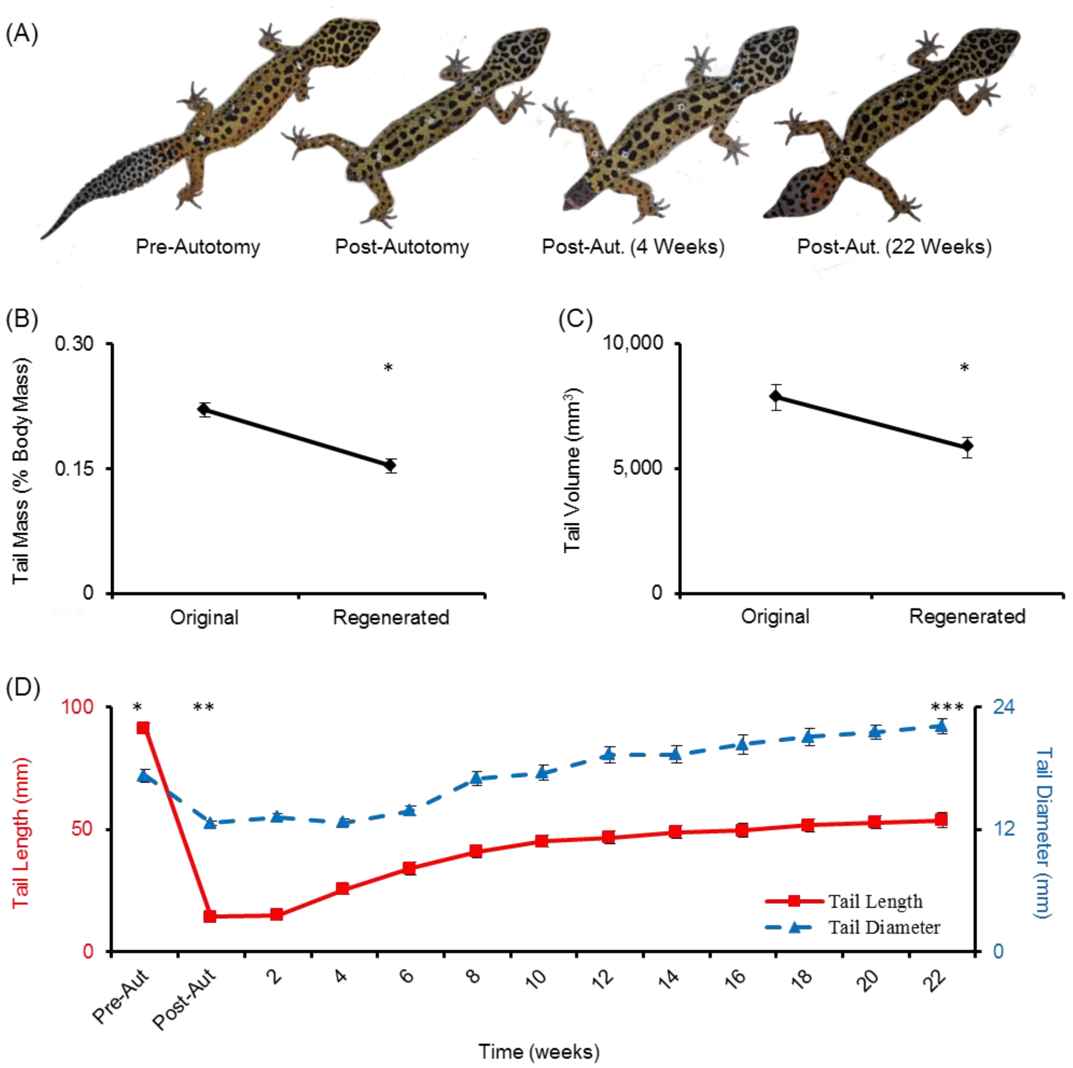

Figure 4. Changes in tail morphology with autotomy and regeneration. (A) Stages of tail loss and re-growth in *Eublepharis macularius*. (B, C) Mean mass and volume of original and regenerated tails. Asterisk indicates significant differences (paired *t*-test, $P<0.05$). (D) Mean tail length (red) and diameter (blue) measured biweekly throughout autotomy and regeneration. Asterisk indicates significant differences in both tail length and diameter between pre-autotomy, post-autotomy, and fully regenerated tails (repeated measures ANOVA, $P<0.05$). Error bars are s.e.m.

