Neuromuscular control of locomotion is altered by tail autotomy in geckos

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List of Symbols and Abbreviations:

CoM	center of mass
EMG	electromyography
GRF	ground-reaction force
RIA	rectified integrated area

SUMMARY STATEMENT

Motor control of locomotor muscles in geckos is altered in response to altered demands that occur with tail autotomy, demonstrating the complex relationship between muscle function and locomotion.

ABSTRACT

Animal locomotion is driven by underlying axial and appendicular musculature. In order for locomotion to be effective, these muscles must be able to rapidly respond to changes in environmental and physiological demands. Although virtually unstudied, muscles must also respond to morphological changes, such as those that occur with tail autotomy in lizards. Tail autotomy in leopard geckos (*Eublepharis macularius*) results in a 25% loss of caudal mass and significant kinematic alterations to maintain stability. To elucidate how motor control of the locomotor muscles is modulated with these shifts, we used electromyography (EMG) to quantify patterns of *in vivo* muscle activity in fore- and hind limb muscles before and after autotomy. Forelimb muscles (biceps brachii and triceps brachii) 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 significant neuromuscular shifts that occur to accommodate dramatic changes in body size and mass distribution, and illuminate the utility of tail autotomy as a system for studying the neuromuscular control of locomotion.

INTRODUCTION

Locomotion is fundamental to animal survival, where it is critical for prey capture and predator evasion. The ability to successfully accomplish these tasks often requires flexible locomotor strategies as animals encounter environmental and physiological demands (Irschick and Garland, 2001). Although strategies for meeting these demands can often be visibly observed through analyses of animal behavior and locomotor kinematics (Russel and Bels, 2001), it is the underlying muscles of the body and limbs that actuate these movements by driving propulsion, 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 (Irschick and Jayne, 1998; Daley and Biewener, 2003), thus requiring an adjustment in the level of muscle activity of hip extensors and femur retractors to function effectively (Pierotti et al., 1989; Carlson-Kuhta et al., 1998; Gillis and Biewener, 2002; Higham and Jayne, 2004a; 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 (Marigold and Patla, 2002; Daley et al., 2009; Daley and Biewener, 2011).

Although the response of locomotor muscle activity and recruitment are well-described in regard to environmental demands, how muscle function can rapidly adapt to alterations in body form, such as changes in mass, is relatively unexplored (Gillis and Biewener, 2003; Gillis and Higham, 2016; Jagnandan and Higham, 2018). Muscles play a pivotal role in the movement and support of body mass (Marsh, 1999), yet few data exist on how limb muscles adapt to changes in loading. The neuromuscular changes that occur with changes in mass have been briefly addressed in animals and humans 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; Griffin et al., 2003; McGowan et al., 2006; McGowan et al., 2009). Although useful for understanding the adaptability of muscle, artificial loading experiments represent unnatural conditions that may limit ecological relevance.

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; Kuo et al., 2012; Gillis et al., 2013), 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 the role of the tail in steady locomotion (Vitt et al., 1977; McElroy and Bergmann, 2013). 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; Jagnandan and Higham, 2017). Autotomy in this species induces a significant anterior shift in the center of mass (CoM) (Jagnandan et al., 2014; Jagnandan and Higham, 2017). 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.

Given the rapid loss of considerable mass, shift in the CoM, and postural changes observed after autotomy in *E. macularius*, we examined the modulation of locomotor muscle activity using electromyography (EMG) in this species to determine if and how the limb muscles are recruited differently after tail loss. We hypothesize that geckos must quickly alter motor output and activation of the limb muscles to compensate for the altered mass and CoM displacement. Specifically, we predict 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, we expect 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, we predict 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 (Fig. 1). The biceps brachii and triceps brachii 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 (linked to retraction of the femur), the puboischiotibialis (linked to depression of the femur and flexion of the knee), and the gastrocnemius (linked to extension of the ankle).

Implantation of the EMG electrodes into each of these muscles followed an established surgical procedure (Higham and Jayne, 2004a). 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 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 voluntarily walked on a level trackway (1.0×0.13 m) with sandpaper substrate to prevent slipping. Geckos were not coaxed to run down the trackway in order to avoid interference with the implanted EMG electrodes. 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 (Appendix I). One forelimb and hind limb stride was obtained from each trial and was representative of an individual moving at a relatively constant speed, at least two strides after the initial acceleration. 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. All animal research was conducted in accordance with the University of California, Riverside Animal Care and Use Protocols (A-20110025 and A-20110038).

