

## RESEARCH ARTICLE

# Tail autotomy and subsequent regeneration alter the mechanics of locomotion in lizards

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## ABSTRACT

Animals can undergo significant weight change for a variety of reasons. Autotomy, the voluntary shedding of an appendage in response to a predator stimulus, provides an effective model for measuring the effects of rapid weight change on locomotor behavior and the responses to more gradual weight gain, particularly in lizards capable of both autotomizing and regenerating their tail. Although the general effects of autotomy on locomotor performance are commonly explored, we 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 hindlimb joint angles signify a more sprawled posture following autotomy. Changes in hindlimb GRFs resulted in an increase in weight-specific propulsive force, without a corresponding change in locomotor speed. Hindlimb 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 variations in morphology, kinematics and force.

**KEY WORDS:** Tail autotomy, Regeneration, Biomechanics, Locomotion, Center of mass, Kinematics, Ground reaction force

## INTRODUCTION

Many animals encounter episodes of substantial weight change throughout their lifetime as a result of famine, obesity, illness, pregnancy and even limb or 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 weight loss or gain. An assessment of the effects of rapid weight 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 (Parry, 1957; Amaya et al., 2001), echinoderms (Emson and Wilkie, 1980), salamanders (Wake and Dresner, 1967), lizards (Congdon et al., 1974; Daniels, 1983; Arnold, 1984; Cooper and Vitt, 1985; Bateman and Fleming, 2009) and even some rodents (Sumner and Collins, 1918; Layne, 1972). 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 (Vitt et al., 1977; Dial and Fitzpatrick, 1983), 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 (Bustard, 1967; Avery, 1970; Congdon et al., 1974), sexual display and reproduction (Smyth, 1974; Vitt et al., 1977). Additionally, the tail can be essential for normal patterns of locomotion and balance (Etheridge, 1967; Daniels, 1983) and thus its loss may markedly affect locomotor behavior and performance (Vitt et al., 1977; Daniels, 1983; Bateman and Fleming, 2009; McElroy and Bergmann, 2013).

It is clear from recent work that the lizard tail is important in a number of locomotor behaviors. Jusufi et al. (Jusufi et al., 2008) showed that the tail functions as an emergency fifth leg and can be used to recover from slips while ascending a vertical surface; they also found that autotomy increases the likelihood of falling. Gillis et al. (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 because of 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 versus tail-less) 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 weight and shift in center of mass (CoM) associated with tail autotomy is crucial to link the radical change in morphology with measured performance

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**List of abbreviations**

CoM	center of mass
GRF	ground reaction force
SVL	snout–vent length

differences. Furthermore, studies considering the time course of locomotor changes associated with regenerating the tail following autotomy are rare (Downes and Shine, 2001; Chapple and Swain, 2002).

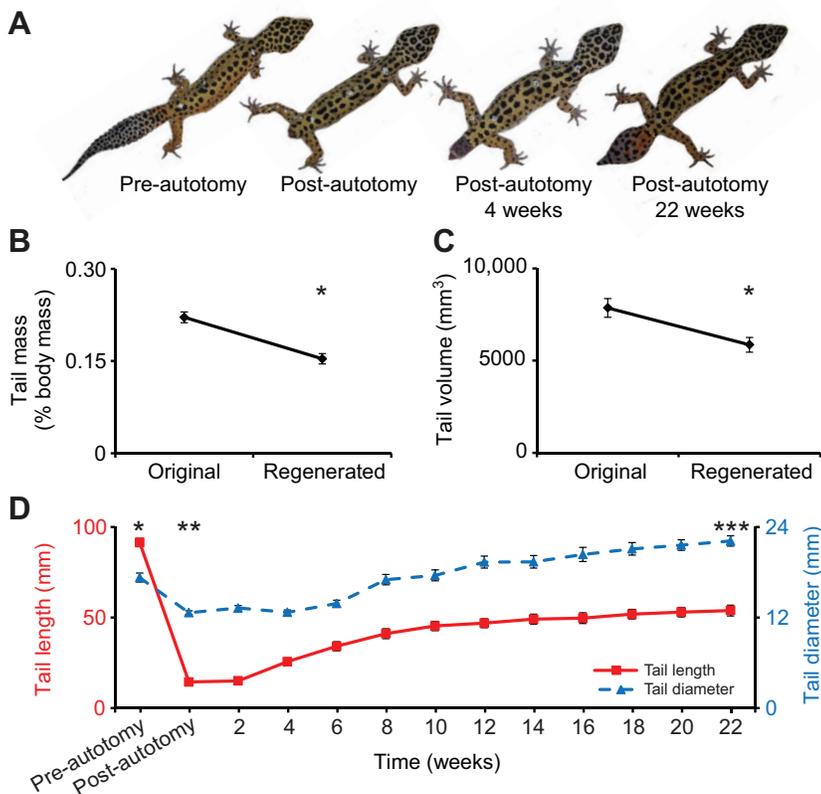
We investigated how locomotor mechanics are affected by the rapid loss and gradual recovery of weight associated with tail autotomy and regeneration in the leopard gecko *Eublepharis macularius* Blyth 1854. The tail of this desert-dwelling species is one of the largest (relative to body size) among geckos, 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). Because losing a relatively large tail is likely to elicit a greater change in locomotion (McElroy and Bergmann, 2013), this species is well-suited for examining 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 (Higham and Russell, 2010; McLean and Vickaryous, 2011; Delorme et al., 2012; Higham and Russell, 2012; Lynn et al., 2013).