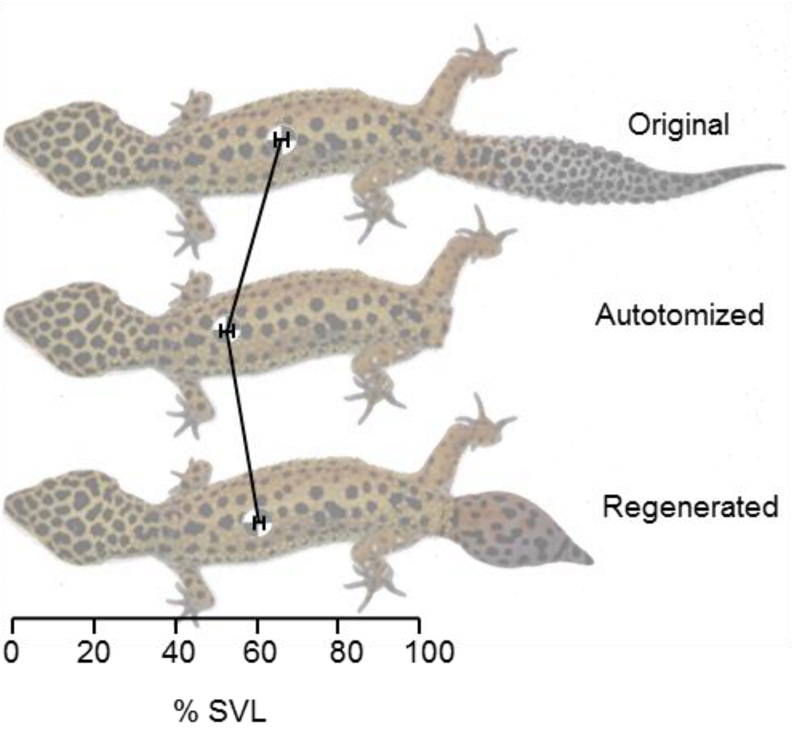

Figure 5. CoM location in lizards with original, autotomized, and fully regenerated tails. Error bars are s.e.m. All three points are significantly different from one another (repeated measures ANOVA, *P*=0.017).

