

Physiological Effects of Tail Regeneration following Autotomy in Italian Wall Lizards, *Podarcis siculus*

PAULA EBERLE,¹ DANIEL HARO,¹ KENDRA REKEVICS,¹ AND HEATHER E. M. LIWANAG^{1,2}

¹California Polytechnic State University, San Luis Obispo, California, 93407-0401, USA

ABSTRACT.—Caudal autotomy (the voluntary loss of a tail) is an important antipredator defense in many lizard species. The subsequent regrowth of the tail, seen in most lizards with the ability to autotomize, may pose some physiological costs both during the regrowth process and during potential repeated autotomy. In this study, we examined the physiology and behavior of the lacertid lizard *Podarcis siculus*, as well as the physiology and activity of the detached tails, following autotomy. Evaporative water loss and respiratory exchange ratio increased after autotomy, showing both an increased loss and a redistribution of important resources inside the animal. The lizards exhibited no change in behavioral thermoregulation during regrowth, as thermal preference did not change. Males and females differed in tail energy content, suggesting differing energy storage needs between the sexes. The rate of regeneration and tail movement between the sexes were not different, but tail movement did vary between previously regenerated tails and original tails, indicating a possible detriment to future autotomy events. Overall, autotomy appears to influence more than just a lizard's ability to escape, as our study provides evidence of potential physiological tradeoffs associated with this antipredator behavior.

Prey species use a variety of defenses to evade the threat of predators. First-line defenses include crypsis, fleeing, and retaliation (Ruxton et al., 2004; Wall and Shine, 2007; Martin et al., 2009). If these defenses fail, however, animals may go to extremes to escape predation. One such extreme, autotomy, is the voluntary loss of a body part of an animal under threat (McVean, 1975). Many different taxa include members that use autotomy to escape predation, namely, snakes (Cooper and Alfieri, 1993), lizards (Downes and Shine, 2001; Brock et al., 2015), amphibians (Dinsmore, 1977), Hemipteran insects (Emberts et al., 2017), and even rodents (Dubost and Gasc, 1987; McKee and Alder, 2010; Seifert et al., 2012). The loss of a tail, limb, or even skin in some cases can help an animal escape alive.

Caudal autotomy (the voluntary loss of a tail) is an ancestral character state of Squamate reptiles (Zani, 1996) and is found in nearly two-thirds of all lizard families (Downes and Shine, 2001; Bateman and Fleming, 2009). During caudal autotomy in Lacertids, the tail detaches at one of the many possible intravertebral fracture planes, which typically begin at the fourth caudal vertebra (Barbadillo et al., 1995). The tail then writhes rapidly to distract a predator while the lizard makes its escape, a behavior that can result in extreme costs associated with appendage loss and subsequent regrowth. Caudal fat bodies comprise approximately 56% of all lipid stores in *Lacerta vivipara* (Avery, 1974). In *Niveoscincus metallicus* and *Takydromus septentrionalis*, the most proximal third of the tail stores the majority of lipid reserves (Chapple and Swain, 2002; Lin and Ji, 2005). Consequently, partial loss has very little effect on lipid storage in the animals; however, near-complete tail loss can be detrimental (Chapple and Swain, 2002; Lin and Ji, 2005). Tail loss can result in changes in sprint speed (Brown et al., 1995; Lin and Ji, 2005; McElroy and Bergmann, 2013) and loss of agility and balance (Brown et al., 1995; Gillis et al., 2008; Jusufi et al., 2008), as well as decreased survival rates in the wild (Downes and Shine 2001; Lin et al., 2017).

The regrowth of a tail is likely to impose some physiological costs to the animal. Most studies investigating costs have dealt with reductions in energy in terms of fat stores, juvenile growth,

reproduction, or some combination of those factors (Dial and Fitzpatrick, 1981; Chapple et al., 2002; Doughty et al., 2003; Chapple and Swain, 2002, 2004; Clause and Capaldi, 2006; Starostová et al., 2017). Some have found that autotomy does not increase preferred temperature or evaporative water loss (EWL) (Zamora-Camacho et al., 2015; Fernández-Rodríguez et al., 2021). Studies investigating the metabolic response to tail regrowth have found conflicting results, with some finding an increase in metabolic rate during regeneration (Dial and Fitzpatrick, 1981; Naya et al., 2007), whereas others have found no change (Congdon et al., 1974; Fleming et al., 2009; Starostová et al., 2017). Others have found costs linked to changes in metabolism, such as a reduction in telomere length in lizards with autotomized tails (Fitzpatrick et al., 2019). To our knowledge, no study has investigated how tail loss affects which substrates are used for metabolism.

We had several hypotheses regarding the physiological response to tail autotomy in lacertid lizards, as follows: 1) lizards adjust preferred temperature for thermoregulation in response to tail autotomy; 2) EWL is altered in response to tail autotomy; and 3) ratios of substrates used for metabolism are altered during tail regrowth. To test our hypotheses, we measured thermal preference (T_{pref}), EWL, and resting rates of oxygen consumption (RMR_{O_2}) and carbon dioxide expiration (RMR_{CO_2}) to calculate respiratory exchange ratio (RER) in an established population of nonnative Italian Wall Lizards (*Podarcis siculus siculus*) collected in San Pedro, California (Deichsel et al., 2010). We measured variables before induced tail autotomy, after tail autotomy, and after tail regrowth, while taking tail type (whether a tail was original or previously regrown), sex, and lizard mass into account. We predicted that behavioral and physiological responses would occur postautotomy and would return to original values after tail regrowth. Specifically, we predicted that after autotomy lizards would increase T_{pref} because of a higher body temperature's potentially beneficial effects on healing (Smith et al., 1988), and faster rate of tail regeneration (Bellairs and Bryant, 1985) but not to a point that would induce thermal stress. We predicted EWL would increase after autotomy because of the change in integument, and we predicted that RER would increase toward a value of 1, reflecting a shift away from lipids in substrate used for

²Corresponding author. E-mail: hliwanag@calpoly.edu
DOI: 10.1670/21-021

TABLE 1. Sample size (n), body mass, and tail lengths of male and female *Podarcis siculus* with original or previously regenerated tails, used in this study. Values in cells are mean \pm SD.

Parameter	Male		Female		Overall
	Original	Regenerated	Original	Regenerated	
n	4	5	6	4	19
Body mass (g)	8.38 \pm 1.00	8.39 \pm 1.43	6.48 \pm 0.54	7.08 \pm 1.25	7.51 \pm 1.32
Preautotomy tail length (cm)	16.2 \pm 1.4	13.6 \pm 2.7	12.5 \pm 1.6	10.2 \pm 1.4	13.1 \pm 2.7
Original portion of tail (cm)	16.2 \pm 1.4	6.2 \pm 4.4	12.5 \pm 1.6	1.8 \pm 8.3	
Regenerated portion of tail (cm)		7.5 \pm 3.8		8.5 \pm 1.1	
Fully removed tail length (cm)	14.3 \pm 1.2	11.9 \pm 2.6	11.1 \pm 1.7	9.0 \pm 1.2	11.5 \pm 2.5

metabolism (Ferrannini, 1988) because lipids are primarily stored in the base of the tail (Chapple and Swain, 2002).

Because tail movement has the potential to distract predators and increase a lizard's escape time (Dial and Fitzpatrick, 1983), we also investigated variation in the energy content and movement of tails postautotomy. We hypothesized that tail type (i.e., original or previously regrown) and the sex of the lizard would influence the amount of energy stored in the tail as well as the vigor of tail movement postautotomy. To quantify tail movement, we specifically measured the degree of bending in tails while moving and the duration of continued tail movement. To determine if original (intact) tails stored more energy and were better able to theoretically distract a predator through movement than previously regenerated tails, we compared tail energy content and movement by tail type. To determine if sexes differed in stored resources and ability to theoretically distract a predator through tail movement, we also investigated whether tail energy content and movement differed by sex.

