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RESEARCH PAPER

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Tail regeneration alters the digestive performance of lizards

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Abstract

Tissue regeneration is a fundamental evolutionary adaptation, which is well known in lizards that can regenerate their entire tail. However, numerous parameters of this process remain poorly understood. Lizard tail serves many functions. Thus, tail autotomy comes with many disadvantages and the need for quick regeneration is imperative. To provide the required energy and materials for caudal tissue building, lizards are expected to undergo a number of physiological and biochemical adjustments. Previous research showed that tail regeneration induces changes in the digestive process. Here, we investigated if and how tail regeneration affects the digestive performance in five wall lizard species deriving from mainland and island sites and questioned whether the association of tail regeneration and digestion is affected by species relationships or environmental features, including predation pressure. We expected that lizards from high predation environments would regenerate their tail faster and modify accordingly their digestive efficiency, prioritizing the digestion of proteins; the main building blocks for tissue repair. Second, we anticipated that the general food shortage on islands would inhibit the process. Our findings showed that all species shifted their digestive efficiency, as predicted. Elongation rate was higher in sites with stronger predation regime and this was also applied to the rate with which protein digestion raised. Gut passage time increases during regeneration so as to improve the nutrient absorbance, but among the islanders, the pace was more intense. The deviations between species should be attributed to the different ecological conditions prevailing on islands rather than to their phylogenetic relationships.

KEYWORDS

digestion, islands, *Podarcis*, tail autotomy, tail regeneration

1 | INTRODUCTION

Saurian tail exhibits a remarkable diversity in functions, serving as a balance and fat storage organ (Doughty et al., 2003), aiding locomotion (McElroy & Bergmann, 2013) and participating in courtship, territorial behaviour and social status (Fox et al., 1990; Maginnis, 2006; Peters et al., 2016). Most notably, tails are associated with predator escape tactics through autotomy, that is, the self-induced breakage of the tail (Bateman & Fleming, 2009). Tail shedding is widespread among lizards, suggesting an ancestral character. Nonetheless, the cost of autotomy may be severe and tail regeneration has to occur swiftly (Bellairs & Bryant, 1985; Lin et al., 2017). The originally bony

vertebrae are replaced by rigid cartilage, the new caudal 'skeleton', a process that is usually completed within some weeks (Barr et al., 2019; Lozito & Tuan, 2017). Tail tissue repair is known to be facilitated by changes in energy allocation (Naya & Božinović, 2006). In fact, during tail regeneration lizards have to cope with the distinct ecological and environmental conditions prevailing in their ecosystems (Simou et al., 2008).

During the elongation phase of the tail, between caudal autotomy and the complete regeneration of the lost part, lizards experience significant costs related to reduce fitness and performance, and hence change appropriately many aspects of their overall biology (Maginnis, 2006). Since wound healing and tail regeneration require the activation of many molecular, cellular and physiological mechanisms (Alibardi, 2010), lizards have to reallocate energy to support the whole process that otherwise would fuel other biological processes (Bernando & Agosta, 2005; Maginnis, 2006). Previous research showed that the performance of digestion is modified to cover the 'new' high requirements of the regenerating tail (Sagonas et al., 2017).

The percentage of energy and nutrients that animals absorb from food and use to fuel their body functions are determined by digestion (Karasov & Martinez Del Rio, 2007). Apparent digestive efficiency (ADE), the relative percentage of ingested energy absorbed through the gut, is typically used to assess digestion success. Digestion, and consequently ADE, is a particularly plastic trait that in lizards is affected by temperature (McConnachie & Alexander, 2004; Pafilis et al., 2007), water availability (Karameta, Gourgouliani, et al., 2017), food abundance and quality (Sagonas et al., 2015), the time food remains in the gut (gut passage time, GPT; Van Damme et al., 1991; Vervust et al., 2010), body size (Pafilis et al., 2016) and age (Durtsche, 2004; Karameta, Mizan, et al., 2017). Insularity is another factor shaping saurian ADE and GPT (Karameta, Gourgouliani, et al., 2017; Pafilis et al., 2007; Sagonas et al., 2015) as islands are characterized by specific values of several of the above parameters: milder environmental temperatures (Schwaner, 1989), lower food availability and less profitable diet (Blondel et al., 2010; Brown & Pérez-Mellado, 1994; Pérez-Mellado & Corti, 1993) as well as lower precipitation (Weigelt et al., 2013). Recently, a number of studies have demonstrated that phylogeny is a significant factor that could explain the variation in the efficiency of digestion among organisms (Karasov & Douglas, 2013; Karasov & Martinez Del Rio, 2007).