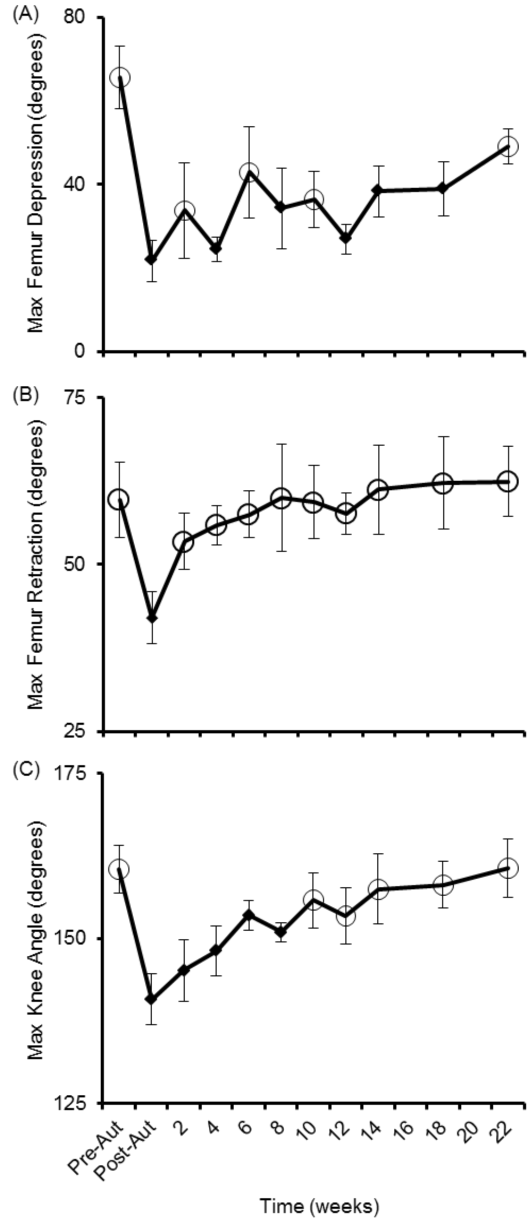

Figure 6. **Means of maximum hind limb joint angles during stance phase measured throughout autotomy and regeneration.** Values for (A) femur depression, (B) femur retraction, and (C) knee angle are means + residuals from seven individuals. Error bars are s.e.m. Open circles indicate values that are not significantly different from values obtained in the pre-autotomy trials (repeated measures ANOVA and *post hoc* tests for multiple comparisons, $P>0.05$).

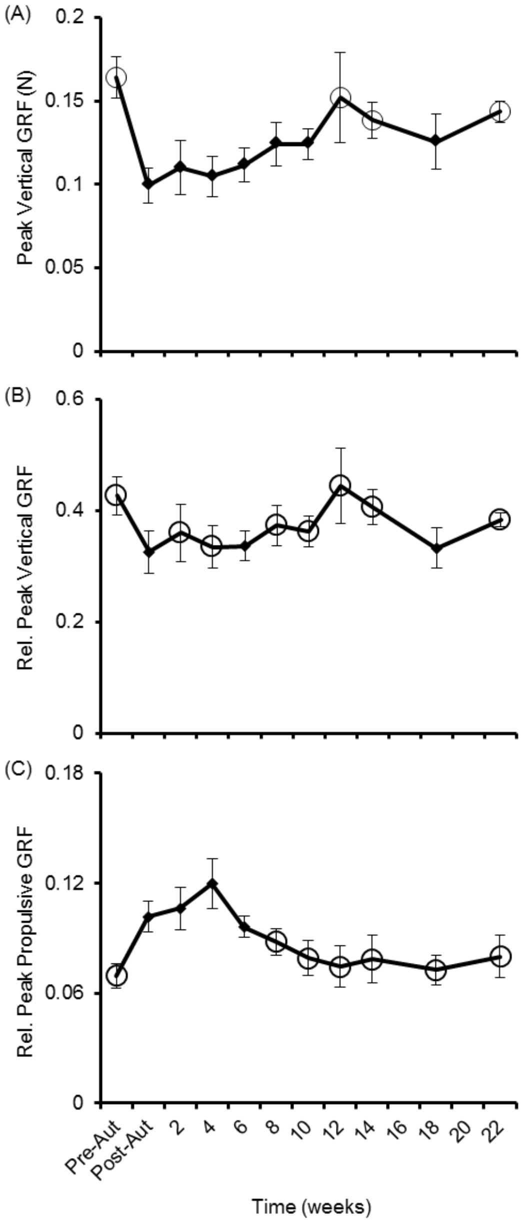

Figure 7. **Means of peak GRFs from the propulsive half of stance phase.** (A) Peak vertical GRF, (B) Weight-specific peak vertical GRF, and (C) Weight-specific peak propulsive GRF. Values for propulsive forces are means + residuals to remove the effect of speed. Error bars are s.e.m. Open circles indicate values that are not significantly different from values obtained in the pre-autotomy trials (repeated measures ANOVA and *post hoc* tests for multiple comparisons, *P*>0.05).