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 samples 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 was 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 was used to generate x, y, and z coordinates for digitizing. Points marked on the animals were digitized using DLTdv5 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. Body speed was calculated using the marker on the midpoint of the back. 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. (Paired *t*-tests were also used to compare pre- and post-surgery trials for five individuals). Assumptions for normality and equal variances were not violated for any of the variables measured based on Shapiro-Wilk tests and Levene's tests, respectively. 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; Jagnandan and Higham, 2017). Despite moving at significantly slower speeds than individuals of the same species in previous studies (Fuller et al., 2011; Jagnandan et al., 2014; Jagnandan and Higham, 2017), 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 (Fig. 2A-B), while significant decreases in femur retraction (Fig. 3A-B), ankle angle (Fig. 4A-B), femur depression (Fig. 5A-B), and knee angle (Fig. 5C-D), were observed after the tail was removed.

Timing, not necessarily the level, of muscle activity was consistent with hypothesized functions of the muscles in relation to movement at the joints (Zaaf et al., 1999) (Fig. 2-5). The biceps brachii exhibited two bursts of activity during the stride (Fig. 2C-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 brachii 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 (Fig. 2E-F). However, the burst in late swing was relatively lower in magnitude than the stance bursts.

The caudofemoralis was characterized by a single prolonged burst of activity beginning at or just before footfall that persisted throughout the entire stance phase (Fig. 3C-D). Higher amplitudes were generally observed near the beginning of the burst, as indicated by a relatively early time of half burst RIA. Caudofemoralis activity occurred as the femur was retracted throughout stance (Fig. 3). The gastrocnemius remained active throughout much of stance as well, but was also characterized by a high-amplitude burst near the end of swing phase (Fig. 4C-D). The ankle was extended by the gastrocnemius in the second half of stance and again near the end of swing (Fig. 4A-B). Activity of the puboischiotibialis 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 (Fig. 5).

Statistical differences in the effects of the removal of the tail via autotomy are summarized in Tables 1-3. Amplitude and timing variables of the forelimb muscles were not significantly affected by the anteriorly shifted CoM due to autotomy. In contrast, the caudofemoralis incurred a significant reduction in maximum amplitude and stance RIA after the tail was removed. A significant decrease in amplitude variables was also evident in the first burst of activity (during stance) in the gastrocnemius. 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 puboischiotibialis were observed.

DISCUSSION

The impact of tail autotomy on muscle activation

Tail autotomy in lizards results 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). *In vivo* muscle activity in *E. macularius* revealed differential impacts of autotomy on locomotor muscles throughout the fore- and hind limbs. Specifically, we 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; Reilly et al., 2005; Higham and Biewener, 2008; 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 expected. 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, we found that motor control patterns of the biceps brachii and triceps brachii were not decoupled from forelimb kinematics in our 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 caudofemoralis is a propulsive muscle of particular interest in regard to autotomy because of its critical role in retracting the femur (Snyder, 1952; Snyder, 1954; Snyder; 1962; Russell and Bauer, 1992; Reilly, 1994; Irschick and Jayne, 1999; Nelson and Jayne, 2001) 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 caudofemoralis, so the muscle remains intact after removal of the tail (Russell and Bauer, 1992). However, our results suggest that its functional role in locomotion may be altered. A notable decrease in the amplitude of activation of the caudofemoralis is present throughout stance phase after autotomy (Fig. 3C-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. We 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 caudofemoralis burst is absent in autotomized geckos.

Additionally, the gastrocnemius exhibits a reduced amplitude and RIA of muscle activity (Tables 1, 3) that is consistent with a reduction in ankle extension in the second half of stance (Fig. 4). This again suggests that with the loss of mass via autotomy, less force is needed to propel the gecko 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 gastrocnemius 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 hindlimb is off the ground is unaltered. Moreover, the high amplitude of this second burst suggests a functional role besides extension of the ankle. We hypothesize that the activity

of the gastrocnemius 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 (Gillis et al., 2010; Ekstrom and Gillis, 2015).

The puboischiotibialis 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,b; 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. Whether the lack of change in muscle activity caused an active change in kinematics, or whether the change in kinematics was a passive result of the inability of the muscle to be modulated, is not currently understood. A future study could artificially impose loads on the geckos in order to determine the extent to which this muscle can be modulated. If it cannot be modulated, then an increased external load will induce a postural shift. If the load is resisted, then the muscle should exhibit a shift in activation pattern without a change in posture.

