Physiology of Original and Regenerated Tails in Aegean Wall Lizard (*Podarcis erhardii*)

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Caudal autotomy is a widespread antipredator mechanism among lizards. Thrashing the tail distracts the predator’s attention, facilitating the lizard’s escape. Regeneration occurs rapidly, but the regenerated tail differs from the original in many ways. In the present study, we examine probable physiological differences between, and the performance of, regenerated and original tails in Aegean Wall Lizard from Aegean islets. Autotomy was induced artificially. There was no difference in duration of tail movement. Moreover, we failed to detect any differences in lactate and glycogen concentrations. Differences in LDH activity and protein levels were not significant. However, lipid levels were higher in regenerated tails. Finally, regenerated tails show higher autotomy percentages when predation was simulated. We failed to detect large-scale physiological differences in regenerated tails (with the exception of lipids) in these particular populations.

Autotomy is one of the most effective antipredatory strategies and is particularly common among lizards (Bellairs and Bryant, 1985; Arnold, 1988). A portion of the tail is shed in response to pressure exerted by the predator (Arnold, 1984). The tail appendage thrashes vigorously in order to distract the predator’s attention from the escaping lizard (Vitt et al., 1977). Tail movement may be prolonged, and this protracted duration has been observed to increase predator-handling time, thus further facilitating escape (Congdon et al., 1974; Dial and Fitzpatrick, 1984; Daniels et al., 1986). Despite its efficiency, tail loss is considered a costly tactic, as it involves many effects (Arnold, 1984, 1988). These include loss of energy reserves (in species that store caudal fat) that are essential for somatic growth and reproductive output (Fox and McCoy, 2000; Doughty et al., 2003), impaired locomotory performance (Martin and Avery, 1998; Chapple and Swain, 2002a), losing social status (Fox et al., 1990; Martin and Salvador, 1995), and increased vulnerability to predators (Althoff and Thompson, 1994).

Most species display tail regeneration after autotomy, and the greater part is replaced rapidly (Arnold, 1988). The duration of tail growth varies considerably (Bryant and Bellairs, 1976; Dial, 1978) depending on the life history and longevity of the species (Arnold, 1988).

Tail regeneration often occurs to the detriment of body growth and reproduction (Dial and Fitzpatrick, 1981; Martin and Salvador, 1993), while also having an impact on behavior, habitat use and activity of tailless individuals (Formanowicz et al., 1990; Martin and Salvador, 1995, 1997). Regeneration is the most effective way to offset autotomy costs (Chapple and Swain, 2002b; Meyer et al., 2002), and once it has been completed, lizards regain lost status, locomotory abilities, and the autotomy mechanism (Vitt, 1981). However, regenerated tails differ in many ways from the originals (Bellairs and Bryant, 1985): morphologically (a cartilaginous tube replaces ossified vertebrae; regenerated muscles are not attached to the skeleton), neurologically (regenerated muscles are supplied with a branch derived from only a single nerve), and physiologically (regenerated tail displays reduced activity, Hughes and New, 1959; Clark, 1971; Duffy et al., 1992).

In the present paper, we attempted to assess whether the regenerated tail functions as an escape device as successfully as the original. In particular we examined the three main factors related to autotomy success: tail loss performance, duration of thrashing time of the tail, and levels of some physiological metabolites supporting postautotomy movement.

**MATERIALS AND METHODS**

**Studied species.**—We conducted the present study on the Aegean Wall Lizard (*Podarcis erhardii*), a small-bodied lacertid (SVL up to 70 mm, body mass 7.58 ± 1.4 g) that feeds on arthropods (mostly insects) and is found in stony places with low and bushy vegetation (Gruber, 1986). Its main predators are snakes (e.g., *Vipera ammodytes*). This species is widely distributed on the southern Balkan Peninsula and shows great differentiation throughout the Aegean islands, with over 25 subspecies. Specimens used in this study (91 total) were collected from six islets (Fig. 1): Dragonada in East Crete (24 animals), Nea Kammeni in Santorini (23 animals), Antikeros in Keros (16 animals), Glaronissi in Koufonisia (17 animals), and Ovriokastro in Paros (11 animals).