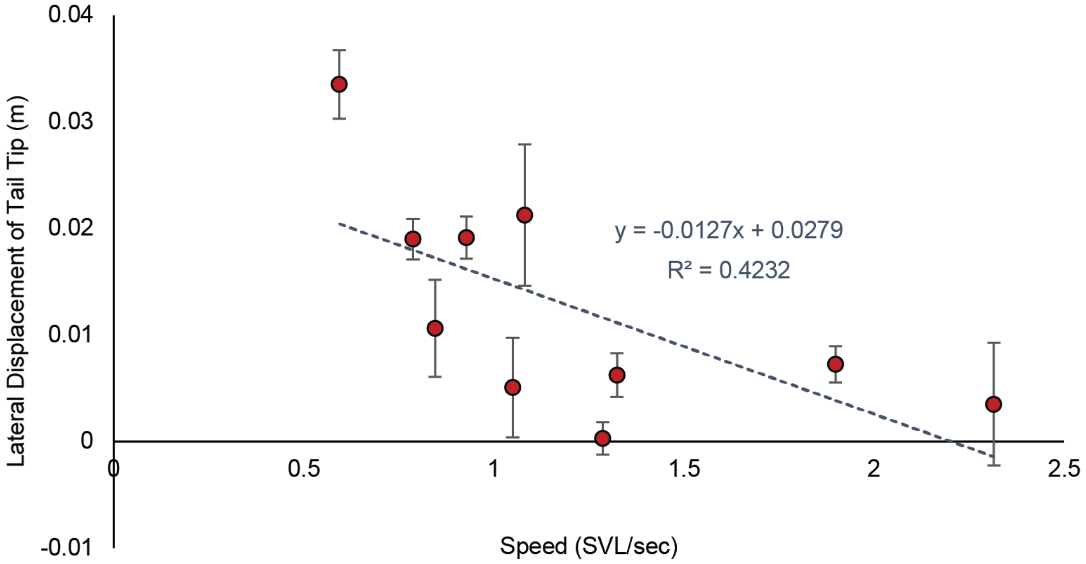

Figure 8. Relationship of lateral displacement of the tail tip with speed. Lateral displacement is measured as the lateral distance of the tail tip relative to the pelvic girdle, as measured on the left side of the body. Data points are means for each individual (n=10). Error bars are s.e.m. Regression analysis demonstrates a significant negative relationship of lateral displacement of the tail tip with speed (*P*=0.042).

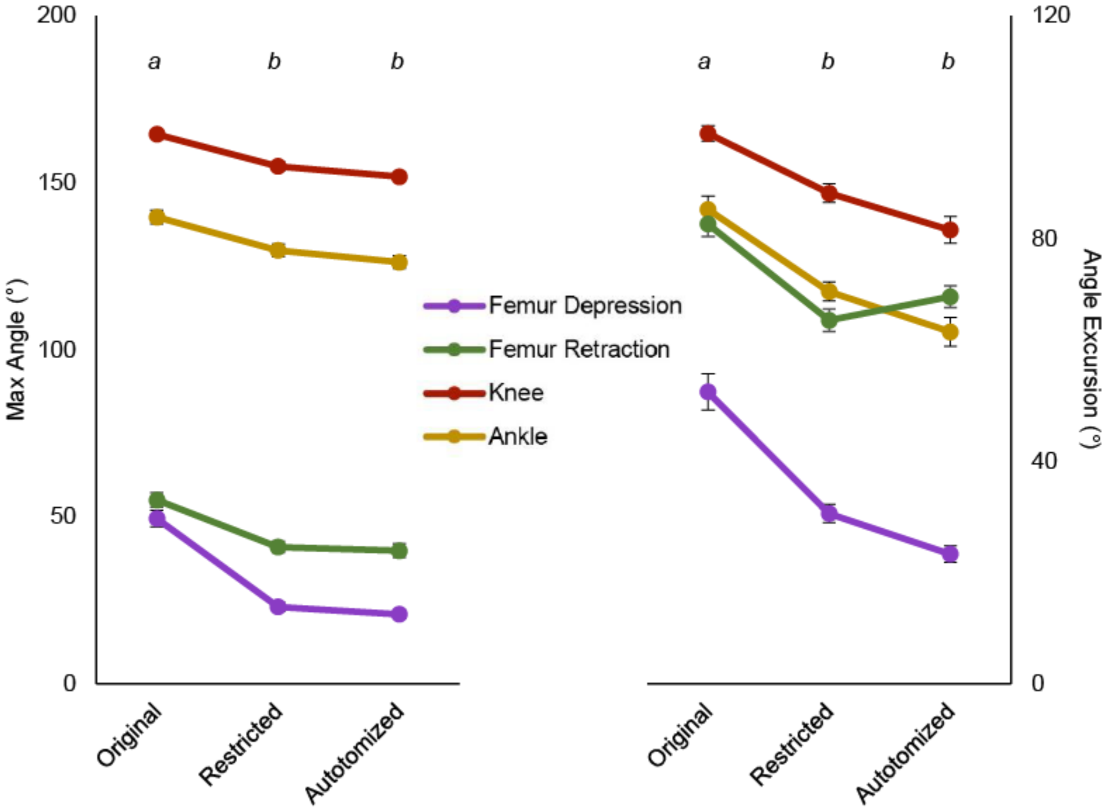

Figure 9. Means of maximum angles (left) and angular excursions (right) of hind limb joints during stance phase. Values for femur depression, femur retraction, knee angle, and ankle angle are means + residuals from 10 individuals. Error bars are s.e.m. Letters above each treatment indicate significant differences (repeated measures ANOVA and *post hoc* tests for multiple comparisons, $P<0.05$).