Comparisons to other lizards

Several studies have examined the activity of limb muscles during lizard locomotion, although they are on fairly distantly related species including *Chamaeleo calyptratus* (Higham and Jayne 2004b), *Anolis carolinensis* (Foster and Higham 2014), *Dipsosaurus dorsalis* (Nelson and Jayne 2001), *Sceloporus clarkii* (Reilly 1995), and *Varanus exanthematicus* (Jayne et al. 1990; Jenkins and Goslow 1983). Additionally, there is considerable variation in the specific muscles examined, often limited to the hind limb (Higham and Jayne 2004b, Reilly 1995, Nelson and Jayne 2001, but see Jenkins and Goslow, 1983).

Two studies have examined the activation patterns of the gastrocnemius in lizards (chameleons: Higham and Jayne 2004b; fence lizards: Reilly 1995). Both studies found that the gastrocnemius was active predominantly throughout the first half of the stance phase, with some activation occurring late in swing phase. Our results are strikingly different from these, with most of the activation occurring in swing. It is unclear what role this suggests, but this could imply that the gastrocnemius simply is not recruited as much during stance to propel the relatively slow-moving leopard geckos. Although chameleons are also quite slow, they are arboreal and may exhibit muscle recruitment patterns related to the specialized grasping and propulsive mechanics (Higham and Jayne 2004a; Higham and Anderson 2013; Fischer et al. 2010; Krause and Fischer 2013; Peterson 1984). Future work should investigate the activation patterns of the gastrocnemius in other geckos, including arboreal and terrestrial species.

The activation patterns of the caudofemoralis have also been examined in several species of lizard, including *A. carolinensis* (Foster and Higham 2014), *C. calyptratus* (Higham and Jayne 2004b), *D. dorsalis* (Nelson and Jayne 2001), and *S. clarkii* (Reilly 1995). Although most of these studies manipulated factors that were not considered in our study (e.g. perch diameter, perch incline, locomotor speed), a general pattern emerges. Caudofemoralis activity begins either in late swing or early stance and is predominantly active during the first half of stance in order to retract the femur. We found the same result in our pre-autotomy trials with leopard geckos, highlighting the conserved activation of this muscle.

Activation patterns of the puboischiotibialis, a knee flexor and femur depressor, have been examined in *C. calyptratus* (Higham and Jayne 2004b) and *A. carolinensis* (Foster and Higham 2014). In most cases, there were two bursts of activity in this muscle - one in early stance and one in early swing. However, this pattern varies among treatments in *A. carolinensis* (Foster and Higham 2014). Our results are consistent with this double burst activation pattern, with a burst typically occurring in early stance and early swing.

The forelimb muscles that we examined (the biceps brachii and triceps brachii) have been examined in *V. exanthematicus* (Jenkins and Goslow 1983). For the biceps brachii of varanids, activity began in late swing but was primarily restricted to the stance phase. However, activity of

both muscles was fairly sporadic throughout the stance phase of the stride (Jenkins and Goslow 1983). We found similar results for the biceps brachii in leopard geckos, with a burst beginning late in swing and continuing into the stance phase, followed by another burst near the end of stance. The triceps of varanids exhibits an isolated burst of activity during swing and another burst within stance. We did not consistently find an isolated burst of activity in swing, which may be related to the slower locomotor speeds of leopard geckos.

Future directions

Tail autotomy in lizards is an effective natural model for investigating the neuromuscular control of locomotion in response to altered demands. Our 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.

COMPETING INTERESTS

The authors declare no competing interests.