All animals were collected in the wild during the non-reproductive period (October–November) and in accordance with Greek National Law (Presidential Decree 67/81). We used only adult males in order to avoid biases due to age or sex (Bellairs and Bryant, 1985). The lizards were held at the laboratory facilities of the Biology Department at the University of Athens. Animals were housed separately in vitreous single terraria (20 cm × 25 cm × 15 cm) on a substrate of sand with stones provided as hiding places. The lizards were held at 25°C, under a controlled photoperiod (12h L:12h D), using fluorescent lights for a period of at least 3 weeks.

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having undergone any movement ("resting tails"). The liquid nitrogen immediately after autotomy and without an alternative treatment group, the tails were placed in concentrations of lactate and other components at time zero ("exhausted" tails). In order to rapidly determine baseline moment of autotomy to cessation of all movement once a tail was shed, we measured the time from the grasping the tail a distance of 25 mm from the cloaca. Each conditions, a pair of calipers acted as the predator’s jaws, reduce pressure variation and standardize experimental flooring, which allowed them to maintain good traction. To lizards were placed in a terrarium with a rough cork mat one end and two ice bags at the other (Van Damme et al., 1986), providing a temperature gradient ranging from 17°C to 55°C. In order to stimulate predation, we followed the technique proposed by Pérez-Mellado et al. (1997). The animals were allowed to thermo-regulate to their preferred body temperature before the experimental procedure, since ease of autotomy is often temperature dependent (Bustard, 1968; Daniels, 1984). We used a specially equipped terrarium (1 m × 20 cm × 25 cm) using two incandescent heating lamps (100 W and 60 W) at one end and two ice bags at the other (Van Damme et al., 1986), providing a temperature gradient ranging from 17°C to 55°C. In order to stimulate predation, we followed the technique proposed by Pérez-Mellado et al. (1997). The lizards were placed in a terrarium with a rough cork mat flooring, which allowed them to maintain good traction. To reduce pressure variation and standardize experimental conditions, a pair of calipers acted as the predator’s jaws, grasping the tail a distance of 25 mm from the cloaca. Each trial lasted a maximum of 15 s, and if by that time autotomy had not occurred, the lizard was returned to its terrarium. Once a tail was shed, we measured the time from the moment of autotomy to cessation of all movement ("exhausted" tails). In order to rapidly determine baseline concentrations of lactate and other components at time zero in an alternative treatment group, the tails were placed in liquid nitrogen immediately after autotomy and without having undergone any movement ("resting tails"). The choice of lizards comprising each group was random. We repeated that particular experimental process after regeneration was completed (nine weeks later) for all individuals that had initially autotomized. Predation simulation was applied only at the front of the regenerated tissue, that is, in the intact part of the tail. As with the original tails, we took precautions to obtain "resting" tails.

**Tissue glycogen determination.**—Glycogen was determined against a glucose standard by the indirect method of Seifert et al. (1950) after modifying the homogenization procedure (Pafilis et al., 2005). Measurements were read at 620 nm using a spectrophotometer (Novaspec II, Pharmacia Bio-tech).

**Tissue LDH activity determination.**—We measured lactate dehydrogenase (LDH) activity following the method of Kornberg (1955). We then homogenized 0.1–0.15 g of a tissue sample in proportion 1:10 with a special solution (0.1 M Tris/HCl pH 7.5, 1 mM EDTA). The homogenate was centrifuged at 4°C and 12,000 rpm for 1 min. We then prepared a reaction mixture (3 ml) containing 0.1 M Tris/HCl pH 7.3, NADH 0.1% (w/v), 0.15 M pyruvate acid, 6 mM KCN, and double distilled water. The reaction was initiated with the addition of 50 ml of the tissue homogenate; we then measured the decrease in absorption at 340 nm in relation to time.

**Tissue lactate determination.**—Initially, muscle tissue was separated from scales and bones under low temperature conditions. We used a special aluminum round table immersed by its shaft in liquid nitrogen (Pafilis et al., 2005). Subsequently, approximately 150 mg of tissue was homogenized (1:3 w/v) with 10% ice-cold perchloric acid in a cold mortar on ice. The homogenate was centrifuged at 4°C and 5,000 rpm for 10 min. The supernatant was then neutralized with 0.5 M Tris/0.5 M KOH and subsequently centrifuged at 4°C and 10,000 rpm for 10 min. The pellet was discarded, and the supernatant was used for the estimation of total lactate concentration according to the method described by Hohorst (1965). Lactate concentration was expressed as mg lactate/mg tissue.