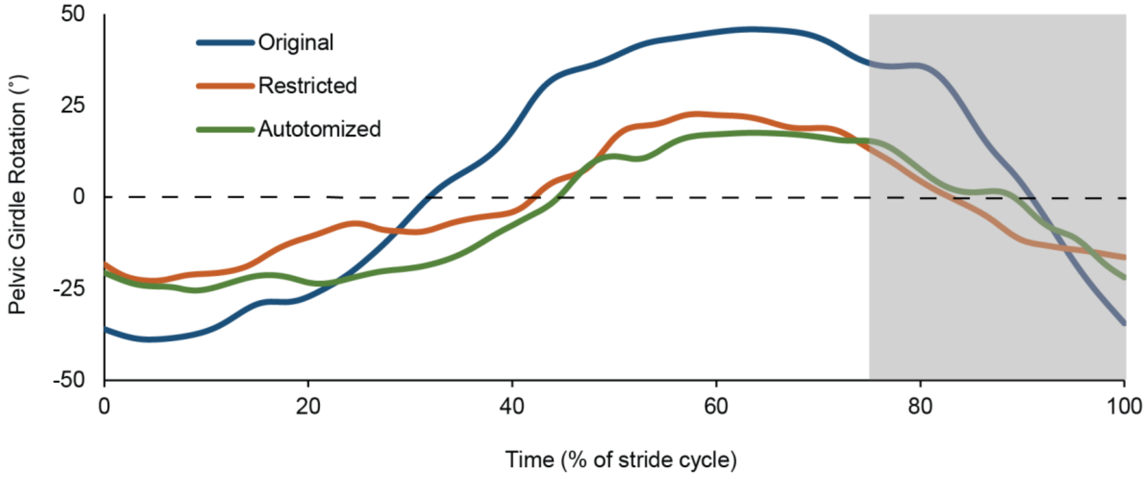

Figure 10. Pelvic girdle rotation throughout a representative hind limb stride. Degree of rotation of the pelvic girdle over time (as a percentage of stride cycle) is provided for a leopard gecko with its original (blue), restricted (orange), and autotomized (green) tail. Negative values indicate that the pelvic girdle is rotated to the right (toward the hind limb being observed) and positive values indicate that the pelvic girdle is rotated to the left (toward the opposite hind limb). The non-shaded region represents the stance phase of the observed hind limb and the area shaded in gray represents the swing phase.

