

## Tail tip removal for tissue sampling has no short-term effects on microhabitat selection by *Podarcis bocagei*, but induced autotomy does

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**Abstract.** Tail tip removal is a common method for obtaining tissue samples for genetics and other studies on lizards. This study evaluates the effect of tail tip autotomy on microhabitat selection in the lacertid *Podarcis bocagei*. Different-length tail fragments were experimentally removed from lizards of a small population. Forcing lizards to autotomise small tail tips (<1 cm) did not affect microhabitat selection. In contrast, a significant negative effect was observed in those lizards which underwent induced autotomy of the entire tail (> 5 cm). After autotomy these lizards were observed to favour more closed habitats, where predator avoidance is expected to be more efficient, although of potentially lower thermal quality.

**Keywords.** *Podarcis*, tip removal; tissue sampling.

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Material for genetic studies in lizards and for physiological and ecotoxicological investigations is usually obtained from tail-tip removal. Many lizard species have the ability to autotomize their tails when seized by predators (Arnold, 1984, 1988; Bellairs et al., 1985), which increases the probability of escape and survival, but may in turn entail long-term costs (Wilson, 1992; Downes and Shine, 2001; Niewiarowski et al., 1997). Since Arnold's (1984) comprehensive review of reptile caudal autotomy as a defensive behaviour over 20 years ago, our understanding of the costs associated with tail loss has increased remarkably (Bateman and Fleming, 2009). Lizards are known to significantly modify their behaviour in response to tail loss. Many of these changes are a by-product of decreased locomotor performance due to the absence of tail (e.g. Chapple and Swain, 2002). However,

altered behaviour has also been recorded without a concomitant measurement of reduced locomotory ability (reviewed in Bateman and Fleming, 2009). Although Arnold (1988) noted that tailless lizards are faced with an increased risk of predation through not having a tail to lose to a subsequent predator, few quantitative data on the behavioural responses to caudal autotomy were available at the time.

Lizards may change their habitat selection post-autotomy, due to locomotor restrictions, energetic requirements and/or the lack or reduced efficiency of this important antipredatory mechanism. For example, due to compromised mobility and balance, tailless lizards may use different habitats from tailed ones (Ballinger, 1973). From a physiological perspective, lizards may select open areas which are more favourable for thermoregulation (Martín and Salvador, 1992), presumably to speed up regeneration and recovery processes. However, lizards may also have to use sub-optimal habitats (Martín and Salvador, 1993), particularly those areas with greater cover, as a mechanism for reducing conflicts with conspecifics and exposure to predators (Martín and Salvador, 1992; Cooper, 2007). These observations are mainly based on studies focusing on natural autotomy of the entire tail or experiments simulating it. However, energetic and behavioural costs generated by tail tip removal cannot be assumed to be insignificant without previous investigation. In this context, as indicated above, behavioural changes in microhabitat use can be indicative of costs associated to overall locomotor performance, physiology and predation risk (Clause and Capaldi, 2006).

We studied a population of *Podarcis bocagei*, a lacertid lizard endemic to the north-western Iberian Peninsula to evaluate the effect of forced tail-tip removal for tissue sampling in relation to microhabitat use. The sampling site (Gião, NW Portugal 41.318°N; 8.676°W) was a small, physically restricted area comprising granite walls where lizards were easy to capture. The surrounding landscape consisted of an agroenvironment dominated by corn fields, patched with eucalypts (*Eucalyptus globulus*) and maritime pines (*Pinus pinaster*) and disseminated houses. We began sampling activities 30 min after the sun reached the rock walls. A total of 27 individuals grouped into three age/sex classes (9 juveniles, 9 adult females and 9 adult males; Table 1) with intact tails, were collected in July 2009. Our field experiment consisted of two steps. In the first step lizards were marked and underwent three different tail manipulative treatments - 0: no manipulation; 1: induced autotomy of the entire tail; 2: removal of a small tail tip (induced by hand; < 1 cm; Cordero et al., 1998). Afterwards, lizards were uniquely marked with a dorsal number painted with a marker pen to facilitate behavioural observations of habitat use and then released in the same microhabitat of capture. Microhabitat was categorised into four classes based on the percentage of vegetation cover - 1: totally covered (> 75% of vegetation); 2: partially covered (50-75%); 3: partially uncovered (25-50%); 4: totally uncovered (<25%). In the second step, one week later and at the same time and weather conditions than in the first survey, lizards were visually identified and the microhabitat at first sight during each transect was recorded. Transects were repeated four times ( $\approx$  one survey per hour) on the same side of the granite wall.