To examine the effects of autotomy and regeneration on locomotor mechanics, we addressed three questions: (1) how do tail loss and regeneration affect the lizard's CoM? We hypothesize that the removal of substantial caudal weight as a result of autotomy will shift the CoM anteriorly and regeneration of the tail will return the CoM to its original position. (2) How does removal of the tail and the associated shift in CoM affect locomotor mechanics while running on a level surface? We hypothesize that a large tail weighs down the posterior end of the trunk and that removal of this weight

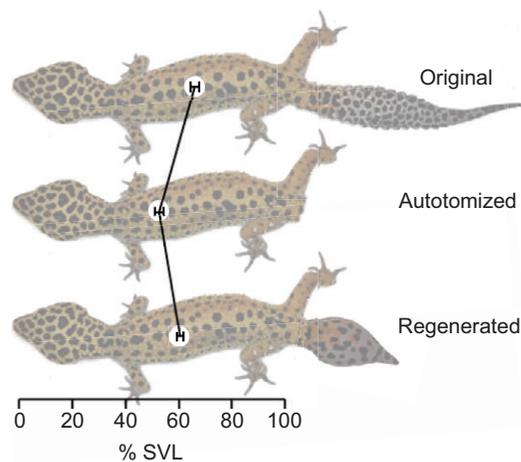
will result in the rising of the posterior end. Therefore, we anticipate an increase in hip height following autotomy, resulting in passive changes in forelimb and hindlimb joint kinematics. Additionally, propulsive hindlimb ground reaction forces (GRFs) are expected to decrease proportionally with the loss of mass. (3) As the tail is regenerated, do joint kinematics and propulsive hindlimb GRFs change continually and ultimately return to the original state? After a shift in kinematics and GRFs immediately following autotomy, we 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.

**RESULTS**

Body weight, tail length and tail diameter of leopard geckos after autotomy ceased showing significant changes after 18 weeks (*post hoc* comparison of weeks 18 and 20: body weight,  $t=-0.842$ , 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 weight (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 weight,  $t=-2.238$ , 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 (Fig. 1). Paired *t*-tests indicated that regenerated tails were significantly smaller in both mass (original, 22% body mass; regenerated, 15% body mass) and volume (original,  $7.8 \times 10^3 \text{ mm}^3$ ; regenerated,  $5.8 \times 10^3 \text{ mm}^3$ ) 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



**Fig. 1. Changes in tail morphology with autotomy and regeneration in the leopard gecko (*Eublepharis macularius*).** (A) Stages of tail loss and re-growth in the leopard gecko. (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 represent s.e.m.