MATERIALS AND METHODS

Animals, Husbandry, and Autotomy Induction.—In July 2018, we collected 19 adult free-ranging *P. siculus* (10 females, 9 males) from residential areas in San Pedro, California, and brought them to California Polytechnic State University (Cal Poly, San Luis Obispo, CA) for experimentation. *Podarcis siculus* is sexually dimorphic; we discerned males from females based on body size, body shape, head shape, color pattern, and size of femoral pores (Liwanag et al., 2018). Four of the females and five of the males had previously regenerated tails (Table 1). Each lizard was housed in a separate 10-L plastic terrarium (25 cm \times 16 cm \times 16 cm, Lee's Aquarium & Pet Products) with approximately 1.5 cm of shredded bark used as a substrate (Zilla bark blend). Each terrarium was placed partially atop a heating pad (Sunbeam) set on "low," such that the pad only heated half of the enclosure, creating a heat gradient of 27–32°C across the terrarium. A water bowl with a hide underneath was placed on the cooler side of the terrarium to reduce evaporation. All enclosures were placed under full-spectrum lighting set to a cycle of 12-h light and 12-h dark. Lizards were given water ad libitum, and twice a week, each lizard was fed four to five crickets, which were dusted with vitamins (calcium powder and D3) every third feeding.

The animals were kept for 2 mo prior to initial testing to ensure complete acclimation to the laboratory setting. Each lizard was tested for all three physiological measurements (T_{pref} , EWL, and RMR) prior to induction of caudal autotomy (preautotomy), after induction of caudal autotomy (postautotomy), and once again after the induction of caudal autotomy (follow-up). We report the exact days of measurement relative to

autotomy for each variable in the following methodology descriptions. Because we could not monitor lizards until full tail regrowth was complete, our follow-up measurements represent partial tail regrowth. Prior to the induction of autotomy, we measured the length of original and previously regrown tail portions, and we measured lizard mass to the closest 0.001 g by using a digital balance (Practicum 213); we then measured tail mass after autotomy. Because metabolism and selected body temperatures can cycle with a lizard's circadian rhythm (Firth and Belan, 1998; Hare et al., 2006; Parker, 2014), all trials were started between 0900 and 1000 h for consistency. Individuals were tested for different variables a minimum of 2 d apart, in an effort to reduce stress on the individual.

After preautotomy measurements, we induced caudal autotomy in each lizard. Tail autotomy was induced by gently pinching the base of the tail, 10-mm distal to the cloacal opening (leaving approximately 12 \pm 2% [mean \pm SD] of the preautotomy tail length). The location of autotomy was chosen as a standard length to ensure uniformity and to induce detachment at the most proximal fracture plane (Brock et al., 2015). Lizards with previously regenerated tails were treated with the same uniformity, as none of them had previously autotomized to the most proximal fracture plane, allowing an additional autotomy event. The lizards were allowed to wriggle while pinched until they voluntarily autotomized their tails. What remained on the lizard was gently blotted with a sterile Kimwipe to clean away any blood present, which was minimal. We then measured the new mass of each animal, as well as the length of the tail remaining on the animal, from vent to fracture plane. For lizards with previously regenerated tails, the length of the regenerated tail was recorded, as well as the length of original tail portion (Table 1). We allowed lizards to recover for 1–2 d before any postautotomy measurements to allow the wound to properly scab (McElroy and Bergmann, 2013).

Thermal Preference.—We fasted lizards for at least 2 d prior to T_{pref} trials to ensure that they were not choosing a temperature to aid in digestion. Because we could only test four lizards per day due to equipment constraints, preautotomy measurements were taken at 63, 62, 54, 49, or 48 d prior to tail loss; postautotomy measurements were taken 42, 43, 44, 45, or 47 d after tail loss; and follow-up measurements were taken 92, 94, 97, 98, or 99 d after tail loss. Each lizard was fitted with a flexible 40-gauge type K thermocouple (Omega Engineering) inserted into the cloaca and secured with 0.5-inch-wide medical tape gently placed around the base of the tail. Each lizard was placed in an individual lane in a linear temperature gradient (163-cm length \times 46-cm width) that ranged 10–40°C. The gradient was split into four lanes along its length, and four lizards were measured simultaneously per trial. The walls of the gradient, as well as the dividers between

the lanes, were opaque to avoid effects of outside stimuli and/or other lizards on behavior. The floor was composed of a metal sheet covered in a thin layer of reptile sand (Zoo Med) to ensure the lizards did not have direct contact with the metal floor. We replaced the sand between measurements to reduce potential effects of odor on temperature selection. A 1-cm-thick plastic sheet was placed above the gradient to restrict air flow between the lanes and reduce any potential influence of neighboring scents on behavior. At the cold end of the gradient, cold water was circulated through copper piping beneath the floor by using a circulating water bath set to 10°C (SD07R-20, PolyScience). At the warm end of the gradient, electric heat strips set to 40°C were mounted beneath the floor. The entire gradient was then fit inside a larger Plexiglass chamber lined with Drierite (W. A. Hammond) to reduce the buildup of condensation.

Lizard internal body temperatures were monitored and recorded every 15 min over a 4-h time period by using a four-channel thermocouple reader (Model RDXL4SD, Omega Engineering). The value of T_{pref} was calculated as the average body temperature of the animal over the last hour of the trial to ensure the animals were calm and were effectively selecting their thermal environment (Hertz et al., 1993; Huey, 1982). Four lizards per day were tested for five consecutive days in a row. Because time of day can influence T_{pref} (Carretero et al., 2006; Cowgell and Underwood, 1979), all trials were conducted at the same time of day (between 0900 and 1000 h) for consistency. Each individual was tested once for T_{pref} under each condition (preautotomy, postautotomy, and follow-up) with 1–2 mo between tests for each individual.

Evaporative Water Loss.—We measured EWL as mass lost over time at 25°C. Lizards were fasted 3 d prior to testing to ensure that any mass loss would be because of EWL rather than defecation. Preautotomy measurements were taken 83 d prior to tail loss, postautotomy measurements were taken 5 d after tail loss, and follow-up measurements were taken 32 d after tail loss. Each lizard was placed into a nylon screen pouch (11 cm × 14 cm, 18 × 16 mesh count, 0.011 ga diameter) that was slightly larger than the lizard to reduce EWL induced by movement (Heatwale and Veron, 1977). Immediately before the trial began, animals were weighed in the pouches to the nearest 0.001 g by using a digital balance. Each lizard-filled pouch was placed atop a dry, clean petri dish on one of three removable shelves and then into a Thermo Scientific Environmental Chamber model 818 set at 25°C. We chose 25°C because through past work we have observed that lizards were more stressed (i.e., attempted to escape pouches) at higher temperatures (Liwanag et al., 2018). Maximum airflow at the air vent within our 504-L chamber was 1.4 km·h⁻¹ and was undetectable at shelf locations. Relative humidity was measured to be 19.8% in the chamber at 25°C. Absolute humidity was estimated to be 0.005 kg m⁻³. Lizards in their pouches were weighed every 1.5 h over a 7.5-h period to determine mass lost, and their positions both within the chamber and across the shelves were rotated at each weighing to control for any inconsistencies inside the closed chamber. Pouches were checked for urination and defecation at each weighing, and we did not observe any excreta during this experiment. Thus, mass lost over the trial time was assumed to be because of EWL, which was calculated as the rate of mass lost per hour. Lizards were tested once for EWL under each condition (preautotomy, postautotomy, and follow-up) with 1–2 mo between tests.

Metabolic Rate.—We measured resting metabolic rate to examine changes in energy requirements associated with tail regrowth. RMR_{O_2} and RMR_{CO_2} were measured at 25°C. Lizards

were fasted for at least 5 d prior to measurements to ensure postabsorptive status. Preautotomy measurements were taken 14 d prior to tail loss, postautotomy measurements were taken 2 d after tail loss, and follow-up measurements were taken 64 d after tail loss. Each lizard was placed into a large 150-mL plastic syringe with an air hole, and the syringe with the lizard was placed into a Thermo Scientific Environmental Chamber model 818 set to 25°C for at least 1 h of acclimation. As with EWL measurements, we chose 25°C to reduce activity and exposure to potentially stressful temperatures. After acclimation to the chamber temperature, each syringe was flushed with ambient air for 5 min by using an aquarium pump. The front of the syringe was closed, and the air hole was sealed before the animal in its syringe was placed back into the environmental chamber for an additional 1 h. Although we were not able to observe the lizards' behavior inside the chamber, the size of the syringe restricted activity to approximate resting behavior. We then injected a 20-mL subsample of air from the syringe into a flow of baseline air that was being analyzed for O₂ and CO₂ concentrations. Using a pump and flowmeter at the end of the system, we pulled air at 100 mL per minute. Air was first scrubbed of all water by using a magnesium perchlorate [Mg(ClO₄)₂] column and then passed through a Sable Systems CA-10 Carbon Dioxide Analyzer. After being scrubbed of remaining CO₂ and water using a Drierite/Ascarite/Drierite column, the air was sent through a Sable Systems FC-10 Oxygen Analyzer. Prior to testing, the carbon dioxide analyzer was calibrated using an Ascarite column to calibrate 0% CO₂ and a known gas concentration to calibrate 5% CO₂ gas; the oxygen analyzer was calibrated by using outside air scrubbed of both water and CO₂ and by assuming scrubbed air has a concentration of 20.95% fractional concentration of O₂. Following the conventions of Lighton (2008), the estimated rate of O₂ consumption for each 20-mL sample of injected air was calculated using ExpeData data analysis software (Sable Systems International) as follows:

$$M_sO_2 = \frac{[100\text{mL min}^{-1}(0.2094 - F'_eO_2)]}{(1 - 0.2094)}$$

where F'_eO_2 is the fractional concentration of O₂ in the sample. The O₂ consumption rate was then plotted as a function of time, and the volume of O₂ for each sample was determined by integrating under the curve of M_sO_2 with respect to time. We estimated the volume of gas consumed in the chamber with the following equation:

$$VolO_{2Final} = VolO_{2Sample} \cdot \left(\frac{Vol_{Syringe} - Vol_{Animal}}{Vol_{Sample}} \right)$$

where $VolO_{2Final}$ is the final volume of gas consumed by the lizard, $VolO_{2Sample}$ is the volume consumed in the sample, $Vol_{Syringe}$ is the syringe volume of 140 mL, Vol_{Animal} is the volume of the animal estimated using the animal's mass and assuming a density of 0.98 g × cm⁻³, and Vol_{Sample} is the sample volume of 20 mL. We then divided this volume of gas by total time sealed to determine the rate of O₂ consumption for each lizard in its container. Carbon dioxide concentration of each sample was used to calculate estimated CO₂ production rate in a similar manner. The following equation was used to determine the uncorrected sample rate of CO₂ production:

$$M_sCO_2 = [100\text{mL min}^{-1}(F'_iCO_2 - F'_eCO_2)]$$

where F'_iCO_2 is the fractional concentration of CO₂ at background levels and F'_eCO_2 is the fractional concentration of CO₂ in

the sample. Because the CO₂ analyzer was the first analyzer in the series, this calculation could not take the effects of CO₂ enrichment by O₂ depletion into account. The uncorrected volume of CO₂ produced (Vol_uCO_2) was calculated using integration as described for O₂ and then corrected for CO₂ enrichment using the following equation:

$$VolCO_2 = \frac{[Vol_uCO_2 - (F'_iCO_2 \cdot VolO_2)]}{(1 - F'_iCO_2)}$$

where $VolCO_2$ is the corrected volume of CO₂ produced in the sample, Vol_uCO_2 is the uncorrected volume of CO₂ produced in the sample, F'_iCO_2 is the fractional concentration of CO₂ at background levels measured for each trial, and $VolO_2$ is the volume of O₂ consumed in the sample. The rate of CO₂ production by each lizard was then calculated as described for O₂, using the corrected volume of CO₂. The CO₂ volume produced was used to define an RER for each lizard as CO₂ volume produced divided by O₂ volume consumed. Each individual was tested once for RMR under each condition (preautotomy, postautotomy, and follow-up) with 1–2 mo between tests for each individual.

Tail Movement.—Immediately after the autotomy process, we placed the autotomized tail onto a large piece of black foam core poster board to provide a dark background for easier viewing. Tail movement was filmed on a camera (Olympus Stylus TG 860) held by hand at a standard 3 feet above the tail. A timer was kept in frame at all times, and a ruler was taped onto the bottom of the board as a reference point for analysis. We stopped recording when the tail no longer moved over a period of 15 sec. The videos were analyzed using Kinovea software (version 0.8.15). Because some tails bent more strongly than others and consequently created more visual movement than others with a similar bend frequency, we measured the angle of the tail at every fifth bend. We defined a bend as the tail moving from a center line (straightened tail) to its furthest contraction on one side. To measure the angle of the tail, a vertex was set at the bottom of the tail base, with one ray extending down the base and the other ray reaching out to the tail tip. Along with the angle of the bend, time since the first bend was also recorded. These measurements were performed until the tail no longer underwent a single bend over a period of 10 sec, and this time was recorded as the end of movement; this was chosen on the assumption that the tail would no longer be an effective predator deterrent at that point and to provide a standardized stopping point.

Energy Stored in Tails.—After filming, we placed tails individually into plastic 15-mL conical-bottom tubes and transferred them to a Chromalox drying oven at a temperature of 60°C (Chromalox). Tails were allowed to dry for 2 mo, after which we homogenized them by first flattening the tails with a hammer and then grinding them using a coffee grinder (model E160B type cm03, Proctor-Silex). Once ground, the tails were placed back into the drying oven for 2 wk, after which we put them into a sealed jar. The jar contained Drierite to minimize moisture build-up during storage.

Prior to calorimetry, the individual tail grounds were pressed into pellets and weighed. We used a bomb calorimeter (IKA C 200H, IKA Works Inc.) to perform bomb calorimetry of the tails. To calibrate the calorimeter, we pressed three pellets of benzoic acid (C₇H₆O₂), weighed them, then burned them in the machine, and recorded the resulting temperature changes. From this information, we were able to find the heat capacity of the bomb calorimeter and its contents (C_v) using the equation

below:

$$-3,226 \text{ kJ mol}^{-1} = -C_v \Delta T + 0.05 \text{ kJ}$$

where $-3,226 \text{ kJ mol}^{-1}$ is the standard molar energy of combustion for benzoic acid, ΔT is the change in temperature shown on the calorimeter over the burn, and 0.05 kJ is the amount of heat liberated by the string within the bomb used to ignite the sample. After calculating the C_v for all three samples, we averaged them and found a value of $10.74 \text{ kJ}^\circ\text{C}^{-1}$, to be used in all further calculations. To get the energy of combustion (ΔU) in kJ for each of the tails, we used the equation:

$$\Delta U = 10.74 \text{ kJ}^\circ\text{C}^{-1} (\Delta T)$$

where ΔT is the change in temperature of the calorimeter during the burning of a tail.

Statistical Analyses.—To analyze differences in T_{pref} , EWL, RMR_{O₂}, RMR_{CO₂}, and RER across autotomy state (preautotomy, postautotomy, and follow-up), we used linear mixed effects models with autotomy state, sex, and preautotomy tail condition (original or previously regrown) as fixed effects, mass as a covariate, and lizard identity as a random effect. We log-transformed EWL (logEWL) before analysis to meet parametric model assumptions. We used linear models to examine if initial bend angle, slope of angle over time, time the tail spent moving, bends per second, and tail energy content differed by sex, preautotomy tail state (original or previously regrown), and tail mass (except for mass-specific energy content). We examined residual plots and used Shapiro–Wilk tests on residuals to determine if data met parametric model assumptions. Lastly, we visually inspected correlations between T_{pref} , EWL, RMR_{O₂}, RMR_{CO₂}, and RER. Likewise, we also investigated whether initial bend angle, angle slope over time, bends per second, time for tails to stop moving, and tail energy content could be related to each other through correlation plots. We report achieved power (β) for our major comparisons (T_{pref} , EWL, RMR_{O₂}, RMR_{CO₂}, and RER across autotomy state; and both tail energy content and tail movement time between sexes and tail type) in the respective results sections, assuming Cohen's recommended medium effect size of $F = 0.15$. All statistical analyses were conducted in the R programming language using the “lme4” package for model building, the “lmerTest” package for model interpretation, the “multcomp” package for post-hoc comparisons, and the “pwr” package for power determinations (R Core Team, 2019). Where unspecified, $P < 0.05$ was considered statistically significant. Numerical values are represented as mean \pm SEM.