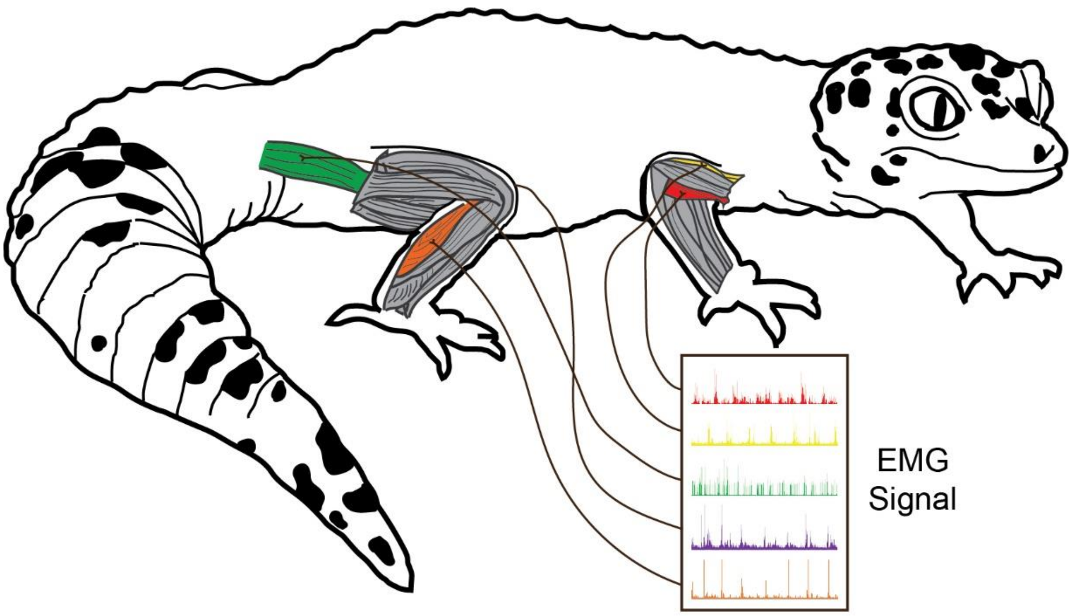

Figure 11. Locations of muscles implanted in *Eublepharis macularius* with EMG electrodes. EMG signals from top to bottom are biceps (red), triceps (yellow), CF (green), PIT (located on ventral surface of proximal hind limb; purple), GAST (orange).

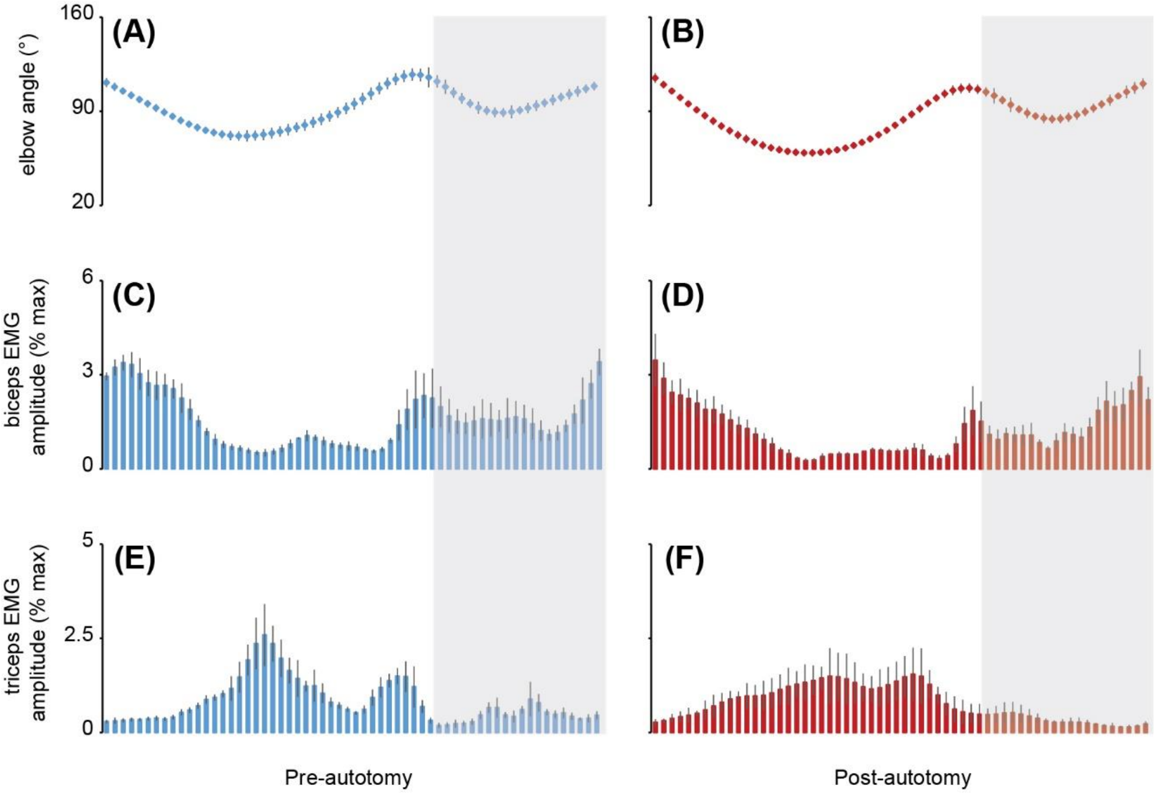

Figure 12. Elbow angle and forelimb muscle activity before and after autotomy. Binned elbow angle (A, B) and binned EMG amplitude of the biceps (C, D) and triceps (E, F) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C, E) and post-autotomy (red; B, D, F) treatments. Values are means + residuals from ten individuals. Error bars are s.e.m.