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Figures
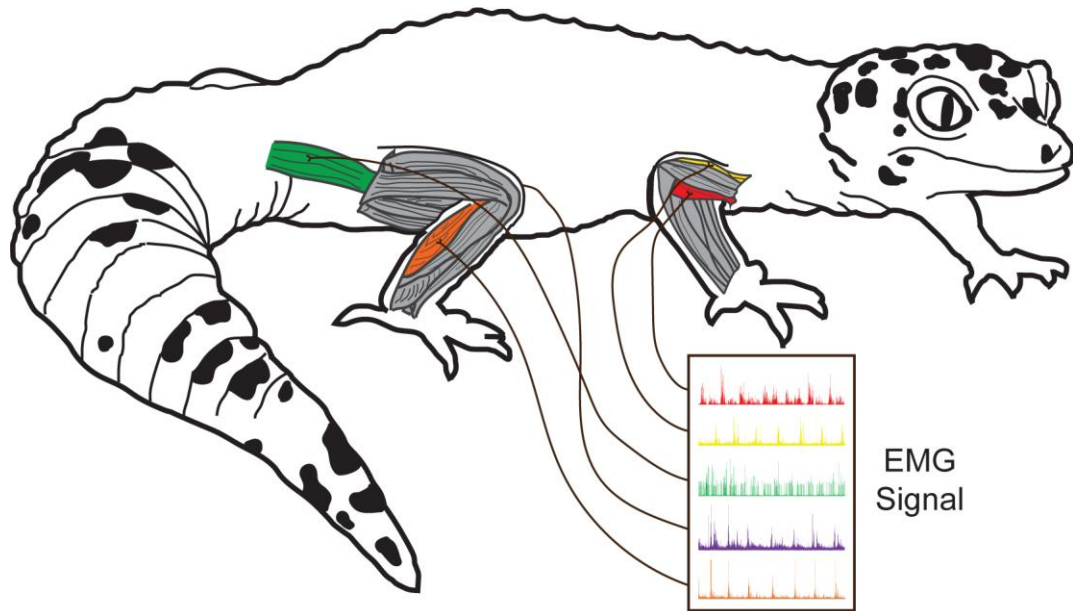
Fig. 1. Locations of muscles implanted in *Eublepharis macularius* with EMG electrodes.
EMG signals from top to bottom are biceps brachii (red), triceps brachii (yellow),
caudofemoralis (green), puboischiotibialis (located on ventral surface of proximal hind limb;
purple), gastrocnemius (orange).
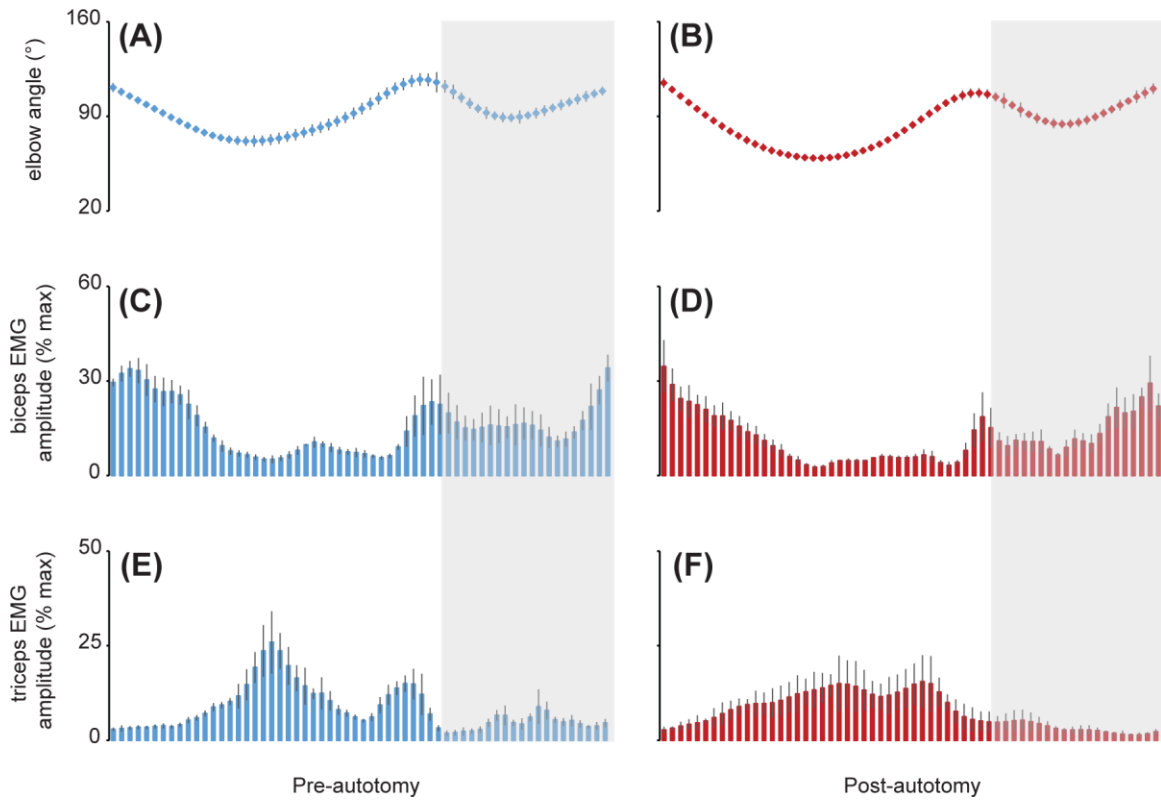
Fig. 2. Elbow angle and forelimb muscle activity before and after autotomy. Binned elbow angle (A, B) and binned EMG amplitude of the biceps brachii (C, D) and triceps brachii (E, F) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C, E) and