

Tail regeneration affects the digestive performance of a Mediterranean lizard

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Abstract In caudal autotomy, lizards shed their tail to escape from an attacking predator. Since the tail serves multiple functions, caudal regeneration is of pivotal importance. However, it is a demanding procedure that requires substantial energy and nutrients. Therefore, lizards have to increase energy income to fuel the extraordinary requirements of the regenerating tail. We presumed that autotomized lizards would adjust their digestion to acquire this additional energy. To clarify the effects of tail regeneration on digestion, we compared the digestive performance before autotomy, during regeneration, and after its completion. Tail regeneration indeed increased gut passage time but did not affect digestive performance in a uniform pattern: though protein income was maximized, lipid and sugar acquisition remained stable. This divergence in proteins may be attributed to their particular role in tail reconstruction, as they are the main building blocks for tissue formation.

Keywords Caudal autotomy · Reptiles · Digestion · Tail regeneration

Introduction

Caudal autotomy, the self-amputation of the tail, is an effective antipredator mechanism that is widespread among lizards (Bateman and Fleming 2009). In brief, the shed tail distracts the predator, allowing the lizard to escape (Arnold 1988). Nevertheless, the lizard tail is a multiuse organ that serves numerous roles (e.g., locomotion, balance, social and sexual interactions, energy storage; Maginnis 2006). Hence, it is imperative for lizards to regenerate their tail to restore their overall fitness rapidly (Bellairs and Bryant 1985). However, tail regeneration is an energy-consuming process (Naya and Božinović 2006) and lizards have to find a way to fuel the extra requirements.

Animals satisfy their energetic demands through the digestive system that enables the efficient absorption and assimilation of food nutrients. The efficacy of digestion determines the amount of energy an animal gains from food and that can be allocated to different biological processes (Karasov and Martinez Del Rio 2007). The reptilian digestive system is highly plastic: reptiles can radically alter their digestive efficiency by controlling the time food remains in the gut (Herrel et al. 2008; Sagonas et al. 2015), shifting digestive enzyme activity and concentration (Pinoni et al. 2011), or by adapting the length of the gastrointestinal tract (Vervust et al. 2010; Sagonas et al. 2015).

Though tail regeneration is known to affect many important biological functions such as immunity (Slos et al. 2009), reproduction (Fox and McCoy 2000), and locomotion (McElroy and Bergmann 2013), the associated energetic trade-offs and the underlying supporting mechanism (i.e.,

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chemical stimuli for tissue reconstruction, energy, and macromolecule flows) remain understudied. Here, we aimed to clarify the impact of tail regeneration on digestion in a Mediterranean lizard (*Podarcis erhardii*). To assess digestive performance, we used two measures: the gut passage time (GPT; the rate with which food passes through the body) and the apparent digestive efficiency (ADE; the relative percentage of ingested energy absorbed through the digestive tract) for proteins, lipids, and sugars. We measured these parameters at three different stages: prior to autotomy, during regeneration, and after the completion of tail restoration. We hypothesized that digestive performance would be higher during regeneration to cover the excessive need for macromolecules and energy. In particular, we expected that GPT would be prolonged to provide extra time to digestive enzymes. We also predicted that ADEs would be higher to fuel the reconstruction of caudal tissues.

Materials and methods

Study species

The Erhard's wall lizard (*P. erhardii*) is a small, diurnal insectivorous lacertid lizard (snout vent length, SVL 50–80 mm), occurring in the southern Balkans and in the majority of the Aegean islands. We worked with 47 adults (SVL 60.95 ± 0.92 mm, mass 5.78 ± 0.25 g), exclusively male individuals (to minimize possible sex effects) from Andros Island ($37^{\circ}51'$ N, $24^{\circ}51'$ E; Cyclades). Captured animals were transferred to the laboratory facilities of the University of Athens and held under controlled conditions (see [ESM](#)).

Gut passage time

Prior to the experiment, food was withheld from the lizards for 3 days, until no feces were found in the terraria. GPT was determined as the time a plastic marker, embedded in a mealworm, remained in the gastrointestinal tract from consumption to defecation (Van Damme et al. 1991). Terraria were inspected every hour for the appearance of the marker. GPT was measured at three independent stages: day 0 (before autotomy), day 15th (during regeneration), and day 90th (end of regeneration). Previous studies reported in detail the duration of regeneration for the focal species (Simou et al. 2008; Tsasi et al. 2009).

Apparent digestive efficiency

Digestive efficiency was estimated separately for each nutrient, namely proteins (ADE_P), lipids (ADE_L), and sugars (ADE_S) (Pafilis et al. 2007). To evaluate ADEs, lizards were fed every other day with identical "twin" mealworms

according to the methodology proposed by Pafilis et al. (2007) (see [ESM](#)). ADEs were measured thrice: before autotomy, during regeneration, and at the completion of tail reconstruction.