**Fig. 2. Location of CoM in lizards with original, autotomized and fully regenerated tails.** Error bars represent s.e.m. All three points are significantly different from one another (repeated measures ANOVA,  $P=0.017$ ).

morphology resulted in a significant anterior shift in location of the CoM from a mean of  $65.9 \pm 1.6\%$  SVL with the original tail to  $52.7 \pm 1.6\%$  SVL with no tail and finally, a posterior shift to  $60.5 \pm 1.3\%$  SVL with a fully regenerated tail (repeated measures ANOVA,  $F_{2,5}=10.174$ ,  $P=0.017$ ) (Fig. 2). A *post hoc* comparison indicated a significant difference in the location of the CoM 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 hindlimbs were not significantly impacted by autotomy and regeneration (Table 1). However, although forelimb joint kinematics were unaffected, changes in hindlimb joint angles were prominent (Fig. 3). 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' 2 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,  $P=0.672$ ) (Fig. 4A). 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,  $F_{2,5}=4.419$ ,  $P=0.078$ ) (Fig. 4B). 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,  $P=0.125$ ) (Fig. 4C).

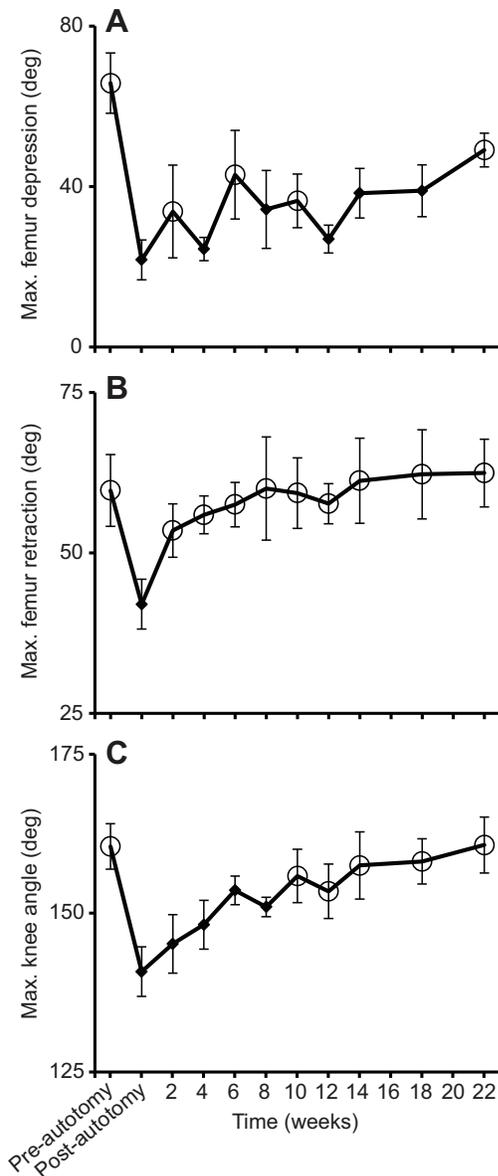
## DISCUSSION

Tail autotomy is a remarkable strategy for escaping predatory attacks. Although survival is likely enhanced, we present several biomechanical ramifications of the associated alteration of both body weight and relative location of the CoM. For this we 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 has 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 (Goss, 1969; McLean and Vickaryous, 2011; Delorme et al., 2012), disparities in size and shape are only briefly mentioned (Whimster, 1978; Lynn et al., 2013) and have not previously been quantified. Additionally, such studies have generally been conducted on juvenile animals and although ontogenetic differences in tail