post-autotomy (red; B, D, F) treatments. Values are means from ten individuals. Error bars are s.e.m.
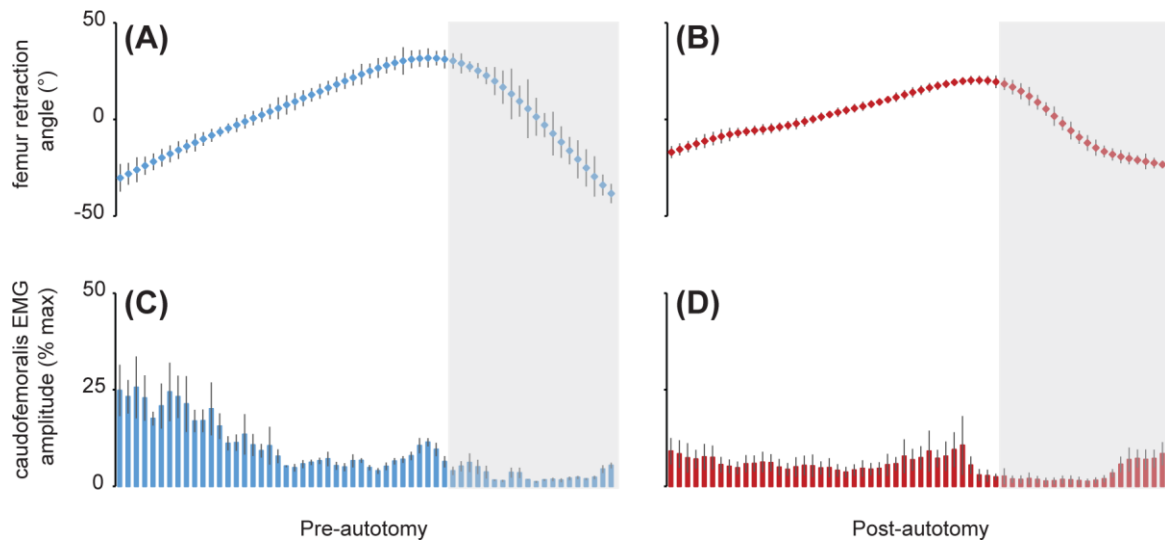
Fig. 3. Femur retraction angle and caudofemoralis muscle activity before and after autotomy. Binned femur retraction angle (A, B) and binned EMG amplitude of the caudofemoralis (C, D) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C) and post-autotomy (red; B, D) treatments. Values are means from ten individuals. Error bars are s.e.m.
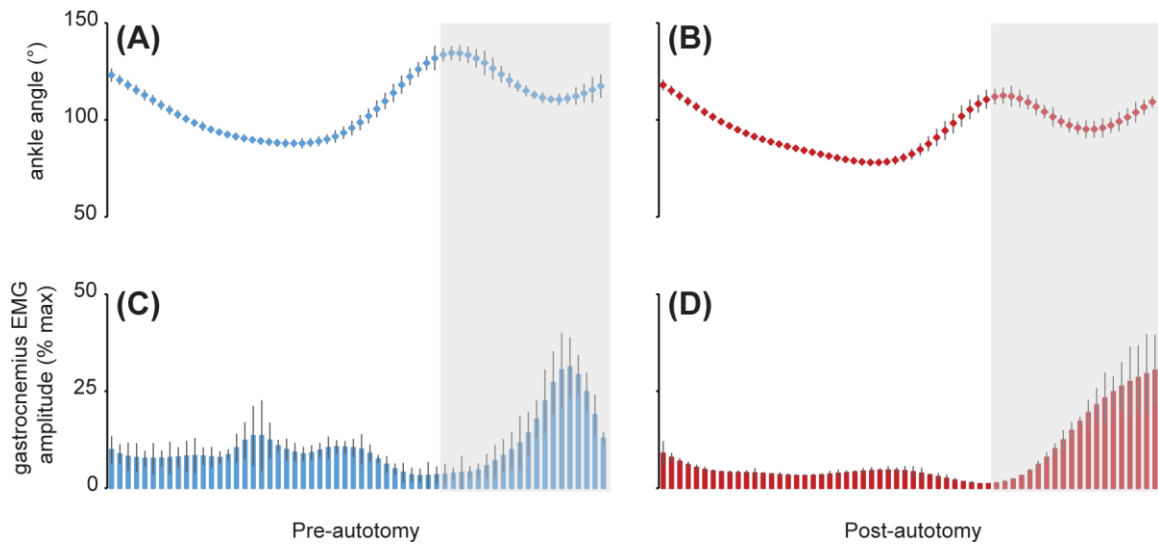
Fig. 4. Ankle angle and gastrocnemius muscle activity before and after autotomy. Binned ankle angle (A, B) and binned EMG amplitude of