RESULTS

Effects of Autotomy and Regrowth on Lizard Physiology.— T_{pref} was not significantly different among preautotomy, postautotomy, or follow-up time points ($F_{2,44} = 0.046$, $P = 0.96$; $\beta = 0.62$), with an overall mean of $33.7 \pm 0.2^\circ\text{C}$ (Table 2). Previous tail state, sex, and mass were not significant predictors of T_{pref} (tail: $F_{1,15} = 0.215$, $P = 0.65$; sex: $F_{1,18} = 0.858$, $P = 0.37$; mass: $F_{1,21} = 1.941$, $P = 0.18$). logEWL was significantly different among preautotomy, postautotomy, and follow-up time points ($F_{2,41} = 51.379$, $P < 0.001$; $\beta = 0.60$; Fig. 1). Post-hoc comparisons revealed that postautotomy logEWL was higher than preautotomy logEWL ($z = -5.881$, $P < 0.001$) and higher than follow-up logEWL ($z = 10.057$, $P < 0.001$). Follow-up logEWL was significantly lower than preautotomy logEWL ($z = 3.430$, $P = 0.002$; Fig. 1). Previous tail condition and mass were not significant predictors of logEWL

TABLE 2. Mean \pm SE (n) of physiological variables measured in *Podarcis siculus* before inducing caudal autotomy (preautotomy), after autotomy (postautotomy), and after partial tail regrowth (follow-up).

Time Point	T _{pref} (°C)	EWL (mg H ₂ O h ⁻¹)	SMR _{O₂} (mL O ₂ h ⁻¹)	SMR _{CO₂} (mL CO ₂ h ⁻¹)	RER
Preautotomy	33.99 \pm 0.46 (18)	19.16 \pm 1.45 (19)	1.56 \pm 0.11 (19)	1.29 \pm 0.08 (19)	0.84 \pm 0.02 (19)
Postautotomy	33.53 \pm 0.39 (19)	34.16 \pm 3.31 (19)	1.32 \pm 0.09 (19)	1.28 \pm 0.07 (19)	0.99 \pm 0.02 (19)
Follow-Up	33.68 \pm 0.36 (19)	13.47 \pm 1.37 (19)	1.32 \pm 0.08 (19)	1.12 \pm 0.06 (19)	0.86 \pm 0.01 (19)

(tail: $F_{1,15} = 4.177$, $P = 0.06$; mass: $F_{1,21} = 0.734$, $P = 0.40$). Sex was a significant predictor of logEWL ($F_{1,18} = 5.044$, $P = 0.04$).

Preautotomy, postautotomy, and follow-up values for RMR_{O₂} were not significantly different from each other ($F_{2,48} = 0.837$, $P = 0.44$; $\beta = 0.6$; Fig. 2), with an overall mean of 1.32 ± 0.09 mL O₂ h⁻¹ (Table 2). Previous tail state, sex, and mass were not significant predictors of RMR_{O₂} (tail: $F_{1,16} = 0.365$, $P = 0.55$; sex: $F_{1,19} = 0.080$, $P = 0.78$; mass: $F_{1,24} = 3.601$, $P = 0.07$). Preautotomy, postautotomy, and follow-up values for RMR_{CO₂} were significantly different from each other ($F_{2,49} = 4.643$, $P = 0.014$; $\beta = 0.68$). Post-hoc comparisons of means corrected for repeated tests using the Holm method showed that postautotomy RMR_{CO₂} was significantly higher than follow-up RMR_{CO₂} ($z = 2.992$, $P = 0.007$; Fig. 2). Preautotomy RMR_{CO₂} did not differ from postautotomy RMR_{CO₂} ($z = -1.248$, $P = 0.42$) or follow-up RMR_{CO₂} ($z = 1.001$, $P = 0.57$). Previous tail state, sex, and mass were not significant predictors of RMR_{CO₂} (tail: $F_{1,16} = 0.200$, $P = 0.66$; sex: $F_{1,19} = 0.017$, $P = 0.90$; mass: $F_{1,25} = 2.818$, $P = 0.11$).

RER (ratio of CO₂ produced and O₂ used) was significantly different among preautotomy, postautotomy, and follow-up points ($F_{2,51} = 18.484$, $P < 0.001$; $\beta = 0.70$; Fig. 3). Post-hoc comparisons of means revealed that postautotomy RER was significantly higher than preautotomy RER ($z = -5.022$, $P < 0.001$) and follow-up RER ($z = 5.530$, $P < 0.001$). Preautotomy RER and follow-up RER were not significantly different from each other ($z = -0.140$, $P = 0.99$). Previous tail state, sex, or mass were not significant predictors of RER (tail: $F_{1,51} = 0.270$, $P = 0.61$; sex: $F_{1,51} = 3.714$, $P = 0.06$; mass: $F_{1,51} = 1.635$, $P = 0.21$).

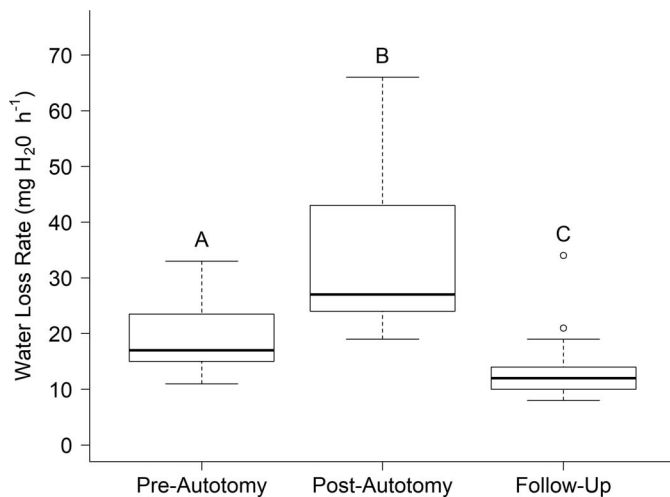


FIG. 1. Evaporative water loss (mg H₂O hr⁻¹) of *P. siculus* ($n = 19$) at 83 d prior to tail autotomy (preautotomy), 5 d after autotomy (postautotomy), and 32 d after autotomy (follow-up). Horizontal lines within each box indicate the median values, and the box boundaries indicate the lower quartile (lower boundary) and upper quartile (upper boundary). Whiskers indicate minimum and maximum values within a quartile ± 1.5 times the interquartile range; individual points are outliers that occurred outside this range. Different letters above each plot represent significant differences among means at those time points.

Correlation plots did not suggest relationships between EWL, metabolic rates, and T_{pref}.

Characteristics of Autotomized Tails.—The initial bend angle of tails immediately following autotomy was significantly higher in original tails ($F_{1,11} = 8.931$, $P = 0.0123$; Table 3). The angle of bend decreased over time in a linear fashion (original tails: $y = -2.083x + 315.59$, $R^2 = 0.405$; regenerated tails: $y = -1.004x + 117.05$, $R^2 = 0.163$). The slope of the bend angle over time did not differ between tail type (original or regenerated; $F_{1,11} = 0.019$, $P = 0.89$), and neither sex nor tail mass were significant predictors of initial bend angle (sex: $F_{1,11} = 0.381$, $P = 0.55$; mass: $F_{1,11} = 0.134$, $P = 0.721$) or change in bend angle over time (sex: $F_{1,11} = 2.014$, $P = 0.18$; mass: $F_{1,11} = 0.641$, $P = 0.44$, respectively). Original tails maintained movement for a significantly longer time than regenerated tails ($F_{1,11} = 5.480$, $P = 0.039$; $\beta = 0.25$; Table 3). Tail bends per second were not significantly different between tail types ($P = 0.12$), and neither time of movement nor bends per second were predicted by sex (time of movement, $P = 0.66$; bends per second, $P = 0.37$) or tail mass (time of movement, $P = 0.37$; bends per second, $P = 0.30$). Tail energy content per gram of tissue was not significantly different between tail types, but the average energy content of tails from females was significantly higher than average energy content of tails from males ($F_{1,14} = 9.968$, $P = 0.007$; $\beta = 0.30$; Table 3). Correlation plots did not

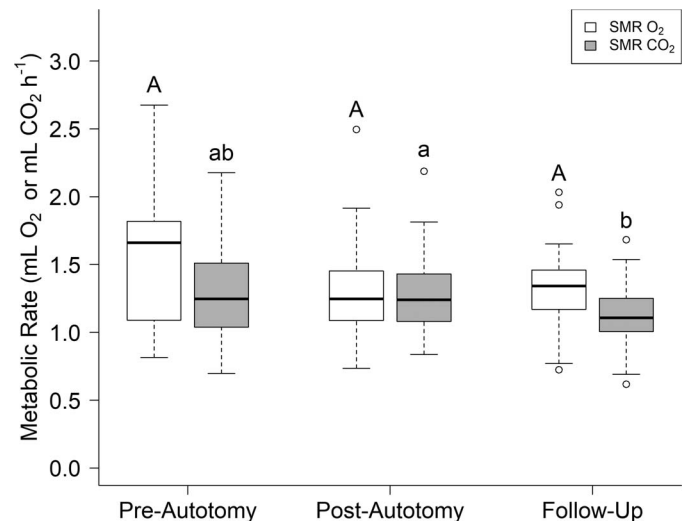


FIG. 2. Standard metabolic rate of *P. siculus* ($n = 19$) at 25°C at 14 d prior to tail autotomy (preautotomy), 2 d after autotomy (postautotomy), and 64 d after autotomy (follow-up). White plots show SMR_{O₂} (mL O₂ hr⁻¹); gray plots show SMR_{CO₂} (mL CO₂ hr⁻¹). Horizontal lines within each box indicate the median values, and the box boundaries indicate the lower quartile (lower boundary) and upper quartile (upper boundary). Whiskers indicate minimum and maximum values within a quartile ± 1.5 times the interquartile range; individual points are outliers that occurred outside this range. Different letters above each plot represent significant differences among means at those time points, with capital letters relating to SMR_{O₂} and lowercase letters relating to SMR_{CO₂}.