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

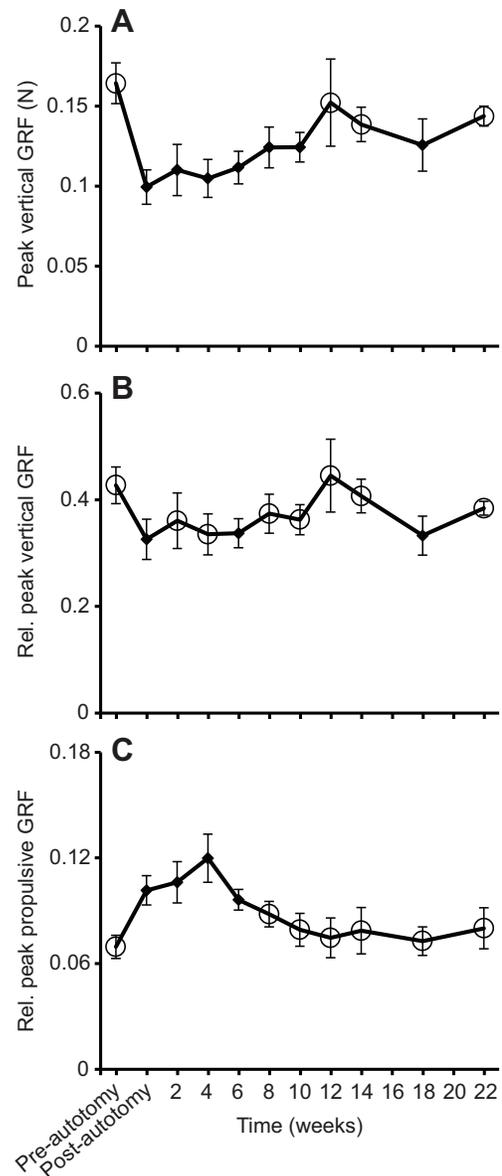
Variable	Pre-autotomy	Post-autotomy	22 weeks post-autotomy	F-ratio	P
Body pitch (deg)	$3.72 \pm 0.61$	$2.44 \pm 0.77$	$2.78 \pm 0.80$	0.867	0.475
Forelimb					
Stride length (SVL)	$0.70 \pm 0.00$	$0.70 \pm 0.00$	$0.70 \pm 0.00$	0.682	0.547
Stance time (s)	$0.33 \pm 0.04$	$0.28 \pm 0.02$	$0.35 \pm 0.02$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

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



**Fig. 3. Means of maximum hindlimb 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 represent 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,  $P > 0.05$ ).

regeneration are unknown, growth and maturity have been suggested to influence regeneration rates in geckos (Congdon et al., 1974; Vitt et al., 1977; Lynn et al., 2013). Here, we show that in mature adult female leopard geckos, fully regenerated tails are smaller in both weight and volume than the original tails and take on a new shape, which is shorter and wider than the original (Fig. 1). Interestingly, the regenerated tails of juveniles of the same species are not smaller in weight (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. We hypothesize that the tail does not return to its original shape because regenerating a replica of the original might not be essential for locomotion in *E. macularius*. In *Aspidoscelis sexlineatus*, the tail is



**Fig. 4. Means of peak GRFs from the propulsive half of stance phase.** (A) Peak vertical GRF. (B) Weight-specific peak vertical GRF. (C) Weight-specific peak propulsive GRF. Values for propulsive forces are means + residuals to remove the effect of speed. Error bars represent 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,  $P > 0.05$ ).

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 (Vitt et al., 1977; Daniels, 1983; Medger et al., 2008), 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 changes in CoM 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 weight (Irschick et al., 2003; Lind et al., 2010; Kuo et al., 2011). 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 hindlimbs) following autotomy, we expect 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). Our prediction that gecko 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 weight. This is supported by the decreases in femoral depression, retraction and knee angles (Fig. 3) observed immediately following autotomy, all of which are indicative of the animal adopting a more sprawled posture (Fuller et al., 2011). Because the hindlimbs are perceived to be the primary generators of locomotor thrust (Russell and Bels, 2001; Chen et al., 2006), it is likely that a locomotor response to autotomy and weight loss would occur here. Thus, the changes in hindlimb kinematics are not surprising. We hypothesize that this sprawled posture may be a means of enhancing stability and balance by lowering the CoM (Rewcastle, 1981; Foster and Higham, 2012) as the animal adjusts to the change in weight distribution during its first steps after losing its tail. Interestingly, each of these hindlimb kinematic parameters begins to return to the pre-autotomy state by the next trial (2 weeks after autotomy) and values are statistically indistinguishable from those 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 weeks 6–8 (Fig. 1D). Thus, it is likely that the increase in tail mass throughout regeneration is not linear, resulting in a non-linear response in hindlimb kinematics. Additionally, frequent observations of hindlimb kinematics over the days, or even hours and minutes following autotomy would likely reveal the amount of time or number of strides necessary for the animal to begin 'recovering'.