the gastrocnemius (C, D) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C) and post-autotomy (red; B, D) treatments. Values are means from ten individuals. Error bars are s.e.m.
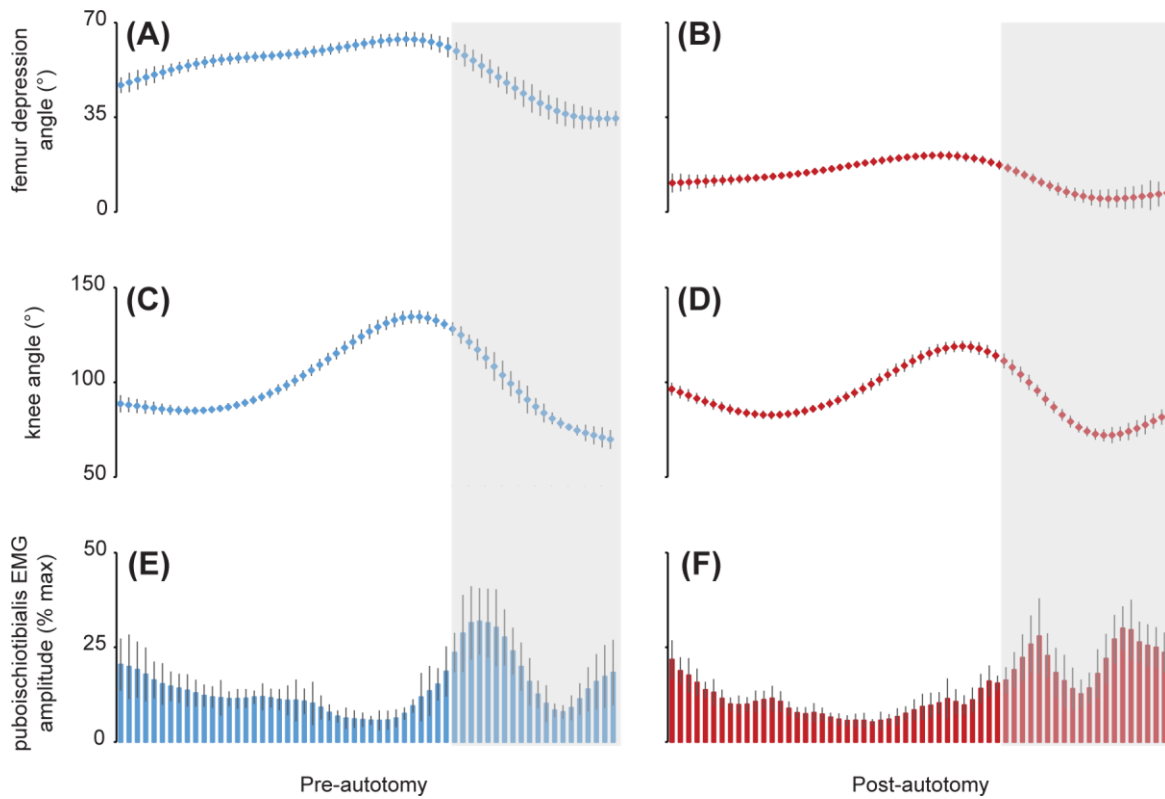
Fig. 5. Femur depression, knee angle, and puboischiotibialis muscle activity before and after autotomy. Binned femur depression angle (A, B), binned knee angle (C, D), and binned EMG amplitude of the puboischiotibialis (E, F) are displayed throughout stance (unshaded) and swing (shaded) phases. Data is shown for pre-autotomy (blue; A, C, E) and post-autotomy (red; B, D, F) treatments. Values are means from ten individuals. Error bars are s.e.m.