Here, we aimed to clarify the impact of tail regeneration on digestive performance in a comparable phylogenetic framework including both insular and mainland species. To this end, we assessed the digestive performance at different stages of tail regeneration (prior to autotomy, during elongation and after the completion of caudal restoration) and recorded regeneration rate in five lacertid lizards from Greece. We expected that previous findings on the effect of tail regeneration on digestion of the insular lizard *P. erhardii* (Sagonas et al., 2017) would apply in our system as well and thus presumed that all species, regardless of their origin, would demonstrate higher ADEs and GPTs during the elongation phase to cope with the increased energy requirements. However, we questioned the evolutionary association between tail regeneration, digestion and species phylogenetic relationships. Two opposite views were assessed. On the one hand, if species inhabiting environments with higher predation (mainland) regenerate their tail faster compare to those living under more relaxed predation pressure (islanders) (Simou, 2009; Tsasi et al., 2009), we anticipated that digestive performance would follow a similar distinct pattern. On the other hand, if phylogenetically related species demonstrate alike digestion efficiency and similar response to tail regeneration, we expected that this association would be independent of the environment.

2 | MATERIALS AND METHODS

2.1 | Study species

This study was conducted in five species of wall lizards (genus *Podarcis*) that occur in mainland and insular Greece. Both mainland species, the widely distributed across Europe common wall lizard (*P. muralis*), and the Peloponnese wall lizard (*P. peloponnesiacus*), endemic to Peloponnese, derived from Lake Doxa (Feneos plateau, Peloponnese; Figure 1) (33 and 38 individuals, respectively). The Erhard's wall lizard (*P. erhardii*) is distributed throughout the southern Balkans and most of the Aegean islands; we captured 47 individuals from Andros Island (Cyclades, Figure 1). The Skyros wall lizard (*P. gaigeae*) is endemic to the Skyros Archipelago and Piperi Island; we sampled 49 lizards from Skyros Island (Sporades, Figure 1). The Milos wall lizard (*P. milensis*) is endemic to Milos Archipelago; we caught 43 individuals from Milos Island (South Cyclades, Figure 1).

To avoid possible sex and age effect, we used exclusively adult males. Lizards were transferred to the laboratory facilities of the Department of Biology at the University of Athens. Lizards were housed individually in plastic terraria (20 × 25×15 cm) with sand and artificial shelters, and room temperature was kept at 25°C thanks to a non-stopping air conditioning unit. A controlled photoperiod (12 light: 12 dark) was provided by fluorescent tube lighting, while additional incandescent lamps (60 W) allowed lizards to thermoregulate behaviourally for 8 hr/d. Lizards had access to water ad libitum and were fed every other day with mealworms (*Tenebrio molitor*), coated with a powder containing vitamin and mineral supplements (TerraVit Powder, JBL GmbH & Co. KG). Lizards were released back to the places they were captured at the end of the experiment.

2.2 | Gut Passage Time (GPT)

We estimated GPT as the time between consumption and defecation of a plastic (indigestible) marker embedded in a mealworm (Van Damme et al., 1991). Prior to the experiment, food was withheld from lizards for three days, until no faeces were found in the terrarium. Once a lizard consumed a marked mealworm, terraria were inspected for the appearance of the marker every hour. Faecal material, where the marker was detected, was placed in liquid nitrogen immediately after collection and was stored at −80°C until later biochemical analysis. Before freezing, urate material was removed from the faecal matter. GPT was measured at three different phases:

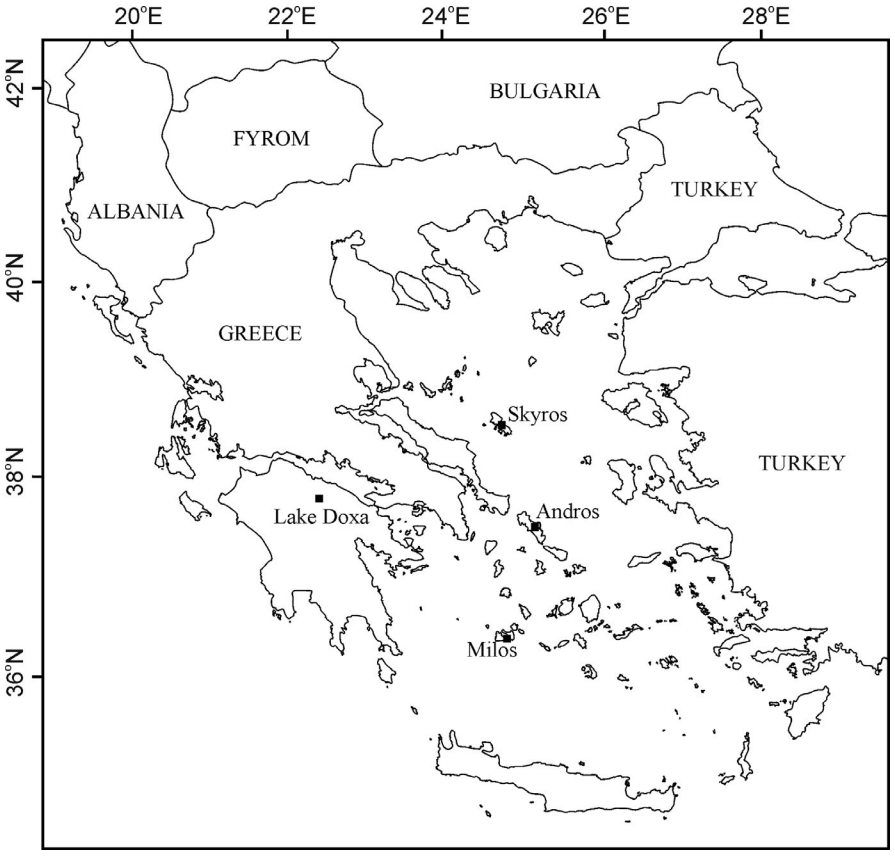

FIGURE 1 Sampling localities for *Podarcis erhardii*, *P. milensis*, *P. tauricus*, *P. gaigeae*, *P. muralis* and *P. peloponnesiacus*

day 0 (just before autotomy), day 15th (during regeneration) and day 90th (end of regeneration for the focal species; Simou, 2009).

2.3 | Apparent digestive efficiency (ADE)

To estimate ADE, we followed the protocol proposed by Harwood (1979). Digestive efficiency was measure, once more, thrice: just before autotomy, during regeneration, and at the completion of tail reconstruction, separately for proteins (ADE_P), lipids (ADE_L) and sugars (ADE_S) (Pafilis et al., 2007). Each lizard was fed with two mealworms of known mass (i500 Backlit Display, My Weight, accurate to 0.01 g) every other day for two months. Two identical mealworms of the exactly same mass and size were stored at −80°C for subsequent biochemical analyses.

We estimated total lipids by homogenizing 30–40 mg of faecal and mealworm material with 1.5 ml of a 2:1 mixture of chloroform and absolute methanol. The homogenate was centrifuged at 3,000 rpm for 10 min in 4°C and the pellet formed was discarded. Total lipid concentration was quantified at the supernatant using diluted phosphovaniline and a standard of olive oil and corn oil mixture (2:1 v/v) (Alexis et al., 1985). Absorbance was read at 530 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech).

Total protein concentration was determined using the pellet obtained from the analysis of lipid using the Biuret method (Layne, 1957). Bovine serum albumin (0.5–10 mg/ml) was used as a standard. The pellet was dissolved with 0.5 ml of 0.1 N NaOH and incubated at 37°C for 30 min. Fifty μl was diluted in 950 ml H_2O, and 4 ml of Biuret Reagent was added. The mixture was incubated for 30 min at room temperature, and then the absorbance was read at 550 nm at a Novaspec II spectrophotometer.

Sugar concentration was estimated following Dubois et al. (1956) protocol. 150 mg of tissue was weighted, homogenized with H_2O at a 1:10 w/v ratio and then boiled for 30 min. Twenty μl of this sample was diluted in H_2O (1:500 v/v), incubated with 1 ml of phenol (5% w/v) and 5 ml of 95% H_2SO_4 for 10 min at room temperature and then 40 min at 30°C. The absorbance was read at 490 nm, and glucose content was estimated against a known glucose standard.

Individual ADEs for proteins, lipids and sugars were calculated according to the following equation:

$$ADE_x = \frac{100\,(I_x - E_x)}{I_x},$$

where I_x is the concentration of ingested (mealworm) nutrient (x = proteins, lipids or sugars) and E_x is the concentration of the nutrient (x = proteins, lipids or sugars) remained in the faeces.

2.4 | Tail autotomy, biochemical analyses and tail growth

Predation-induced autotomy was simulated with the method proposed by Pérez-Mellado et al. (1997). Since caudal autotomy is

affected by body temperature (Bustard, 1967, 1968), lizards were allowed to thermoregulate for two hours prior to the beginning of the experiment. After achieving their preferred body temperature, we placed lizards on a cork substrate that allowed them maintain traction during the predation simulation. A pair of calipers was used to simulate the bite of a predator and grasped the tail 15 mm behind the cloaca. Shed tails were preserved into liquid N_2 immediately after autotomy. Protein and lipid concentrations in each individual tail were evaluated following the same protocols used in ADEs estimation. Lastly, we estimated the concentration of glycogen using the indirect method of Seifter and Dayton (1950) against a glucose standard. Tail muscle tissue was minced, the pieces were boiled for 20 min in the presence of 30% KOH, and measurements were read at 620 nm.

The length of regenerated tails was recorded weekly using a digital caliper (Silverline 380 244, accurate to 0.01 mm). Measurements began the first week after autotomy and were taken till the end of regeneration for all species (Simou, 2009).