Furthermore, the changes we observed in the location of CoM are thought to have significant effects on hindlimb 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 (Fig. 4A). Although this relationship vanished when peak vertical GRF was corrected for weight, the hindlimbs were still found to produce significantly less weight-specific vertical force after autotomy (Fig. 4B). 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 hindlimbs, 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 hindlimbs can apply a greater propulsive force to the substrate during locomotion (Snyder, 1949). Thus, by shifting the CoM anteriorly, tail autotomy should decrease hindlimb propulsive force, which would, in turn, reduce sprint speed (Ballinger et al., 1979; Punzo, 1982). However, we did not find any changes in speed following autotomy and perhaps more interestingly, our findings show that weight-specific hindlimb propulsive GRFs follow the opposite of the expected trend (Fig. 4C). Because the hindlimbs are actually producing more propulsive force without an increase in speed, we 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 hindlimbs. However, data on the forelimb GRFs are needed to support this hypothesis.

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. (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 our 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 our decrease in mass, especially because tail autotomy in leopard geckos results in a greater than 10% reduction in body mass. This may explain why we found differences in kinematics between the conditions. Addition of 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 allowed us to assess GRFs over a period of time – something that is rarely (if ever) done in studies that manipulate the location of the CoM by adding mass.

Morphological variation has been proposed to drive kinematic variation, which then drives force variation in terrestrial running (McElroy and Reilly, 2009). Our findings provide support for this and form a baseline for future work in determining the effects of rapid weight 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 weight associated with autotomy and regeneration are available. Such observations will provide insight into how lizards 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 a non-autotomizing species to determine whether there is a lack of compensation as a result of the lack of a post-autotomic period for selection to act on.

Rapid weight 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 gravity 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 weight loss. Autotomy and regeneration studies conducted on lizards allow for precise manipulations of weight change and mass distribution without detrimental effects to the study organisms.

## 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 (l×w×h) 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 hindlimbs. 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<sup>-1</sup>). All sacrificed animals and autotomized tails were frozen for later use. All animal research was conducted in accordance with the University of California, Riverside Animal Care and Use Protocols (A-20110025 and A-20110038).

### Measurement of 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 weight, 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 weight 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 ~30°C when filming. Following the pre-autotomy trials, autotomy was induced and each animal was allotted 20–30 min to rest in a cloth bag with restricted movement. This ensured that the post-autotomy trial was representative of the first strides an animal made 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 2 week intervals until week 14 and 4 week intervals thereafter, until regeneration ceased at 22 weeks, yielding a total of 11 trials.

### Stride kinematics

Lizards were filmed at 250 frames s<sup>-1</sup> 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 deg) 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 hindlimb 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). We 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).

### Hindlimb locomotor forces

Ground reaction forces 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 (0.10×0.05 m) flush with the latter. This allowed ample room for the gecko to move over the force plate with only one hindlimb making contact. Only runs in which the gecko stepped completely onto the force platform with one hindlimb (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 ground reaction forces were sampled at 5000 Hz using AcqKnowledge 4.0.0 software (BIOPAC Systems, University of Cambridge, UK). Peak vertical and peak propulsive GRFs were calculated from the accelerative phase of each hindlimb 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 whereas 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 pair-wise 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.

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### Competing interests

The authors declare no competing financial interests.

### Author contributions

K.J. made substantial contributions to the design and execution of the experiments, interpretation of the findings and drafting and revision of the manuscript. T.E.H. assisted with the design and execution of the experiments, and T.E.H. and A.P.R. were involved in the conception of the study, interpretation of the findings and revising the manuscript.