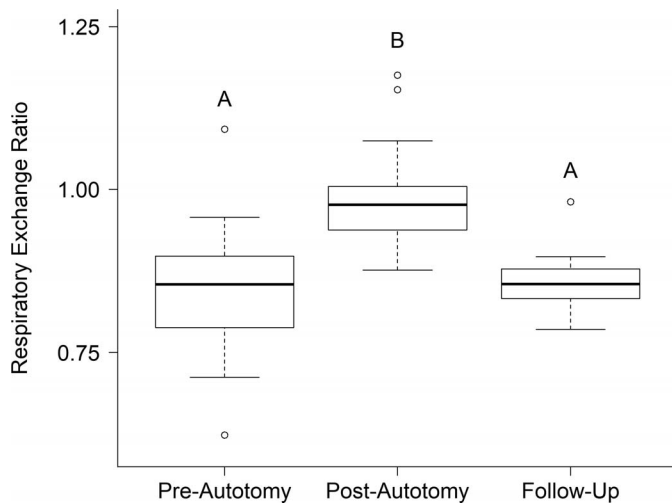


FIG. 3. Respiratory exchange ratio ($\text{mL CO}_2 \cdot \text{mL O}_2^{-1}$) of *P. siculus* ($n = 19$) at 14 d prior to tail autotomy (preautotomy), 2 d after autotomy (postautotomy), and 64 d after autotomy (follow-up). Horizontal lines within each box indicate the median values, and the box boundaries indicate the lower quartile (lower boundary) and upper quartile (upper boundary). Whiskers indicate minimum and maximum values within a quartile ± 1.5 times the interquartile range; individual points are outliers that occurred outside this range. Different letters above each plot represent significant differences among means at those time points.

suggest relationships with tail energy content and bends per second or tail movement over time.

DISCUSSION

Effects of Autotomy and Regrowth on Lizard Physiology.—Our study shows that Italian Wall Lizards respond physiologically to tail autotomy but do not adjust behavioral thermoregulation. EWL and RER increased postautotomy and then decreased (Figs. 1 and 2). Although we expected T_{pref} to increase because of the perceived benefits of an increased body temperature for wound healing (Smith et al., 1988) and increased rate of tail regeneration (Bellairs and Bryant, 1985), we did not find that T_{pref} changed after tail autotomy. Other studies have found similar results in reptiles (Martin and Salvador, 1993; Chapple and Swain, 2004; Zamora-Camacho et al., 2015; Fernández-Rodríguez et al., 2021) and amphibians (Tattersall et al., 2012) during appendage regrowth, despite having similar predictions.

With the increase in water loss during the regrowth process (Fig. 1), lizards may avoid a higher T_{pref} in an attempt to balance costs. Given that higher body temperatures would induce a greater rate of EWL and thermal stress (Kay and Nedderman, 1984; Liwanag et al., 2018), the costs may simply outweigh the benefit of faster healing and tissue growth, assuming these

benefits indeed occur. Our findings are consistent with other studies that have shown open and healing wounds have higher rates of EWL than fully intact tissues (Lamke, 1971; Lamke et al., 1977; Jonkman et al., 1987). Although increased respiration rate can lead to increased total EWL, we did not observe an increase in metabolic rate following autotomy, and in various reptiles, it has been estimated that over two-thirds of total EWL is cutaneous (Bentley and Schmidt-Nielsen, 1966). Consequently, we believe that the EWL change we measured reflects a change in epidermis permeability to water as opposed to a change in respiratory EWL. Because the impermeability of the epidermis is largely determined by the thickness of materials present (Maderson et al., 1978), both currently growing tissues and newly regenerated tissues may be too thin to effectively prevent water loss compared to older, intact tissues. Our lizards did indeed have open wounds immediately postautotomy; however, the regrowth process consists of continuously growing tissues rather than open wounds. Open wounds readily lose water, and even fully healed new tissues retain significantly higher rates of water loss than baseline measurements of healthy skin for some time posthealing (Surinchak et al., 1985). Although the scales of reptiles provide an extra barrier to prevent water loss, the tips of regenerating tails have a “prescale” region that does not have this extra barrier (Alibardi, 1995; Wu et al., 2013). Interestingly, Fernández-Rodríguez et al. (2021) found no difference in EWL between tailed and tailless *Podarcis bocagei* despite using similar methods. Because our study examined only three time points during the postautotomy and regeneration processes, we did not observe how EWL changed throughout the entire period of growth. As not much is known on this subject, and it would be an interesting direction for future studies.

Metabolic rate during tail regrowth has been studied in multiple lizard species with varied results. Geckos have been shown to both increase metabolic rate after autotomy (Dial and Fitzpatrick, 1981) and to show no change (Congdon et al., 1974; Fleming et al., 2009; Starostová et al., 2017), whereas iguanid species tend to show an increase in metabolic rate postautotomy (Naya et al., 2007). In this study, we found that O_2 consumption did not change significantly, although CO_2 production did decrease throughout the experiment (Fig. 2). It is difficult to determine a common trend of metabolic rate during regrowth across all lizards, as relatively few species have been documented thus far (Starostová et al., 2017). Interestingly, in the previously mentioned studies in which mealworms (*Tenebrio molitor*) were fed to lizards (Dial and Fitzpatrick, 1981; Naya et al., 2007), an increase in metabolic rate was observed after autotomy. In the studies in which no change was observed (Fleming et al., 2009; Starostová et al., 2017), lizards were offered crickets (undefined species) ad libitum, as in our study. Congdon et al. (1974) mentioned ad libitum feeding but did not

TABLE 3. Tail movement traits (postautotomy) and energy content for *Podarcis siculus* with respect to sex and tail condition. Values in cells are mean \pm SEM, with sample size reported in parentheses for tail energy content. Sample size in column n is reported for tail movement traits. Four tails were excluded from movement analyses, as follows: two from females with previous regeneration because of lack of autotomy, one from a male with previous regeneration because of a regrowth pattern that limited tail movement, and one from a male with an original tail because of an error in recording methodology.

Sex	Tail condition	n	Initial tail angle ($^\circ$)	Slope of angle change ($\Delta^\circ \Delta s^{-1}$)	Movement time (s)	Bend speed (bends s^{-1})	Energy content (J mg^{-1})
Female	Original	6	249 ± 38	-2.49 ± 0.57	97.5 ± 8.2	1.9 ± 0.1	24.0 ± 1.0 ($n = 5$)
	Regenerated	2	160 ± 94	-1.15 ± 0.83	71 ± 8.6	2.3 ± 0.4	24.3 ± 0.9 ($n = 4$)
Male	Original	3	312 ± 9	-0.58 ± 0.34	99.6 ± 8.2	1.8 ± 0.1	21.7 ± 0.6 ($n = 4$)
	Regenerated	4	113 ± 37	-1.56 ± 0.73	76.7 ± 11.6	2.0 ± 0.1	21.5 ± 0.5 ($n = 4$)

specify the type of food offered. Maginnis (2006) noted that multiple studies have found that the allocation costs of autotomy are visible only under limiting resources (low food levels). Crickets and mealworms have similar protein, lipid, and carbohydrate percentages (crickets: 66% protein, 16% lipid, 4% carbohydrate; mealworms: 66% protein, 14% lipid, 5% carbohydrate) (Stone et al., 2019). Consequently, the amount of food offered may have prevented us from seeing an increase in metabolic rate, although the specific use of crickets likely did not have an effect other than providing lipids that the lizards had lost. Specifically, lizards in our study may have responded to the increased energy demands for the tail regrowth process with increased food intake under ad libitum conditions instead of an increase in metabolic rate. Interestingly, an increase in RER and an apparent shift toward more use of carbohydrates as an energy source was still observed, even though lipids and proteins were available through food.