2.5 | Statistical analysis

To assess the normality and heteroscedasticity of the data, we applied the Kolmogorov–Smirnov and Lilliefors tests and Levene's test. Generalized linear mixed model (GLMM) was then used to test for differences in ADEs, GPT and tail metabolites across different time intervals and between species. When necessary and to reduce the within-group error in digestive performance caused by SVL and GPT, the two variables were used as a covariate. Likewise, ANCOVA was performed to account for body size effects in GPT. Paired *t* test was used to compare the concentration of proteins, lipids and glycogen between intact and fully regenerated tails within each species, while ANOVA was used to compare between species. ANOVA was also used to examine whether different species have different rate of tail growth. For multiple comparisons, we applied Tukey *HSD* post hoc test. In addition, we calculated the percentage of change for GPT (*Δ*GPT) as well as the changed ratio for ADE (*R*ADE) and GPT (*R*GPT), and compared them between species using ANOVA. Principal component analysis (PCA) was applied to analyse the structure of interrelationships among ADEs and GPT before and after autotomy as well as tail growth for the five studied species. Lastly, we used a Mantel test to examine if differences in ADEs are correlated to differences in tail growth.

Yet, because conventional statistical methods tend to produce inflate Type I errors as they assume star phylogenetic relationships, we run a phylogenetic generalized linear mixed model (PGLMM) repeating all former analyses taking into account the phylogenetic status of the five lizard species (Poulakakis et al., 2005). Furthermore, partial Mantel test was conducted to assess whether the distances in ADE and tail growth are related to the genetic distances of species (i.e. whether the differences are the effect of species phylogeny). All analyses were performed in R 4.0.2 (R Development Core Team, 2019).

3 | RESULTS

3.1 | Digestive performance

Snout–vent length showed significant differences between species ($F_{4,205} = 106.45$, $p < 0.001$), with *P. milensis* being the smallest (Tukey *HSD* post hoc; all *p*s < 0.001) and *P. peloponnesiacus* the largest species (Tukey *HSD* post hoc; all *P*s < 0.001). The comparison of GPT among the three phases (before autotomy, during elongation and after regeneration completion) across species yielded significant differences (GLMM; $F_{8,410} = 2.92$, $p = 0.003$), even when SVL taken into account (GLMM with covariate; $F_{8,400} = 2.91$, $p = 0.004$). Tukey *HSD* post hoc analysis showed that island and mainland lizards slowed down significantly GPT during the elongation phase compared to the phases before and after autotomy by 23% and 13% respectively (Table 1). In other words, post hoc analysis revealed the existence of two groups in day 0 and 90 with island (39 hr on average) and mainland (44 hr on average) taxa grouping together (GLMM; $F_{8,410} = 2.92$, $p = 0.003$; see also Table 1). Furthermore, the island species, *P. milensis* and *P. gaigeae* (but not *P. erhardii*), showed significantly higher *Δ*GPT (the percentage of GPT ratio before autotomy and during elongation; 8.74 vs. 6.1; $F_{4,250} = 3.89$, $p = 0.005$) and *R*GPT (the percentage of GPT change ratio before autotomy and during elongation; 22.9% vs. 13.7%; $F_{4,250} = 5.81$, $p < 0.001$) than the mainland species, *P. peloponnesiacus* and *P. muralis*.

The two-way interaction between tail condition and species showed significant differences for ADE for proteins (GLMM; ADE_P: $F_{8,410} = 3.09$, $p = 0.002$), with all species achieving the highest ADE values during the second phase (elongation) compared to the other two phases (before autotomy and after regeneration completion) (Table 1). Furthermore, post hoc analysis showed that the five species differ in their efficiency to digest proteins, with ADE_P values being significantly higher on insular compared to mainland species before autotomy (66.7 vs. 58.8) and after regeneration completion (67.3 vs. 58.4). Yet, during the process of elongation, besides the differences between *P. muralis* and *P. erhardii* (Tukey *HSD* test; $p < 0.001$) no further differences were revealed between mainland and island species (74.0 vs. 77.2; Tukey *HSD* test, all pairwise *p*s > 0.05). When GPT (GLMM; $F_{8,409} = 3.85$, $p < 0.001$) or SVL (GLMM; $F_{8,409} = 3.15$, $p < 0.001$) was used as covariates, we ended up to the same results.

On the other hand, ADE_L (GLMM; ADE_S: $F_{8,410} = 1.16$, $p = 0.324$) and ADE_S (GLMM; ADE_S: $F_{8,410} = 1.86$, $p = 0.066$) showed no significant changes among the three tail phases, although some significant, though marginal, differences appeared for *P. milensis* (GLMM; ADE_S: $F_{2,84} = 3.19$, $p = 0.046$ and ADE_L: $F_{2,84} = 8.51$, $p < 0.001$) and *P. peloponnesiacus* (GLMM; ADE_L: $F_{2,74} = 4.01$, $p = 0.02$) during the elongation phase (Table 1).