Tail autotomy and biochemical analysis

Predation was simulated in the laboratory using the method proposed by Pérez-Mellado et al. (1997) (see [ESM](#)). Shed tails were preserved in liquid N_2 immediately after autotomy. Protein, lipid, and glycogen (the storable form of sugars) concentrations in tail tissues were estimated following the same biochemical protocols used in ADE evaluation (see [ESM](#)).

Statistical analysis

We applied the Kolmogorov-Smirnov and Lilliefors tests and Levene test to examine the normality and heteroscedasticity of our data. Repeated measured analysis of variance (ANOVA) was used to compare ADEs and GPT among different time intervals. Because of the strong positive effects of GPT on digestive efficiency (assessed through regression analysis between GPT and ADEs for proteins, lipids, and sugars; all $P < 0.001$), we repeated the aforementioned analysis when significant differences were obtained, taking into account (as a covariance) the GPT of each individual (repeated measured analysis of covariance; ANCOVA). A paired t test was conducted to examine for differences between the intact and fully regenerated tails on protein, lipid, and glycogen concentrations. For multiple comparisons, we applied the Tukey honest significant difference (HSD) post hoc test. All analyses were performed in R 3.2.5 (R Development Core Team 2015).

Results

GPTs differed significantly among the three stages (before autotomy = 41 h, during regeneration = 49 h, completion of regeneration = 40 h; $F_{2,92} = 85.93$, $P < 0.001$). The Tukey HSD test revealed that lizards slow down food passage during regeneration by almost 22% compared to the stages before and after regeneration completion (Fig. 1 and Table 1).

ADE_L ($F_{2,92} = 2.17$, $P = 0.120$) and ADE_S ($F_{2,92} = 2.16$, $P = 0.122$) showed no differences among the three groups. On the other hand, lizards achieved higher values for ADE_P during tail regeneration than before autotomy and after regeneration ($F_{2,92} = 47.74$, $P < 0.001$) (Fig. 1 and Table 1). When GPT was used as covariate, the differences disappeared ($F_{2,90} = 0.46$, $P = 0.634$).

The comparison between the intact and fully regenerated tails revealed that the latter had higher protein ($t = -4.39$, $df = 74$, $P < 0.001$) and lipid ($t = -3.32$, $df = 74$, $P = 0.001$)

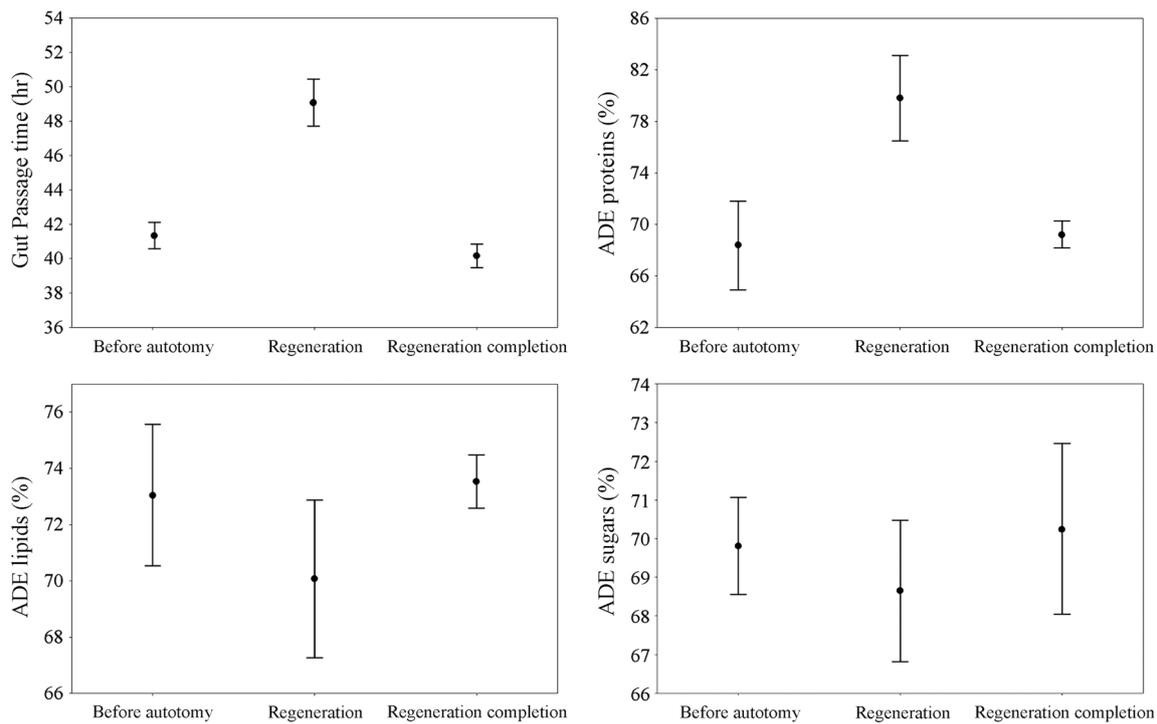


Fig. 1 Repeated measured ANOVA results for digestive performance traits: GPT and ADE for proteins, lipids, and sugars

but not glycogen ($t = -0.84, df = 30, P = 0.409$) levels (Table 1).