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## References

- Alexander, R. M. (1983). *Animal Mechanics*. Oxford: Blackwell Scientific Publication.
- Amaya, C. C., Klawinski, P. D. and Formanowicz, D. R. (2001). The effects of leg autotomy on running speed and foraging ability in two species of wolf spider, (Lycosidae). *Am. Midl. Nat.* **145**, 201-205.
- Arnold, E. N. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *J. Nat. Hist.* **18**, 127-169.
- Avery, R. A. (1970). Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. *Comp. Biochem. Physiol.* **37**, 119-121.
- Gillis, G. B., Kuo, C. Y. and Irschick, D. (2013). The impact of tail loss on stability during jumping in green anoles (*Anolis carolinensis*). *Physiol. Biochem. Zool.* **86**, 680-689.
- Ballinger, R. E., Nietfeldt, J. W. and Krupa, J. J. (1979). An experimental analysis of the role of the tail in attaining high running speed in *Cnemidophorus sexlineatus* (Reptilia: Squamata: Lacertilia). *Herpetologica* **35**, 114-116.
- Bateman, P. W. and Fleming, P. A. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *J. Zool.* **277**, 1-14.
- Bennett, D. B. (1973). The effect of limb loss and regeneration on the growth of the edible crab, *Cancer pagurus*, L. *J. Exp. Mar. Biol. Ecol.* **13**, 45-53.
- Bustard, H. R. (1967). Geckonid lizards adapt fat storage to desert environments. *Science* **158**, 1197-1198.
- Chapple, D. G. and Swain, R. (2002). Effect of caudal autotomy on locomotor performance in a viviparous skink, *Niveoscincus metallicus*. *Funct. Ecol.* **16**, 817-825.
- Chen, J. J., Peattie, A. M., Autumn, K. and Full, R. J. (2006). Differential leg function in a sprawled-posture quadrupedal trotter. *J. Exp. Biol.* **209**, 249-259.
- Congdon, J. D., Vitt, L. J. and King, W. W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science* **184**, 1379-1380.
- Cooper, W. E. and Vitt, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Z. Tierpsychol.* **70**, 265-276.
- Daniels, C. B. (1983). Running – an escape strategy enhanced by autotomy. *Herpetologica* **39**, 162-165.
- Delorme, S. L., Lungu, I. M. and Vickaryous, M. K. (2012). Scar-free wound healing and regeneration following tail loss in the leopard gecko, *Eublepharis macularius*. *Anat. Rec.* **295**, 1575-1595.
- Dial, B. E. and Fitzpatrick, L. C. (1983). Lizard tail autotomy: function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science* **219**, 391-393.
- Downes, S. and Shine, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* **82**, 1293-1303.
- Emson, R. H. and Wilkie, I. C. (1980). Fission and autotomy in echinoderms. *Oceanogr. Mar. Biol.* **18**, 155-250.
- Etheridge, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**, 699-721.
- Finkler, M. S., Sugalski, M. T. and Claussen, D. L. (2003). Sex-related differences in metabolic rate and locomotor performance in breeding spotted salamanders (*Ambystoma maculatum*). *Copeia* **2003**, 887-893.
- Fleming, P. A., Muller, D. and Bateman, P. W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biol. Rev. Camb. Philos. Soc.* **82**, 481-510.
- Foster, K. L. and Higham, T. E. (2012). How forelimb and hindlimb function changes with incline and perch diameter in the green anole, *Anolis carolinensis*. *J. Exp. Biol.* **215**, 2288-2300.
- Fuller, P. O., Higham, T. E. and Clark, A. J. (2011). Posture, speed, and habitat structure: three-dimensional hindlimb kinematics of two species of padless geckos. *Zoology* **114**, 104-112.
- Gillis, G. B., Bonvini, L. A. and Irschick, D. J. (2009). Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis*. *J. Exp. Biol.* **212**, 604-609.
- Goss, R. J. (1969). Heads and tails. In *Principles of Regeneration*, pp. 191-222. New York, NY: Academic Press.
- Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* **3**, 034001.
- Higham, T. E. and Russell, A. P. (2010). Flip, flop and fly: modulated motor control and highly variable movement patterns of autotomized gecko tails. *Biol. Lett.* **6**, 70-73.
- Higham, T. E. and Russell, A. P. (2012). Time-varying motor control of autotomized leopard gecko tails: multiple inputs and behavioral modulation. *J. Exp. Biol.* **215**, 435-441.
- Higham, T. E., Russell, A. P. and Zani, P. A. (2013). Integrative biology of tail autotomy in lizards. *Physiol. Biochem. Zool.* **86**, 603-610.