Because we measured both O_2 consumption and CO_2 production, we were also able to examine RER to provide insight into how the substrates used for metabolism change during the regrowth process. Average RER tends to stay between the values of 0.7 and 1.0; a ratio closer to 0.7 suggests a metabolic breakdown of mostly fats and lipids, whereas RER closer to 1.0 suggests a metabolic breakdown of more carbohydrates (Ferrannini, 1988). Intermediate values indicate that some mixtures of fats, carbohydrates, and proteins are being metabolized by the body (Ferrannini, 1988). For our lizards, the RER increased and closely approached 1.0 post-autotomy and then returned to the preautotomy level (about 0.85) as the tails regrew (Fig. 3), suggesting that the lizards are catabolizing more carbohydrates and may also be synthesizing lipids during regrowth, as lipogenesis is associated with RER values at or above 1.0 (Ferrannini, 1988). Talal et al. (2021) found that a high carbohydrate diet in locusts drove RER over 1 and that lipid accumulation rates were strongly correlated with dietary carbohydrate content and ingestion. Lipid synthesis would help restore reserves in the new tail. Because tails hold 40–80% of all fat stores in lizards (Bernardo and Agosta, 2005) and 56% of fat stores in *Lacerta vivipara* (Avery, 1974), tail autotomy likely depletes most of *P. siculus* lipid stores. In our study, lizards lost approximately 88% of their tails, suggesting they did indeed lose many lipids. It is likely quite important to replenish those reserves so they can rely on them in times of need. Fleming et al. (2009) hypothesized that tail loss led to reduced stamina by way of a loss of fat reserves. They consequently predicted a change in substrate use for energy following autotomy, which our findings partially support. Unfortunately, the literature on how limb loss in general affects RER is lacking. Most studies investigate how diet affects RER, although a study on hibernating Arctic ground squirrels suggested that changes in RER can occur because of changes in substrate use during different physiological states (during torpor in their study) (Karpovich et al., 2009). Most RER studies regarding animals focus on diet, whereas some human-based studies have found interesting results regarding RER in hospital patients. While looking at patients supported with mechanical ventilation, Hunker et al. (1980) found that patients who were fully supported by the hospital, from ventilation to parenteral diet, showed a mean RER of 1.23, whereas patients not requiring the same level of hospitalization had a mean RER of 0.88. Studies have found similarly high RER results in hospitalized patients with postoperative complications (Bar et al. 2020, 2021), indicating that RER can reach close to or surpass

the value of 1 in organisms at rest undergoing physiological processes that differ from the organisms' normal state.

Characteristics of Autotomized Tails.—We observed differences in autotomized tail activity between previously regrown tails and first-time losses. Although both tail types had the same number of bends per second and similar changes in bend angle over time, the previously regrown tails had a smaller initial bend angle and moved for a shorter amount of time than the original tails (Table 3). Our result contrasts with a previous study conducted on a congeneric species (*Podarcis erhardii*), which found no difference in the time it took for the tails to stop moving when compared by regeneration status (Simou et al., 2008). Following autotomy, tails grow back differently compared with original tails. The regenerated tail has a partially ossified cartilage rod when compared to articular vertebrae, muscles are thinner, and ganglia along the spinal cord are not regrown but, instead, spinal nerves are forced to sprout from the point of autotomy and travel into the new tail (Fisher et al., 2012; Gilbert et al., 2013). The aforementioned factors may contribute to the smaller bend angles exhibited by the previously regrown tails, with weaker muscles and weaker nerves resulting in less extreme movement. New tails have also been found to accumulate less lactate per gram of tissue during thrashing than original tails (Meyer et al., 2002). As movement in tails is maintained by energy supplied through the anaerobic conversion of glycogen into lactate (Dial and Fitzpatrick, 1981; Gleeson, 1996; Pafilis et al., 2007), a reduced accumulation of lactate is consistent with reduced activity in previously regenerated tails. Indeed, other studies have suggested using lactate accumulation as an index for the vigor of tail thrashing because of the important task of lactate creation to send required energy to the tail muscles (Meyer et al., 2002; Pafilis et al., 2007). In addition to tail physiology, lizard behavior postautotomy is well studied, demonstrating that lizards can learn and remember how to adjust their movement following subsequent tail losses and that lizards may flee more readily after a first autotomy (Brown et al., 1995; Cooper, 2007), which can result in increased predation by actively foraging predators (Downes and Shine, 2001). The ecological consequences of tail movement itself, however, are not fully understood. Previous research has shown that autotomized tails can increase a lizard's escape time from mammalian and snake predators by 40% in *Scincella lateralis* (Dial and Fitzpatrick, 1983). Although tail movement helps distract would-be predators, more work needs to be done to determine if differences in tail vigor influence escape success.

Although structural and biological differences may explain why original and regrown tails differed in movement, we found little difference in energy contained in the autotomized tail between these two tail types (Table 3). Our results contrast with previous findings that regenerated tails have a higher lipid and energy content than original tails in various gecko species (Vitt et al., 1977). One would expect that the original and regrown tails would differ in their energy content because of their dissimilarities in composition, as follows: bone versus cartilage, thickness of muscles, and other tissue differences (Woodland, 1920; Bellairs and Bryant, 1985; McLean and Vickaryous, 2011; Gilbert et al., 2013). However, as tails store important lipid reserves, it makes sense for lizards to store similar amounts of energy in their tails before and after autotomy. This may explain trends found in lizards that experience caudal autotomy at a young age; despite having smaller tails in adult life, the lizards grow at similar rates and to similar adult sizes as those that never underwent autotomy in their youth (Vitt and Cooper,

1986; Starostová et al., 2017). If regenerated tails stored less energy content than original tails, it would be difficult for these growing juveniles to keep up with lizards possessing original tails. As we could not control for consistent lengths of previous tail autotomy, it would be interesting to see future studies examining previous regeneration versus first-time loss by using controlled lab autotomy as opposed to wild autotomy.

An interesting outcome from the bomb calorimetry trials came in the form of sex differences. Females had a higher average amount of energy content (J mg^{-1}) in their tails than males (Table 3). Differences in energy needs between the two sexes may provide an explanation for this phenomenon. Studies have shown that females that undergo caudal autotomy tend to produce smaller clutch sizes and smaller massed eggs than those with intact tails (Dial and Fitzpatrick, 1981; Chapple et al., 2002). As females require large amounts of energy during reproduction, it is important that they have enough fat stores to both produce eggs and survive in the wild. The need for sufficient energy reserves for reproduction would require females to have more stored energy than males and consequently more stored energy in their tails, as they have larger overall energetic expenses.

Conclusions.—Our study elucidates some of the physiological tradeoffs associated with the antipredator strategy of caudal autotomy. Increased EWL postautotomy is a potential cost to healing lizards during the regeneration process. Changes in RER indicate a change in substrates used for maintenance. It is possible that observed RER reflected a loss of lipids, an effort to replenish lipid stores, or both scenarios. We also found that females stored more energy in tails per gram tissue, likely because females have different energetic demands than males. Additionally, through our investigation of tail movement, we found potential detriments of repeated autotomy because of reduced tail movement in previously autotomized tails, which may affect predator responses. Overall, autotomy appears to have physiological costs, revealing tradeoffs associated with this antipredator behavior.

Acknowledgments.—We thank Gregory Pauly, Riley Williams, Bree Putnam, Neftali Camacho, and Estella Hernandez for helping to collect the animals in San Pedro under a Scientific Collecting Permit issued by the California Department of Fish and Wildlife to Gregory Pauly (SCP-4307). All animal use protocols were evaluated and approved by the Institutional Animal Care and Use Committee at Cal Poly (protocol 1514). Special thanks to Jason Blank for his guidance and for allowing us to use his metabolic equipment. We thank Madeline McDonald for her assistance with analyzing tail movement. This study was funded by the College of Science and Mathematics at California Polytechnic State University, San Luis Obispo, and a Bill and Linda Frost Undergraduate Student Research Scholarship to Paula Eberle.