**Tissue lipid determination.**—Extraction of total lipids was performed by homogenizing muscle tissue (30–40 mg) with 1.5 ml of a mixture containing two volumes of chloroform and one volume of absolute methanol. The homogenate was then centrifuged at 4°C and 3,000 rpm for 10 min. The pellet was used for protein analysis (see below), and the supernatant was used for the determination of total lipid concentration, using an appropriate kit (Chromatetest) according to the method described by Alexis et al. (1985). A mixture of olive oil and corn oil (2:1 v/v) was used as the standard.

**Tissue protein determination.**—Determination of total protein levels was performed using the Biuret method (Layne, 1957). Briefly, the centrifugation pellet obtained from the lipid analysis (see above) was dissolved in 0.5 ml of 0.1 N NaOH and incubated at 37°C for 30 min with occasional vortexing. We diluted 50 ml of the sample with 950 ml of H2O and added 4 ml of the Biuret Reagent. The mixture was incubated for 30 min at room temperature and the absorbance was read at 550 nm using a spectrophotometer.
Original and regenerated tails moved until exhaustion for an average 260 s and 261 s, respectively (Table 1). There was no statistically significant difference between original and regenerated tails ($t = 0.049$, df $= 71$, $P = 0.96$).

Lactate accumulation was correlated negatively to glycogen accumulation ($r = -0.72$, $P < 0.05$). Lactate concentration was increased in the exhausted tails in comparison to the resting tails, whereas glycogen concentration decreased respectively (MANCOVA Wilks’ $\Lambda_{4,40} = 0.198$, $P < 0.05$). We did not detect significant differences in lactate and glycogen accumulations between original and regenerated tails, either in resting or in exhausted tails (MANCOVA Wilks’ $\Lambda_{2,20} = 0.901$, $P = 0.35$). We failed to detect any difference in LDH activity between original and regenerated tails ($t = 0.126$, df $= 10$, $P = 0.90$; Table 1).

Protein concentration was the same in original and regenerated tails, independent of tail movement (ANCOVA: condition of tail $F_{1,55} = 0.198$, $P = 0.88$; movement status $F_{2,55} = 2.31$, $P = 0.11$). As expected, the differences in lipid concentrations between resting and exhausted tails were insignificant ($F_{1,51} = 2.18$, $P = 0.12$). However, the regenerated tails had higher lipid concentration than the original ones ($F_{2,51} = 7.29$, $P = 0.009$).

**DISCUSSION**

Rapid tail regeneration minimizes the complications caused by tail breakage and is therefore essential for the survival of tailless individuals. Tail autotomy ease was two-fold higher when predation was simulated for a second time in regenerated tails (87.18%) than in original tails (42.85%). It seems that the overall cost of autotomy is reduced after a prior tail loss, and lizards autotomize easier. Fox et al. (1998) reported that males that shed their tail previously have already fallen in social status and thus they have little to lose by proceeding in further autotomy. This could be the underlying explanation in our case where only males were used.

Average duration of postautotomy tail movement was similar in both cases. Our results agree with some previous studies (Meyer et al., 2002), while conflicting with others (Rumping and Jayne, 1996) due to differences in method-
We measured the major metabolites of postautotomy movement without detecting any significant difference between original and regenerated tails. Glycogen is the primary substrate for muscle anaerobiosis (Gleeson, 1982). Tail motion should cease when glycogen levels are insufficient to support it. Thus, it can be predicted that the same values will be obtained in both cases, since movement stops because of the lack of fuel. Our findings were similar to previous studies for tail (Pafilis et al., 2005) and whole-body tissues (Haley et al., 1987).