- Irschick, D. J., Vanhooydonck, B., Herrel, A. and Andronescu, A. (2003). Effects of loading and size on maximum power output and gait characteristics in geckos. *J. Exp. Biol.* **206**, 3923-3934.
- Jusuifi, A., Goldman, D. I., Revzen, S. and Full, R. J. (2008). Active tails enhance arboreal acrobatics in geckos. *Proc. Natl. Acad. Sci. USA* **105**, 4215-4219.
- Kelehear, C. and Webb, J. K. (2006). Effects of tail autotomy on anti-predator behavior and locomotor performance in a nocturnal gecko. *Copeia* **2006**, 803-809.
- Kuo, C. Y., Gillis, G. B. and Irschick, D. J. (2011). Loading effects on jump performance in green anole lizards, *Anolis carolinensis*. *J. Exp. Biol.* **214**, 2073-2079.
- Langkilde, T. and Shine, R. (2006). How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. *J. Exp. Biol.* **209**, 1035-1043.
- Layne, J. N. (1972). Tail autotomy in the Florida mouse, *Peromyscus floridanus*. *J. Mammal.* **53**, 62-71.
- Lee, S. J., Witter, M. S., Cuthill, I. C. and Goldsmith, A. R. (1996). Reduction in escape performance as a cost of reproduction in gravid starlings, *Sturnus vulgaris*. *Proc. Biol. Sci.* **263**, 619-623.
- Lee, D. V., Stakebake, E. F., Walter, R. M. and Carrier, D. R. (2004). Effects of mass distribution on the mechanics of level trotting in dogs. *J. Exp. Biol.* **207**, 1715-1728.
- Lind, J., Jakobsson, S. and Kullberg, C. (2010). Impaired predator evasion in the life history of birds: behavioral and physiological adaptations to reduced flight ability. In *Current Ornithology*, Vol. 17 (ed. C. F. Thompson), pp. 1-30. New York, NY: Springer.
- Lynn, S. E., Borkovic, B. P. and Russell, A. P. (2013). Relative apportioning of resources to the body and regenerating tail in juvenile leopard geckos (*Eublepharis macularius*) maintained on different dietary rations. *Physiol. Biochem. Zool.* **86**, 659-668.
- McElroy, E. J. and Bergmann, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiol. Biochem. Zool.* **86**, 669-679.
- McElroy, E. J. and Reilly, S. M. (2009). The relationship between limb morphology, kinematics, and force during running: the evolution of locomotor dynamics in lizards. *Biol. J. Linn. Soc. Lond.* **97**, 634-651.
- McLean, K. E. and Vickaryous, M. K. (2011). A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius*. *BMC Dev. Biol.* **11**, 50.
- Medger, K., Verburt, L. and Bateman, P. W. (2008). The influence of tail autotomy on the escape response of the cape dwarf gecko, *Lygodactylus capensis*. *Ethology* **114**, 42-52.
- Parry, D. A. (1957). Spider leg-muscles and the autotomy mechanism. *Q. J. Microsc. Sci.* **98**, 331-340.
- Punzo, F. (1982). Tail autotomy and running speed in the lizards *Cophosaurus texanus* and *Uma notata*. *J. Herpetol.* **16**, 329-331.
- Rewcastle, S. C. (1981). Stance and gait in tetrapods: an evolutionary scenario. *Symp. Zool. Soc. Lond.* **48**, 239-267.
- Russell, A. P. and Bels, V. (2001). Biomechanics and kinematics of limb-based locomotion in lizards: review, synthesis and prospectus. *Comp. Biochem. Physiol.* **131A**, 89-112.
- Scales, J. and Butler, M. (2007). Are powerful females powerful enough? Acceleration in gravid green iguanas (*Iguana iguana*). *Integr. Comp. Biol.* **47**, 285-294.
- Shine, R. (1980). 'Costs' of reproduction in reptiles. *Oecologia* **46**, 92-100.
- Shine, R. (2003). Effects of pregnancy on locomotor performance: an experimental study on lizards. *Oecologia* **136**, 450-456.
- Smyth, M. (1974). Changes in the fat stores of the skinks *Morethia boulengeri* and *Hemiergis peronii* (Lacertilia). *Aust. J. Zool.* **22**, 135-145.
- Snyder, R. C. (1949). Bipedal locomotion of the lizard *Basiliscus basiliscus*. *Copeia* **1949**, 129-137.
- Sumner, F. B. and Collins, H. H. (1918). Autotomy of the tail in rodents. *Biol. Bull.* **34**, 1-6.
- Vitt, L. J., Congdon, J. D. and Dickson, N. A. (1977). Adaptive strategies and energetics of tail autotomy in lizards. *Ecology* **58**, 326-337.
- Wake, D. B. and Dresner, I. G. (1967). Functional morphology and evolution of tail autotomy in salamanders. *J. Morphol.* **122**, 265-305.
- Whimster, I. W. (1978). Nerve supply as a stimulator of the growth of tissues including skin. II. Animal evidence. *Clin. Exp. Dermatol.* **3**, 389-410.