The comparison of the percentage of ADE_P change ($RADE_P$) revealed that mainland species increased their ADE_P with significantly higher values compared to island species (25% vs. 15% increase; $F_{4,250} = 5.81$, $p < 0.001$). The results of PCA analyses for ADEs and GPT before autotomy and during elongation yielded similar results

TABLE 1 Values of body length (SVL), gut passage time (GPT), apparent digestive efficiency for each nutrient separately and tail metabolites for the three time periods: before autotomy (phase 1), during the elongation phase (phase 2) and after the completion of tail restoration (phase 3). For each value, we provide the mean ± *SD* and the sample size in parenthesis

	Traits	Phase 1	Phase 2	Phase 3	Statistics
P. erhardii	SVL (mm)	60.95 ± 6.30	60.95 ± 6.30	60.95 ± 6.30	
	GPT (hours)	41.32 ± 1.67	49.07 ± 6.42	40.15 ± 1.97	$F_{2,92} = 85.93$, $p < 0.001$
	ADE proteins (%)	68.40 ± 12.98	79.80 ± 11.56	69.21 ± 3.23	$F_{2,92} = 47.74$, $p < 0.001$
	ADE lipids (%)	73.05 ± 8.10	70.07 ± 12.41	73.53 ± 2.94	$F_{2,92} = 2.171$, $p = 0.119$
	ADE sugars (%)	69.81 ± 4.67	68.65 ± 6.90	70.78 ± 8.73	$F_{2,92} = 3.11$, $p = 0.05$
	Proteins (tail conc.)	248.47 ± 49.22		302.56 ± 57.66	$t = 4.73$, $df = 37$, $p < 0.001$
	Lipids (tail conc.)	176.52 ± 33.77		219.39 ± 72.01	$t = 3.14$, $df = 37$, $p = 0.003$
	Glycogen (tail conc.)	4.02 ± 2.59		4.74 ± 2.26	$t = 0.93$, $df = 37$, $p = 0.366$
P. milensis	SVL (mm)	55.63 ± 4.23	55.63 ± 4.23	55.63 ± 4.23	
	GPT (hours)	38.79 ± 3.73	48.02 ± 7.82	40.63 ± 3.68	$F_{2,84} = 36.58$, $p < 0.001$
	ADE proteins (%)	67.23 ± 4.15	76.57 ± 3.47	68.08 ± 3.29	$F_{2,84} = 88.44$, $p < 0.001$
	ADE lipids (%)	74.65 ± 9.52	68.04 ± 9.92	74.84 ± 6.51	$F_{2,84} = 8.51$, $p < 0.001$
	ADE sugars (%)	73.6 ± 7.15	71.45 ± 4.68	74.37 ± 5.14	$F_{2,84} = 3.19$, $p = 0.046$
	Proteins (tail conc.)	228.4 ± 38.67		252.31 ± 36.38	$t = 2.29$, $df = 27$, $p = 0.030$
	Lipids (tail conc.)	180.4 ± 22.11		207.18 ± 44.37	$t = 3.46$, $df = 27$, $p = 0.002$
	Glycogen (tail conc.)	3.4 ± 0.64		3.50 ± 0.96	$t = 0.41$, $df = 27$, $p = 0.687$
P. gaigeae	SVL (mm)	61.76 ± 2.74	61.76 ± 2.74	61.76 ± 2.74	
	GPT (hours)	37.54 ± 3.5	45.79 ± 7.87	35.35 ± 4.75	$F_{2,96} = 45.96$, $p < 0.001$
	ADE proteins (%)	64.38 ± 4.58	75.31 ± 8.13	64.62 ± 4.49	$F_{2,96} = 56.29$, $p < 0.001$
	ADE lipids (%)	84.58 ± 1.91	82.47 ± 7.77	84.45 ± 6.64	$F_{2,96} = 2.02$, $p = 0.138$
	ADE sugars (%)	75.16 ± 4.60	72.77 ± 5.94	74.01 ± 4.28	$F_{2,96} = 3.02$, $p = 0.053$
	Proteins (tail conc.)	177.18 ± 28.9		271.5 ± 80.88	$t = 7.19$, $df = 29$, $p < 0.001$
	Lipids (tail conc.)	181.23 ± 29.97		204.6 ± 28.06	$t = 3.26$, $df = 29$, $p = 0.003$
	Glycogen (tail conc.)	2.37 ± 0.45		1.98 ± 0.7	$t = 2.02$, $df = 13$, $p = 0.065$
P. muralis	SVL (mm)	68.2 ± 5.74	68.2 ± 5.74	68.2 ± 5.74	
	GPT (hours)	44.22 ± 2.13	49.51 ± 2.14	43.62 ± 2.03	$F_{2,64} = 78.81$, $p < 0.001$
	ADE proteins (%)	55.41 ± 7.19	69.85 ± 6.81	54.78 ± 5.46	$F_{2,64} = 58.81$, $p < 0.001$
	ADE lipids (%)	80.84 ± 5.24	78.97 ± 7.61	80.96 ± 6.13	$F_{2,64} = 1.65$, $p = 0.199$
	ADE sugars (%)	77.82 ± 6.29	78.17 ± 8.89	79.3 ± 6.14	$F_{2,64} = 0.38$, $p = 0.686$
	Proteins (tail conc.)	203.69 ± 23.46		292.83 ± 27.7	$t = 9.74$, $df = 19$, $p < 0.001$
	Lipids (tail conc.)	167.73 ± 14.2		163.3 ± 22.15	$t = 0.68$, $df = 19$, $p = 0.507$
	Glycogen (tail conc.)	2.4 ± 0.55		2.58 ± 0.89	$t = 0.90$, $df = 19$, $p = 0.380$
P. peloponnesiacus	SVL (mm)	74.81 ± 2.28	74.81 ± 2.28	74.81 ± 2.28	
	GPT (hours)	44.77 ± 2.94	51.68 ± 3.05	45.26 ± 3.95	$F_{2,74} = 50.52$, $p < 0.001$
	ADE proteins (%)	62.21 ± 7.37	77.61 ± 7.1	62.04 ± 6.38	$F_{2,74} = 63.34$, $p < 0.001$
	ADE lipids (%)	76.92 ± 8.91	74.55 ± 8.39	79.26 ± 4.16	$F_{2,74} = 4.01$, $p = 0.022$
	ADE sugars (%)	77.16 ± 7.96	79.18 ± 8.02	76.27 ± 8.37	$F_{2,74} = 1.44$, $p = 0.243$
	Proteins (tail conc.)	245.2 ± 19.18		312.9 ± 35.71	$t = 6.32$, $df = 17$, $p < 0.001$
	Lipids (tail conc.)	170.47 ± 22.03		175.82 ± 13.96	$t = 0.89$, $df = 17$, $p = 0.383$
	Glycogen (tail conc.)	3.64 ± 0.76		3.85 ± 0.99	$t = 1.01$, $df = 17$, $p = 0.325$