Table 1. 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
Caudofemoralis	74.85 ± 4.58	30.85 ± 9.58	6.450	**< 0.001**
Gastrocnemius	72.22 ± 3.43	57.79 ± 3.68	8.281	**< 0.001**
Puboischiotibialis	53.43 ± 7.02	48.89 ± 8.93	0.180	0.861

Means (± 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 2. 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 (± 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 3. 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*
Caudo-femoralis	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
Gastroc-nemius	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
Puboischio-tibialis	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 (± 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.

Appendix I. Trials completed for each individual used in this study

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
1	Pre-Autotomy	1	3.660	0.332
		2	3.311	0.301
		3	3.171	0.288
		4	3.428	0.311
		5	2.388	0.217
		6	2.929	0.266
	Post-Autotomy	1	3.928	0.357
		2	3.960	0.360
		3	3.525	0.320
		4	3.928	0.357
		5	3.960	0.360
		6	3.525	0.320
2	Pre-Autotomy	1	3.743	0.352
		2	2.950	0.277
		3	2.828	0.266
		4	2.628	0.247
		5	2.798	0.263
	Post-Autotomy	1	4.109	0.386
		2	3.742	0.352
		3	3.205	0.301
		4	3.504	0.330
		5	3.092	0.291
3	Pre-Autotomy	1	6.172	0.580
		2	6.207	0.583
		3	5.586	0.525
		4	5.150	0.484
		5	4.298	0.404
	Post-Autotomy	1	5.486	0.515
		2	5.419	0.509
		3	5.255	0.494
		4	5.503	0.517
		5	4.773	0.448

Appendix I. Trials completed for each individual used in this study (continued)

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
	Pre-Autotomy	1	2.612	0.225
		2	2.804	0.242
		3	2.231	0.192
		4	3.027	0.261
		5	3.064	0.264
		6	2.532	0.218
4				
	Post-Autotomy	1	3.134	0.270
		2	3.655	0.315
		3	3.933	0.339
		4	4.670	0.402
		5	4.360	0.376
		6	4.058	0.350
		7	2.282	0.197
	Pre-Autotomy	1	8.698	0.818
		2	8.256	0.776
		3	7.561	0.711
		4	7.712	0.725
		5	7.335	0.690
5				
	Post-Autotomy	1	5.107	0.480
		2	4.579	0.431
		3	5.082	0.478
		4	5.110	0.480
		5	5.029	0.473
		6	4.177	0.393
	Pre-Autotomy	1	3.000	0.261
		2	3.063	0.267
		3	3.148	0.274
		4	3.152	0.275
		5	2.910	0.253
6				
	Post-Autotomy	1	3.436	0.299
		2	3.633	0.316
		3	3.725	0.324
		4	3.499	0.305
		5	3.700	0.322

Appendix I. Trials completed for each individual used in this study (continued)

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
7	Pre-Autotomy	1	7.242	0.596
		2	7.184	0.591
		3	6.113	0.503
		4	5.248	0.432
		5	6.174	0.508
	Post-Autotomy	1	8.182	0.673
		2	8.008	0.659
		3	10.285	0.846
		4	8.748	0.720
		5	4.971	0.409
8	Pre-Autotomy	1	6.821	0.640
		2	5.249	0.493
		3	5.363	0.503
		4	4.211	0.395
		5	4.645	0.436
		6	3.693	0.347
	Post-Autotomy	1	3.219	0.302
		2	2.922	0.274
		3	2.932	0.275
		4	2.611	0.245
		5	2.255	0.212
9	Pre-Autotomy	1	4.157	0.386
		2	4.011	0.372
		3	3.389	0.314
		4	2.781	0.258
		5	3.007	0.279
		6	3.415	0.317
		7	3.468	0.322
	Post-Autotomy	1	3.003	0.279
		2	2.955	0.274
		3	2.230	0.207
		4	1.633	0.152
		5	2.197	0.204
		6	2.899	0.269
		7	3.250	0.302
		8	3.040	0.282

Appendix I. Trials completed for each individual used in this study (continued)