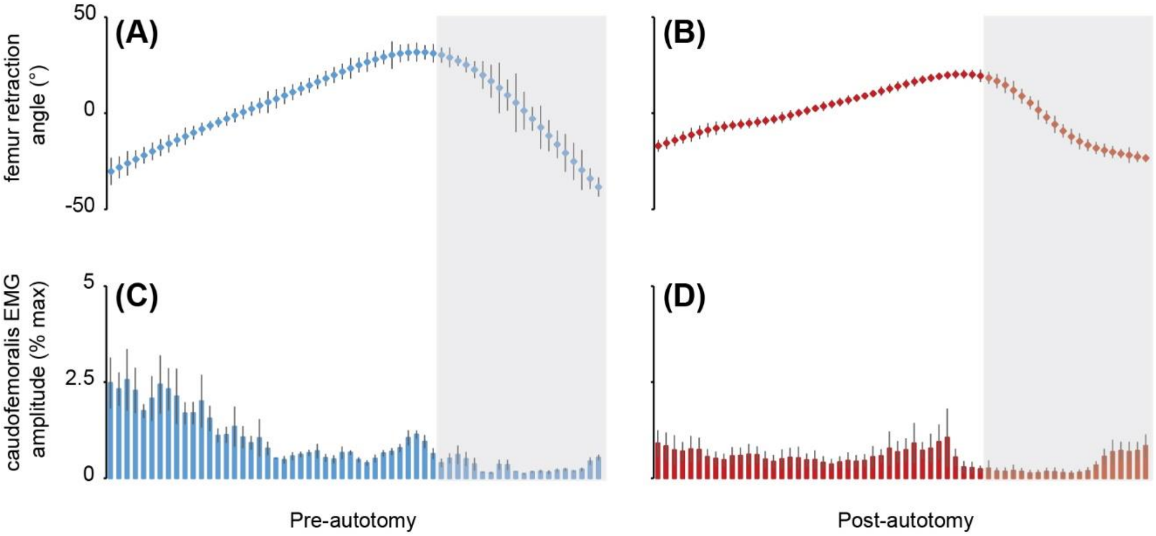

Figure 13. Femur retraction angle and CF muscle activity before and after autotomy. Binned femur retraction angle (A, B) and binned EMG amplitude of the caudofemoralis (C, D) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C) and post-autotomy (red; B, D) treatments. Values are means + residuals from ten individuals. Error bars are s.e.m.