with the two main axes explaining together 60.6% and 65.1% of the variation in the data set, respectively. In both cases, island and mainland species separated along PC1 (Figure 2a,b and Table 2). Repetition of the analysis at the stage of fully regenerated tail provided similar findings.

3.2 | Tail growth and metabolites

The comparison of intact and fully regenerated tails (see Table 1 for statistics) revealed that for all five species, the latter had significantly higher protein concentration (all *p*s < 0.01). Lipid concentration on

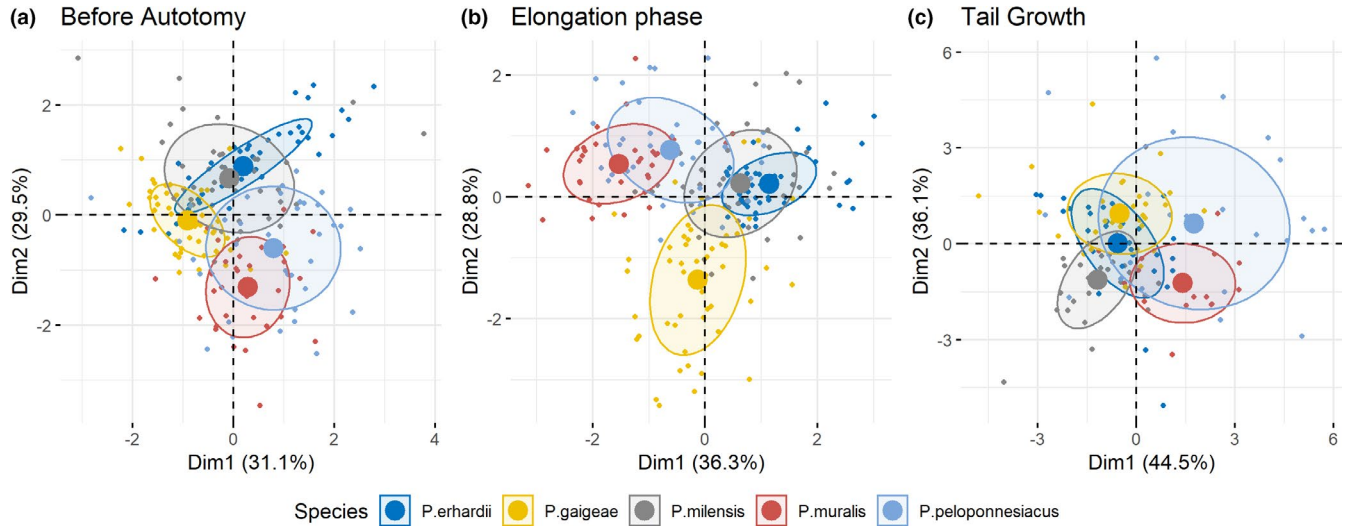

FIGURE 2 Principal component analysis (PCA) for ADEs and GPT for two time periods (a) before autotomy and (b) during the elongation phase. (c) PCA for tail growth