Discussion

Digestion is a dynamic process that responds readily to inner changes or external stimuli related to energy demands (Sagonas et al. 2015; Pafilis et al. 2016; Karameta et al. 2017). Our findings suggested that digestive performance is affected by tail regeneration as well. However, the nature of this relation was rather complex: whereas regeneration increased GPT and ADE_P, digestive efficiencies for lipids and sugars remained unchanged.

Tail regeneration is an energetically costly process requiring the tuning of many molecular, cellular, and physiological mechanisms (Alibardi 2010). This could deprive energy and nutrients from other important biological processes (Maginnis 2006). To compensate for these increased requirements, lizards undergoing regeneration adjusted its digestion by extending GPT (Fig. 1). By increasing the period food remains in the gastrointestinal tract, *P. erhardii* offers more time for digestive enzymes to act and thus may obtain higher nutrient absorbance (McConnachie and Alexander 2004; Sagonas et al. 2015).

Sugars and lipids represent the main and more direct energy sources (Karasov and Martinez Del Rio 2007). Thus, we

Table 1 Values of body size, ADE_S, and GPT before autotomy, during regeneration, and after regeneration completion are given in the first five rows. Protein, lipid, and glycogen concentrations (mg/g tissue) of the intact and fully regenerated tails are given in the last three rows. Means ± standard deviation; sample size in parentheses

Traits	Intact tail	Elongation phase	Fully regenerated tail
SVL (mm)	60.95 ± 6.30; (47)	60.95 ± 6.30; (47)	60.95 ± 6.30; (47)
GPT (hours)	41.32 ± 1.67; (47)	49.07 ± 6.42; (47)	40.15 ± 1.97; (47)
ADE proteins (%)	68.40 ± 12.98; (47)	79.80 ± 11.56; (47)	69.21 ± 3.23; (47)
ADE lipids (%)	73.05 ± 8.10; (47)	70.07 ± 12.41; (47)	73.53 ± 2.94; (47)
ADE sugars (%)	69.81 ± 4.67; (47)	68.65 ± 6.90; (47)	70.35 ± 8.71; (47)
Proteins (tail conc.)	248.47 ± 49.22 (38)		302.56 ± 57.66 (38)
Lipids (tail conc.)	176.52 ± 33.77 (38)		219.39 ± 72.01 (38)
Glycogen (tail conc.)	4.02 ± 2.59 (16)		4.74 ± 2.26 (16)

expected that tail regeneration would favor higher ADE_S and ADE_L . Nevertheless, this assumption was not confirmed and digestive efficiencies for sugars and lipids showed no increase during regeneration (Fig. 1). On the contrary, ADE_P was considerably higher during tail reconstruction (Fig. 1). Proteins serve as building blocks for the formation of tissues (Karasov and Martinez Del Rio 2007). As such, proteins are essential in caudal regeneration since they contribute to the construction of tail muscles, growth of cartilage and axial skeleton, and formation of tail skin (Alibardi 2010). Protein digestion is also time-consuming (da Diefenbach 1975; Pafilis et al. 2007). By prolonging the gut passage time, *P. erhardii* allows proteolytic enzymes to process and extract proteins more effectively, increasing ADE_P (Skoczylas 1978).

Tail constitutes an important energy storage organ in lizards and, typically, contains high fat levels (Doughty et al. 2003). Following caudal autotomy, more lipids and proteins are accumulated in the tail to support its regeneration (Vitt et al. 1977; Boozalis et al. 2012). Our results on *P. erhardii* are in agreement with those of previous studies on this species (Simou et al. 2008). The higher ADE_P detected supports this increased protein concentration per gram of tissue. On the other hand, the higher lipid levels of the fully regenerated tail cannot be explained by a higher ADE_L . This finding should be rather attributed to a metabolic alteration of the regenerated tail tissues (Meyer et al. 2002; Simou et al. 2008).

To sum up, our study provides the first evidence regarding the impact of tail regeneration on digestion. During regeneration, lizards need additional amounts of nutrients to rebuild the amputated tail. Therefore, *P. erhardii* processes the food at a slower pace offering proteolytic enzymes more time to act. This increases the digestive efficiency of proteins that are required to build up the new tissue. In order to confirm the generality of this pattern, further research that will include more species is required.

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