LITERATURE CITED

- ALIBARDI, L. 1995. Electron microscope analysis of the regenerating scales in lizards. *Italian Journal of Zoology* 62:109–120.
- EVERY, R. A. 1974. Storage lipids in the lizard *Lacerta vivipara*: a quantitative study. *Journal of Zoology* 173:419–425.
- BAR, S., C. GRENEZ, M. NGUYEN, B. DE BROCA, E. BERNARD, O. ABOU-ARAB, B. BOUHEMAD, E. LORNE, AND P. G. GUINOT. 2020. Predicting postoperative complications with the respiratory exchange ratio after high-risk noncardiac surgery: A prospective cohort study. *European Journal of Anaesthesiology* 37:1050–1057.
- BAR, S., D. SANTARELLI, AND E. LORNE. 2021. Predictive value of the respiratory exchange ratio for the occurrence of postoperative complications in laparoscopic surgery: a prospective and observational study. *Journal of Clinical Monitoring and Computing* 35:849–858.
- BARBADILLO, L. J., D. BAUWENS, F. BARAHONA, AND M. J. SÁNCHEZ-HERRAÍZ. 1995. Sexual differences in caudal morphology and its relation to tail autotomy in lacertid lizards. *Journal of Zoology* 236:83–93.
- BATEMAN, P. W., AND P. A. FLEMING. 2009. To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology* 277:1–14.
- BELLAIRS, A., AND S. V. BRYANT. 1985. Autotomy and regeneration in reptiles. *Biology of the Reptilia* 15:303–410.
- BENTLEY, P. J., AND K. SCHMIDT-NIELSEN. 1966. Cutaneous water loss in reptiles. *Science* 151:1547–1549.
- BERNARDO, J., AND S. J. AGOSTA. 2005. Evolutionary implications of hierarchical impacts of nonlethal injury on reproduction, including maternal effects. *Biological Journal of the Linnean Society* 86:309–331.
- BROCK, K. M., P. A. BEDNEKOFF, P. PAFILIS, AND J. FOUFOPOULOS. 2015. Evolution of antipredator behavior in an island lizard species, *Podarcis erhardii* (Reptilia: Lacertidae): the sum of all fears? *Evolution* 69:216–231.
- BROWN, R. M., D. H. TAYLOR, AND D. H. GIST. 1995. Effect of caudal autotomy on locomotor performance of wall lizards (*Podarcis muralis*). *Journal of Herpetology* 29:98–105.
- CARRTERO, M. A., E. MARCOS, AND P. DE PRADO. 2006. Intraspecific variation of preferred temperatures in the NE form of *Podarcis hispanica*. Pp. 55–64 in C. Corti, P. Lo Cascio, and M. Biaggini (eds.), *Mainland and Insular Lacertid Lizards: A Mediterranean Perspective*. Firenze University Press, Italy.
- CHAPPLE, D. G., AND R. SWAIN. 2002. Distribution of energy reserves in a viviparous skink: does tail autotomy involve the loss of lipid stores? *Austral Ecology* 27:565–572.
- CHAPPLE, D. G., AND R. SWAIN. 2004. Caudal autotomy does not influence thermoregulatory characteristics in the metallic skink, *Niveoscincus metallicus*. *Amphibia-Reptilia* 25:326–333.
- CHAPPLE, D. G., C. J. MCCOULL, AND R. SWAIN. 2002. Changes in reproductive investment following caudal autotomy in viviparous skinks (*Niveoscincus metallicus*): lipid depletion or energetic diversion? *Journal of Herpetology* 36:480–486.
- CLAUSE, A. R., AND E. A. CAPALDI. 2006. Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 305:965–973.
- CONGDON, J. D., L. J. VITT, AND W. W. KING. 1974. Geckos: adaptive significance and energetics of tail autotomy. *Science* 184:1379–1380.
- COOPER, W. E. 2007. Compensatory changes in escape and refuge use following autotomy in the lizard *Sceloporus virgatus*. *Canadian Journal of Zoology* 85:99–107.
- COOPER, W. E., AND K. J. ALFIERI. 1993. Caudal autotomy in the Eastern Garter Snake, *Thamnophis s. sirtalis*. *Amphibia-Reptilia* 14:86–89.
- COWGELL, J., AND H. UNDERWOOD. 1979. Behavioral thermoregulation in lizards: a circadian rhythm. *Journal of Experimental Zoology* 210:189–194.
- DEICHEL, G., G. NAFIS, AND J. HAKIM. 2010. Geographic distribution: *Podarcis siculus*. *Herpetological Review* 41:513–514.
- DIAL, B. E., AND L. C. FITZPATRICK. 1981. The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia* 51:310–317.
- DIAL, B. E., AND L. C. FITZPATRICK. 1983. Lizard tail autotomy: function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science* 219:391–393.
- DINSMORE, C. E. 1977. Tail regeneration in the plethodontid salamander, *Plethodon cinereus*: induced autotomy versus surgical amputation. *Journal of Experimental Zoology* 199:163–175.
- DOUGHTY, P., R. SHINE, AND M. S. LEE. 2003. Energetic costs of tail loss in a montane scincid lizard. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 135:215–219.
- DOWNES, S., AND R. SHINE. 2001. Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* 82:1292–1303.
- DUBOST, G., AND J. GASC. 1987. The process of total tail autotomy in the South-American rodent, *Proechimys*. *Journal of Zoology* 212:563–572.
- EMBERTS, Z., C. W. MILLER, D. KEIHL, AND C. M. MARY. 2017. Cut your losses: self-amputation of injured limbs increases survival. *Behavioral Ecology* 28:1047–1054.
- FERNÁNDEZ-RODRÍGUEZ, I., F. M. BARROSO, AND M. A. CARRTERO. 2021. An integrative analysis of the short-term effects of tail autotomy on