TABLE 2 Results for principal component analysis (PCA) for ADEs and GPT before autotomy (A) and during the elongation phase (B). Variables loading strongly on each principal component are given in bold

A. Component	Eigenvalues	% Variance	Cumulative %
1	1.24	31.09	31.09
2	1.18	29.53	60.61
3	0.89	22.35	82.96
4	0.68	17.04	100.00
	PC1	**PC2**	**PC3**
GPT	−0.243	**0.808**	−0.079
$ADE_{PROTEINS}$	**0.571**	−0.165	−0.693
ADE_{LIPIDS}	−0.530	0.565	0.069
ADE_{SUGARS}	−0.478	0.016	**−0.714**
B. Component	**Eigenvalues**	**% Variance**	**Cumulative %**
1	1.45	36.28	36.28
2	1.16	28.80	65.08
3	0.84	21.00	86.06
4	0.57	13.92	100.00
	PC1	**PC2**	**PC3**
GPT	**0.634**	−0.358	0.367
$ADE_{PROTEINS}$	0.278	**0.650**	−0.549
ADE_{LIPIDS}	−0.556	−0.475	−0.387
ADE_{SUGARS}	0.459	−0.473	**−0.642**

the other hand differed significantly only on island (all *p*s < 0.01) but not in the mainland species (both *p*s > 0.05). Lastly, the levels of glycogen showed no differences between the two tail conditions for all species (all *p*s > 0.05).

GLMM for the interaction between species and tail condition showed significant differences for proteins ($F_{4,258}$ = 5.18, p <0.001) and lipids ($F_{4,258}$ = 3.11, p =0.016) but not for glycogen ($F_{4,182}$ = 0.793, p =0.531). The comparison among species showed that the fully regenerated ($F_{4,129}$ = 6.18, p <0.001) but not intact ($F_{4,129}$ = 1.31, p =0.345) tail in island species had significantly higher levels of lipids compared to mainland (Table 1).

Pairwise comparison of the growth rate (i.e. the curve of tail growth) indicated the presence of two groups, one comprising the mainland species (*P. peloponnesiacus* and *P. muralis*) and another with

the island species (*P. gaigeae*, *P. milensis* and *P. erhardii*) ($F_{4,130} = 9.415$, $p < 0.001$). In general, mainland species regenerate their tail faster than islanders. Likewise, PCA analysis for tail growth showed that the first two principal components could explain together 80.6% of the variation, with island species separating from the mainland ones along PC1 (Figure 2c).

Mantel test between tail growth distances and $R\text{ADE}_P$ distance among species showed a significant positive correlation ($r = 0.97$, $p = 0.008$), suggesting that the faster growth rate of mainland species could be attributed to the higher changes of ADE_P.

3.3 | Testing for phylogenetic effects

We found no phylogenetic effect regarding the differences observed for ADE_P. As such, the comparison between species showed that island species demonstrated significantly higher ADE_P compared to the mainland ones ($F_{1,3} = 7.02$, $p = 0.044$). Likewise, and in agreement to GLMM, PGLMM showed that tail growth rate was significantly higher in mainland species compare to islanders ($F_{1,3} = 315.81$, $p < 0.001$), suggesting no phylogenetic impact on tail growth. Finally, Mantel test between species genetic distances, tail growth differences and ADEs showed no significant correlation ($r = 0.53$, $p = 0.133$).

4 | DISCUSSION

Caudal autotomy is an effective last-line, anti-predatory mechanism, due to the high costs associated with tail loss that impose quick tail regeneration (Bateman & Fleming, 2009; Maginnis, 2006). Here, we focused on tail regeneration and its implications on digestive performance in a comparative phylogenetic framework, including both insular and mainland *Podarcis* species. In accordance with our initial hypothesis, our findings in regard to digestive performance corroborate previous research: all species shifted certain digestion traits to offset tail regeneration. Thus, lizards increased their GPT and ADE_P during the elongation phase in response to higher requirements, while tail regeneration was significantly faster in mainland species. Furthermore, the comparison between mainland and island species yielded interesting differences. In particular, we found that the differences observed between ADEs and GPT across the three phases of tail regeneration among species had no phylogenetic signal but were rather positively related to the faster growth rate that mainland *Podarcis* achieved.

Tail regeneration is an energetically costly process, essential though for the survival of tailless lizards. As caudal autotomy increases the risk of subsequent predation, rapid regeneration is favoured when the benefits outweigh the costs (Arnold, 1984). Populations inhabiting islands experience relaxed predation pressure and limited food resources compared to their mainland kin (Cooper et al., 2014; Itescu et al., 2017; Pérez-Mellado et al., 1997). In our study system, predator diversity varies substantially between locations, with mainland sites hosting more diverse and higher predator abundances than island sites (Brock et al., 2014; Pafilis et al., 2009). Furthermore, as in most Mediterranean islands, arthropod prey availability is lower compared to mainland (Brown & Pérez-Mellado, 1994; Schwarz et al., 2020), and thus resources available to fuel tail regeneration are limited. Taken together, these two factors could explain the significantly steeper regeneration growth curve in mainland lizards: living under high predation regime mainland lizards evolved a faster caudal regeneration that will provide important survival advantages (Lin et al., 2017). On the other hand, islanders that live in lower predation environments (compared to the mainland) that in addition lack sufficient energy flow cannot afford to support a swift, but costly, caudal regeneration.