Average lactate concentration in tails after tail movement cessation ranged from 1.65 to 2.08 mg/g tissue. This value is lower in comparison to some geckos (2.71 mg/g tissue, Dial and Fitzpatrick, 1983; 2.25 mg/g tissue, Meyer et al., 2002) but comparable to other lacertids (Pafilis et al., 2005). Lactic acid, the by-product of anaerobic glycolysis, lowers pH in the tissues where it accumulates (Hogan et al., 1994). Muscle locomotory abilities are widely dependent on pH (Withers, 1992). The tail appendage would thrash until either glycogen is expended, or lactate levels reach a threshold inconducive to muscle activity levels (Brooks and Gaesser, 1980; Pafilis et al., 2005).

According to our results, which agree with previous studies (Meyer et al., 2002), LDH activity showed no difference between original and regenerated tails. The average for LDH activity was 314.61 mol/min/g, close to those that were estimated elsewhere (250 µmol/min/g, Meyer et al., 2002; 305 µmol/min/g, Pafilis et al., 2005). However metabolic changes during regeneration have been reported as impacting seriously the efficiency of other enzymes (Shah and Ramachandran, 1976). Stability in LDH performance may be attributed to the regulatory role it plays in anaerobic metabolism (Somerov, 1973; Bennett, 1974).

Our results are in full accordance with the only other study comparing the physiology of regenerated and original tails (Meyer et al., 2002), apart from the case of lactate accumulation reported to be lower in regrown tissues. Meyer et al. (2002) cited numerous possible explanations for that finding, including differences in protein content, nervous stimulation, concentration of anaerobic products, and substrate levels (e.g., glycogen). Lactate production depends mainly on continuous feed of substrate, LDH activity, duration of anaerobic metabolism, and secondary factors (e.g., temperature; Karlson, 1980). Duration is an extremely important factor in the case of an autotomized tail, because Cori's cycle (in which lactate converts back to pyruvate) is not possible since it occurs only in the liver (Gaesser and Brooks, 1980). Glycogen levels were similar in both cases, while LDH shows the same activity and thrashing duration did not differ as shown above. Although we found no differences in lactate levels, we believe that Meyer et al. (2002) are correct in seeking a measurement of thrashing intention and extension among anaerobic metabolites. Biochemical pathways and enzymes involved in anaerobic metabolism supporting tail motion remain unexplored and further extensive research is needed.

We failed to detect any significant difference in protein concentrations between original and regenerated tails. Protein concentration could be used as an indicator of tail content in contractile and non-contractile tissue (Meyer et al., 2002). Meyer et al. (2002) reported that original tails had higher protein levels and correlated that fact with lower contractibility of regrown tissues. We used the classical biuret method, which provides a general estimation of protein concentration, while Meyer et al. (2002) applied a more specific protein assay (Bradford Biorad kit). Our results for higher lipid levels in regenerated tails support the idea of a less contractile tail.

Contrary to the rest of our results, lipid levels were higher in the case of regenerated tails. That was certainly not an unexpected finding. Caudal fat is used as an energy store in many lizards (Daniels, 1984; Arnold, 1988). Morphology and structure are affected considerably by tail regeneration (Schall et al., 1989). Tissue content is altered, and more lipids are accumulated in the regenerated tail (Congdon et al., 1974; Vitt et al., 1977; Pond, 1981) to cover the increased needs of a tailless lizard to support the energy expense required in regeneration. Pianka and Vitt (2003) suggested that metabolism might be altered due to hormonal postautotomy changes and thus affect feeding rate. In our case there is one more underlying reason: in addition to the energy-consuming process of regeneration, lizards have to deal with the limited food availability of Mediterranean islets (Brown and Pérez-Mellado, 1994). A higher lipid level is an advantage offering increased chances for survival.

Comparing the two distinct situations in terms of postautotomy movement (exhausted and resting tails) we detected significant differences. Original and regenerated tails showed the same pattern in which exhausted tails differed from resting in the accumulation of higher and lower concentrations of lactate and glycogen, respectively. Not surprisingly, glycogen concentration was higher in resting tails, since no postautotomy movement was allowed and hence no anaerobic glycolysis occurred. On the contrary, in exhausted tails (in both cases) glycogen levels were expended in as much as it fuelled tail thrashing. Lactate reaches higher concentrations after prolonged exercise (Gleeson, 1996). Glycogen was converted into lactate in exhausted tails, and that fact explains the higher lactate levels in comparison to resting tails.

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