- thermoregulation and dehydration rates in wall lizards. *Journal of Thermal Biology* 99:102976.
- FERRANNINI, E. 1988. The theoretical bases of indirect calorimetry: a review. *Metabolism* 37:287–301.
- FIRTH, B. T., AND I. BELAN. 1998. Daily and seasonal rhythms in selected body temperatures in the Australian lizard *Tiliqua rugosa* (Scincidae): field and laboratory observations. *Physiological Zoology* 71:303–311.
- FISHER, R. E., L. A. GEIGER, L. K. STROIK, E. D. HUTCHINS, R. M. GEORGE, D. F. DENARDO, K. KUSUMI, J. A. RAWLS, AND J. WILSON-RAWLS. 2012. A histological comparison of the original and regenerated tail in the Green Anole, *Anolis carolinensis*. *The Anatomical Record* 295:1609–1619.
- FITZPATRICK, L. J., M. OLSSON, L. M. PARSLEY, A. PAULINY, G. M. WHILE, AND E. WAPSTRA. 2019. Tail loss and telomeres: consequences of large-scale tissue regeneration in a terrestrial ectotherm. *Biology Letters* 15: 20190151.
- FLEMING, P. A., L. VERBURGT, M. SCANTLEBURY, K. MEDGER, AND P. W. BATEMAN. 2009. Jettisoning ballast or fuel? Caudal autotomy and locomotor energetics of the Cape dwarf gecko *Lygodactylus capensis* (Gekkonidae). *Physiological and Biochemical Zoology* 82:756–765.
- GILBERT, E. A. B., S. L. PAYNE, AND M. K. VICKARYOUS. 2013. The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological and Biochemical Zoology* 86:631–644.
- GILLIS, G. B., L. A. BONVINI, AND D. J. IRSCHICK. 2008. Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis*. *The Journal of Experimental Biology* 212:604–609.
- GLEESON, T. T. 1996. Post-exercise lactate metabolism: a comparative review of sites, pathways, and regulation. *Annual Review of Physiology* 58:565–581.
- HARE, K. M., S. PLEDGER, M. B. THOMPSON, J. H. MILLER, AND C. H. DAUGHERTY. 2006. Daily patterns of metabolic rate among New Zealand lizards (Reptilia: Lacertilia: Diplodactylidae and Scincidae). *Physiological and Biochemical Zoology* 79:745–753.
- HEATWOLE, H., AND J. E. N. VERON. 1977. Vital limit and evaporative water loss in lizards (Reptilia, Lacertilia): a critique and new data. *Journal of Herpetology* 11:341–348.
- HERTZ, P. E., R. B. HUEY, AND R. D. STEVENSON. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *The American Naturalist* 142:796–818.
- HUEY, R. B. 1982. Temperature, physiology, and the ecology of reptiles. *Biology of the Reptilia* 12:25–91.
- HUNKER, F. D., C. W. BRUTON JR., E. M. HUNKER, R. M. DURHAM, AND C. L. KRUMDIECK. 1980. Metabolic and nutritional evaluation of patients supported with mechanical ventilation. *Critical Care Medicine* 8: 628–632.
- JONKMAN, M. F., I. MOLENAAR, P. NIEUWENHUIS, AND H. J. KLASSEN. 1987. Evaporative water loss and epidermis regeneration in partial-thickness wounds dressed with a fluid retaining versus a clot inducing wound covering in guinea pigs. *Scandinavian Journal of Plastic and Reconstructive Surgery* 23:29–34.
- JUSUFI, A., D. I. GOLDMAN, S. REVZEN, AND R. J. FULL. 2008. Active tails enhance arboreal acrobatics in geckos. *Proceedings of the National Academy of Sciences of the United States of America* 105:4215–4219.
- KARPOVICH, S. A., Ø. TØIEN, C. L. BUCK, AND B. M. BARNES. 2009. Energetics of arousal episodes in hibernating arctic ground squirrels. *Journal of Comparative Physiology B* 179:691–700.
- KAY, J. M., AND R. M. NEDDERMAN. 1984. *An Introduction to Fluid Mechanics and Heat Transfer*. Cambridge University Press, UK.
- LAMKE, L. 1971. The influence of different “skin grafts” on the evaporative water loss from burns. *Scandinavian Journal of Plastic and Reconstructive Surgery* 5:82–86.
- LAMKE, L., G. E. NILSSON, AND H. L. REITHNER. 1977. The evaporative water loss from burns and the water-vapour permeability of grafts and artificial membranes used in the treatment of burns. *Burns* 3:159–165.
- LIGHTON, J. R. 2008. *Measuring Metabolic Rates: A Manual for Scientists*. Chapter 4: Constant Volume Techniques Using Gas Analysis (pp. 23–46). Oxford University Press, UK.
- LIN, J., Y. CHEN, Y. WANG, K. HUNG, AND S. LIN. 2017. Tail regeneration after autotomy revives survival: a case from a long-term monitored lizard population under avian predation. *Proceedings of the Royal Society B: Biological Sciences* 284:20162538.
- LIN, Z., AND X. JI. 2005. Partial tail loss has no severe effects on energy stores and locomotor performance in a lacertid lizard, *Trakydromus septentrionalis*. *Journal of Comparative Physiology* 175:567–573.
- LIWANAG, H. E. M., D. HARO, B. CALLEJAS, G. LABIB, AND G. B. PAULY. 2018. Thermal tolerance varies with age and sex for the nonnative Italian Wall Lizard (*Podarcis siculus*) in Southern California. *Journal of Thermal Biology* 78:263–269.
- MADERSON, P. F., A. H. ZUCKER, AND S. I. ROTH. 1978. Epidermis regeneration and percutaneous water loss following cellophane stripping of reptile epidermis. *Journal of Experimental Zoology* 204: 11–32.
- MAGINNIS, T. L. 2006. The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology* 17: 857–872.
- MARTIN, J., AND A. SALVADOR. 1993. Thermoregulatory behavior of rock lizards in response to tail loss. *Behaviour* 124:123–136.
- MARTIN, J., J. J. LUQUE-LARENA, AND P. LOPEZ. 2009. When to run from an ambush predator: balancing crypsis benefits with costs of fleeing in lizards. *Animal Behavior* 78:1011–1018.
- MC ELROY, E. J., AND P. J. BERGMANN. 2013. Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* 86:669–679.
- McKEE, R. C., AND G. H. ALDER. 2010. Tail autotomy in the central American spiny rat, *Proechimys semispinosus*. *Studies on Neotropical Fauna and Environment* 37:181–185.
- McLEAN, K. E., AND M. K. VICKARYOUS. 2011. A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius*. *BMC Developmental Biology* 11:50.
- McVEAN, A. 1975. Autotomy. *Comparative Biochemistry and Physiology Part A: Physiology* 51:497–498.
- MEYER, V., M. R. PREEST, AND S. M. LOCHETTO. 2002. Physiology of original and regenerated lizard tails. *Herpetologica* 58:75–86.
- NAYA, D. E., C. VELOSO, J. L. P. MUNOZ, AND F. BOZINOVIC. 2007. Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology* 146:189–193.
- PAFILIS, P., V. PÉREZ-MELLADO, AND E. VALAKOS. 2007. Postautotomy tail activity in the Balearic lizard, *Podarcis lilfordi*. *Naturwissenschaften* 95:217–221.
- PARKER, S. L. 2014. Physiological ecology of the ground skink, *Scincella lateralis* in South Carolina: thermal biology, metabolism, water loss and seasonal patterns. *Herpetological Conservation and Biology* 9: 309–321.
- R CORE TEAM. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- RUXTON, G. D., T. N. SHERRATT, AND M. P. SPEED. 2004. *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals, and Mimicry*. Oxford University Press, UK.
- SEIFERT, A. W., S. G. KIAMA, M. G. SEIFERT, J. R. GOHEEN, T. M. PALMER, AND M. MADEN. 2012. Skin shedding and tissue regeneration in African Spiny Mice (*Acomys*). *Nature* 489:561–565.
- SIMOU, C., P. PAFILIS, A. SKELLA, A. KOURKOULI, AND E. D. VALAKOS. 2008. Physiology of original and regenerated tails in Aegean Wall Lizard (*Podarcis erhardii*). *Copeia* 2008:504–509.
- SMITH, D. A., I. K. BARKER, AND O. B. ALLEN. 1988. The effect of ambient temperature and type of wound on healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). *Canadian Journal of Veterinary Research* 52:120–128.
- STAROSTOVÁ, Z., L. GVOZDÍK, AND L. KRATOCHVÍL. 2017. An energetic perspective on tissue regeneration: The costs of tail autotomy in growing geckos. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 206:82–86.
- STONE, A. K., T. TANAKA, AND M. T. NICKERSON. 2019. Protein quality and physicochemical properties of commercial cricket and mealworm powders. *Journal of Food Science and Technology* 56:3355–3363.
- SURINCHAK, J. S., J. A. MALINOWSKI, D. R. WILSON, AND H. I. MAIBACH. 1985. Skin wound healing determined by water loss. *Journal of Surgical Research* 38:258–262.
- TALAL, S., A. CEASE, R. FARINGTON, H. E. MEDINA, J. ROJAS, AND J. HARRISON. 2021. High carbohydrate diet ingestion increases post-meal lipid synthesis and drives respiratory exchange ratios above 1. *Journal of Experimental Biology* 224:jeb240010.
- TATTERSALL, G. J., T. M. TYSON, J. R. LENCHYSHYN, AND R. L. CARLONE. 2012. Temperature preference during forelimb regeneration in the Red-Spotted Newt *Notophthalmus viridescens*. *Journal of Experimental Zoology* 317:248–258.
- VITT, L. J., J. D. CONGDON, AND N. A. DICKSON. 1977. Adaptive strategies and energetics of tail autotomy in lizards. *Ecology* 58:326–337.
- VITT, L. J., AND W. E. COOPER. 1986. Tail loss, tail color, and predator escape in *Eumeces* (Lacertilia: Scincidae): age-specific differences in costs and benefits. *Canadian Journal of Zoology* 64:583–592.

- WALL, M, AND R. SHINE. 2007. Dangerous food: lacking venom and constriction, how do snake-like lizards (*Lialis burtonis*, Pygopodidae) subdue their lizard prey? *Biological Journal of the Linnean Society* 91:719–727.
- WOODLAND, W. N. F. 1920. Some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Ruppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Quarterly Journal of Microspacial Science* 65:63–100.
- WU, P., L. ALIBARDI, AND C. CHUONG. 2013. Regeneration of reptilian scales after wounding: neogenesis, regional difference, and molecular modules. *Regeneration* 1:15–26.
- ZAMORA-CAMACHO, F. J., S. REGUERA, AND G. MORENO-RUEDA. 2015. Does tail autotomy affect thermoregulation in an accurately thermoregulating lizard? Lessons from a 2200-m elevational gradient. *Journal of Zoology* 297:204–210.
- ZANI, P.A. 1996. Patterns of caudal autotomy evolution in lizards. *Journal of Zoology* 240:201–220.

Accepted: 12 April 2022.

Published online: 30 November 2022.