Following caudal autotomy, lizards face the high energy costs associated with wound healing and tail regeneration (Alibardi, 2010). To deal with these extraordinary requirements, lizards may modify their digestive efficiency (Sagonas et al., 2017). We found no differences in the ADEs for lipids and sugars (Table 1). Nonetheless, digestive efficiency for proteins increased significantly during the elongation for all species, verifying our initial hypothesis. Proteins are essential for the formation of tail skin, muscles and cartilage and represent the building blocks for tail reconstruction (Alibardi, 2010; Karasov & Martinez Del Rio, 2007). As such, all the species, irrespective of origin, increased their ADE_P during the elongation phase (Figure 2). Nonetheless, the mainland species adopted a more intense rise in protein digestion as evaluated by $R\text{ADE}_P$. Previous studies have recognized four main factors influencing the efficiency of digestion: food availability and food quality (Karasov et al., 2011; Sagonas et al., 2015), individual characteristics (Karameta, Mizan, et al., 2017; Pafilis et al., 2016), environmental conditions (McConnachie & Alexander, 2004; Pafilis et al., 2007) and phylogeny (Karasov & Douglas, 2013; Pérez-Barberia et al., 2004). Here, mainland lizards face a higher cost of predation compare to islanders and thus, to survive, a higher regeneration rate is needed. Faster tissue repair demands higher building block supplies that are provided through effective protein digestion.

The time food remains into the gastrointestinal tract is crucial for the effective catabolism of macromolecules and higher energy absorption (McConnachie & Alexander, 2004; Sagonas et al., 2015). All lizards increased their GPT during the elongation phase so as to improve energy gains and ADEs. However, there was a clear grouping between mainland and island lizards, independent of their phylogenetic relationships that slowed down the food passage rate by approximately 13% and 23%, respectively. Island lizards tend to have longer gastrointestinal tracts leading to higher GPTs (Herrel et al., 2008; Sagonas et al., 2015). However, this pattern is not uniform and deviations with lower GPTs than mainland lizards have been reported (Pafilis et al., 2007). In this study, islanders maximized their GPT (as shown by ΔGPT and RGPT) to improve energy and macromolecule acquisition. In the poor insular habitats, the further retention of food will do the trick under the limited energy flow (Pafilis et al., 2007).

The biochemical composition of the regenerated tails differed from that of the original ones. In particular, protein concentration increased in regenerated tails in all species examined. This uniform pattern comes as no surprise since ADE_p grew up considerably in all species during the elongation phase (Table 1), indicating protein importance in tail reconstruction (Alibardi, 2010; Boozalis et al., 2012). However, lipid concentration was higher in regenerated tails only among islanders (*P. gaigeae*, *P. milensis* and *P. erhardii*). Tails are widely used as lipid storage tissue in lizards (Doughty et al., 2003; Pianka & Vitt, 2003; Pond, 1981) and higher lipid accumulation supports caudal regeneration (Boozalis et al., 2012; Simou et al., 2008). Lizards living on the unpredictable Mediterranean islands where food availability is spatially and seasonally clustered (Lymberakis et al., 2016; Pafilis et al., 2007; Pérez-Mellado & Corti, 1993) have to store energy for harder periods. On the other hand, this finding might reflect the higher probability for autotomy because of the higher predation pressure mainland species experience (Pafilis et al., 2009) that prevents them from storing lipids into their tail.

Overall, our study shed further light on the effects of tail regeneration on lizards' physiology and suggests an important role on the efficacy of digestive system. In particular, we reported that like other physiological mechanisms, digestion is a plastic trait and increase in digestive performance can compensate for the higher requirements for energy and nutrients a lizard might have during tail regeneration. Furthermore, the differences in tail growth and digestive changes that were recorded between mainland and island *Podarcis* species reflect the different environmental conditions prevailing on mainland and islands, but also possible differences in the regeneration process that deserve future investigation.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

K.S., P.P. and E.D.V. conceived and designed the study. All authors carried out the field work. K.S., A.R., I.D., M.P., K.S., I.P., A.V., A.B. and N.K. carried out the laboratory experiments. K.S. analysed the data. K.S., P P. and E.D.V. wrote the manuscript. All coauthors contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data associated with this study are available on the Dryad Digital Repository with DOI https://doi.org/10.5061/dryad.f7m0cfxv9.

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