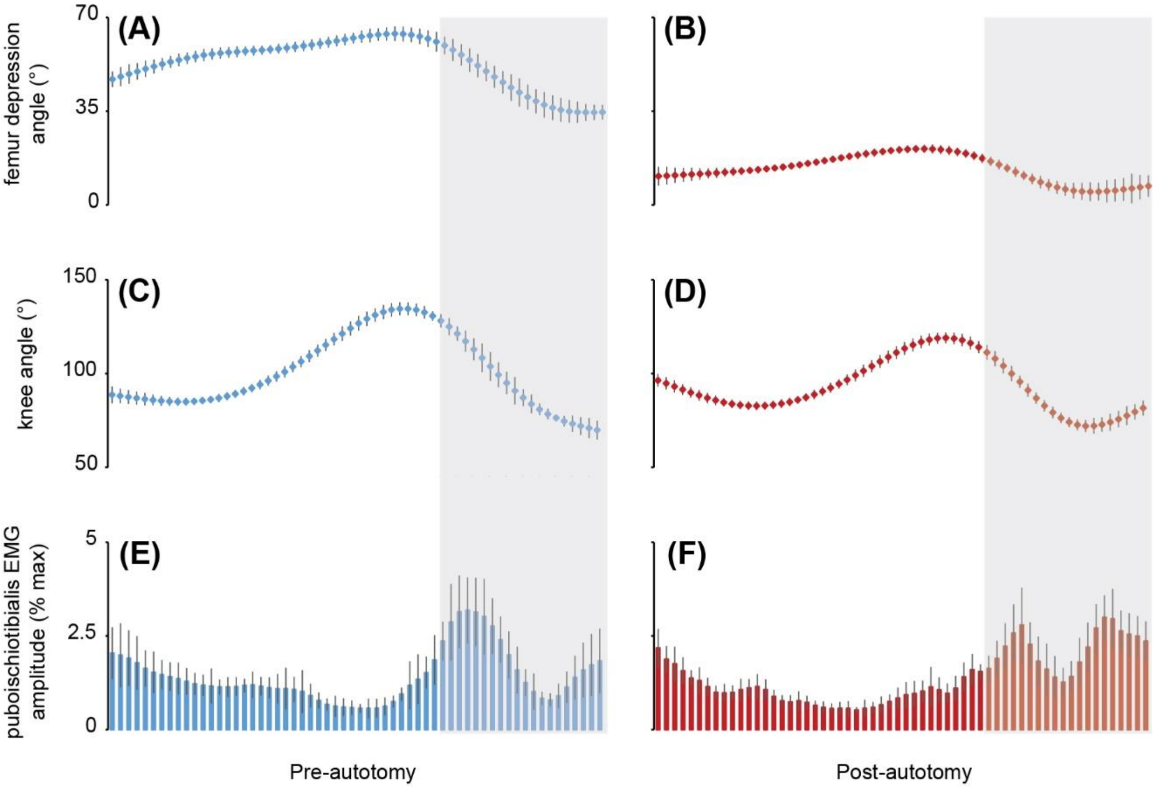

Figure 14. Ankle angle and GAST muscle activity before and after autotomy. Binned ankle angle (A, B) and binned EMG amplitude of the gastrocnemius (C, D) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C) and post-autotomy (red; B, D) treatments. Values are means + residuals from ten individuals. Error bars are s.e.m.

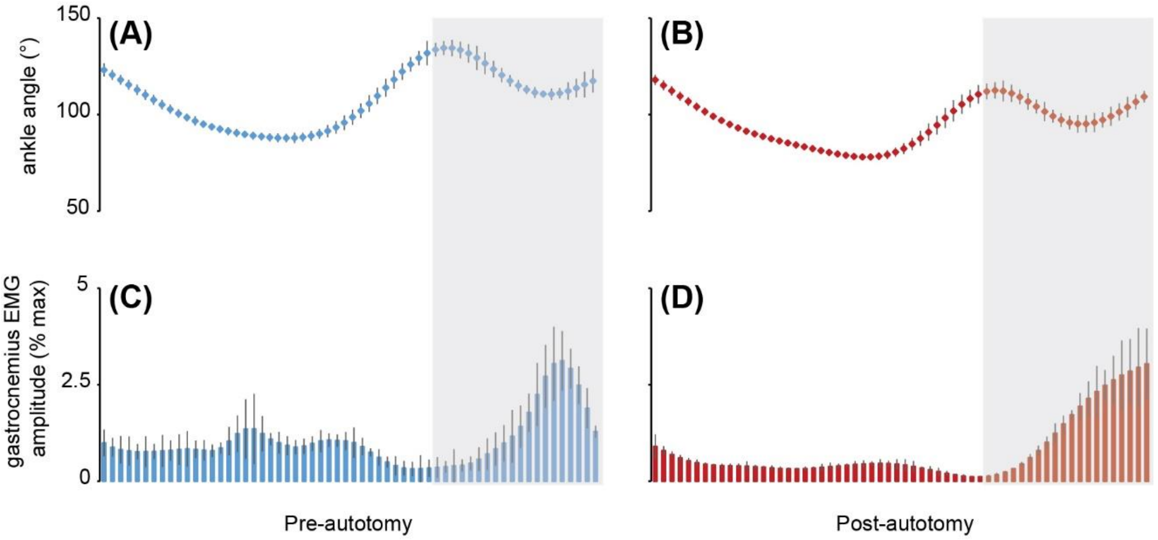

Figure 15. Femur depression, knee angle, and PIT muscle activity before and after autotomy. Binned femur depression angle (A, B), binned knee angle (C, D), and binned EMG amplitude of the puboischiotibialis (E, F) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C, E) and post-autotomy (red; B, D, F) treatments. Values are means + residuals from ten individuals. Error bars are s.e.m.