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
		1	6.714	0.613
		2	6.623	0.604
	Pre-Autotomy	3	5.936	0.541
		4	5.407	0.493
		5	5.861	0.535
		6	5.437	0.496
10				
		1	4.086	0.373
		2	4.016	0.366
		3	4.055	0.370
	Post-Autotomy	4	4.002	0.365
		5	4.176	0.381
		6	4.086	0.373
		7	4.585	0.418

Table S1. Trials completed for each individual used in this study

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
1	Pre-Autotomy	1	3.660	0.332
		2	3.311	0.301
		3	3.171	0.288
		4	3.428	0.311
		5	2.388	0.217
		6	2.929	0.266
	Post-Autotomy	1	3.928	0.357
		2	3.960	0.360
		3	3.525	0.320
		4	3.928	0.357
		5	3.960	0.360
		6	3.525	0.320
2	Pre-Autotomy	1	3.743	0.352
		2	2.950	0.277
		3	2.828	0.266
		4	2.628	0.247
		5	2.798	0.263
	Post-Autotomy	1	4.109	0.386
		2	3.742	0.352
		3	3.205	0.301
		4	3.504	0.330
		5	3.092	0.291
3	Pre-Autotomy	1	6.172	0.580
		2	6.207	0.583
		3	5.586	0.525
		4	5.150	0.484
		5	4.298	0.404
	Post-Autotomy	1	5.486	0.515
		2	5.419	0.509
		3	5.255	0.494
		4	5.503	0.517
		5	4.773	0.448

Table S1. Trials completed for each individual used in this study (continued)

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
4	Pre-Autotomy	1	2.612	0.225
		2	2.804	0.242
		3	2.231	0.192
		4	3.027	0.261
		5	3.064	0.264
		6	2.532	0.218
	Post-Autotomy	1	3.134	0.270
		2	3.655	0.315
		3	3.933	0.339
		4	4.670	0.402
		5	4.360	0.376
		6	4.058	0.350
		7	2.282	0.197
5	Pre-Autotomy	1	8.698	0.818
		2	8.256	0.776
		3	7.561	0.711
		4	7.712	0.725
		5	7.335	0.690
	Post-Autotomy	1	5.107	0.480
		2	4.579	0.431
		3	5.082	0.478
		4	5.110	0.480
		5	5.029	0.473
		6	4.177	0.393
6	Pre-Autotomy	1	3.000	0.261
		2	3.063	0.267
		3	3.148	0.274
		4	3.152	0.275
		5	2.910	0.253
	Post-Autotomy	1	3.436	0.299
		2	3.633	0.316
		3	3.725	0.324
		4	3.499	0.305
		5	3.700	0.322

Table S1. Trials completed for each individual used in this study (continued)

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
7	Pre-Autotomy	1	7.242	0.596
		2	7.184	0.591
		3	6.113	0.503
		4	5.248	0.432
		5	6.174	0.508
	Post-Autotomy	1	8.182	0.673
		2	8.008	0.659
		3	10.285	0.846
		4	8.748	0.720
		5	4.971	0.409
8	Pre-Autotomy	1	6.821	0.640
		2	5.249	0.493
		3	5.363	0.503
		4	4.211	0.395
		5	4.645	0.436
		6	3.693	0.347
	Post-Autotomy	1	3.219	0.302
		2	2.922	0.274
		3	2.932	0.275
		4	2.611	0.245
		5	2.255	0.212
9	Pre-Autotomy	1	4.157	0.386
		2	4.011	0.372
		3	3.389	0.314
		4	2.781	0.258
		5	3.007	0.279
		6	3.415	0.317
		7	3.468	0.322
	Post-Autotomy	1	3.003	0.279
		2	2.955	0.274
		3	2.230	0.207
		4	1.633	0.152
		5	2.197	0.204
		6	2.899	0.269
		7	3.250	0.302
		8	3.040	0.282

Table S1. Trials completed for each individual used in this study (continued)

Individual	Treatment	Trial	Speed (cm/s)	Speed (SVL/s)
		1	6.714	0.613
		2	6.623	0.604
	Pre-Autotomy	3	5.936	0.541
		4	5.407	0.493
		5	5.861	0.535
		6	5.437	0.496
10				
		1	4.086	0.373
		2	4.016	0.366
		3	4.055	0.370
	Post-Autotomy	4	4.002	0.365
		5	4.176	0.381
		6	4.086	0.373
		7	4.585	0.418