A General Linear Model (GLM) was performed in order to evaluate the effect of tail manipulation and age/sex (*Class*) on microhabitat selection. A post-hoc Duncan test was used to detect significant differences of tail manipulations and differences in habitat used by class.

Autotomy of a small segment of the tail (<1 cm) had no detectable effects on microhabitat selection, but autotomy of the entire tail had (Tables 1 and 2). This pattern was

**Table 1.** Differences in microhabitat selection [1: total cover (> 75% of vegetation); 2: partial cover (50-75%); 3: mostly uncovered (25-50%); 4: totally uncovered (<25%)] before and after tail manipulation (Treatment) among the three age/sex (Class) studied (M, adult males; F, adult females; J, juvenile).

Code	Class	SVL	Treatment	Before	After
				Microhabitat	Microhabitat
#1	M	56.11	Tail tip	3	4
#2	M	44.75	Tail tip	3	3
#3	M	57.14	No treatment	3	3
#4	M	50.24	No treatment	4	4
#5	M	50.76	No treatment	4	4
#6	M	50.23	Autotomy	4	2
#7	M	49.71	Autotomy	4	2
#8	M	49.19	Autotomy	3	1
#9	M	44.37	Tail tip	4	4
#10	F	54.23	Tail tip	4	3
#11	F	48.77	Tail tip	3	2
#12	F	46.90	Tail tip	2	2
#13	F	41.04	No treatment	3	3
#14	F	43.55	No treatment	3	3
#15	F	46.06	Autotomy	2	1
#16	F	46.64	Autotomy	3	1
#17	F	41.32	Autotomy	3	1
#18	F	40.75	No treatment	3	2
#19	J	38.17	Autotomy	3	1
#20	J	35.26	Tail tip	1	1
#21	J	32.35	Autotomy	1	1
#22	J	31.04	Autotomy	1	1
#23	J	30.38	Tail tip	1	3
#24	J	32.8	Tail tip	1	3
#25	J	30.11	No treatment	3	3
#26	J	31.72	No treatment	2	2
#27	J	31.87	No treatment	2	2

independent from *Class*, as the *Class\*Treatment* interaction term had no significant effect on microhabitat selection (Table 2). Results showed that independently of the *Class* all individual that we induced autotomy selected more cover microhabitats. In addition, results showed intrinsic differences before tail manipulation in microhabitat use among the three *Class* groups studied. At our study site, males selected more open microhabitats, juveniles used microhabitats with higher cover, with potentially lower thermal advantages and females displayed an intermediate microhabitat selection. The differences observed among the three *Class* groups in terms of microhabitat selection are in accordance with previous observations on this species (Galán, 1994).

Results suggest that collecting a small piece of tail (<1 cm), as usually carried out for genetic, physiological and ecotoxicological studies, has a negligible effect on the lizards'

**Table 2.** Results of General Linear Model (GLM) performed to evaluate the effect of tail manipulation and age and sex class (Class) in microhabitat selection before and after (Time) tail amputation or autotomy treatments.

	SS	df	MS	F	P
Intercept	337.500	1	337.500	569.531	< 0.001
Class	20.333	2	10.167	17.156	< 0.001
Treatment	9.333	2	4.667	7.875	< 0.01
Class*Treatment	0.667	4	0.167	0.281	> 0.05
Error	10.667	18	0.593		
Time	2.241	1	2.241	10.083	< 0.01
Time*Class	2.926	2	1.463	6.583	< 0.01
Time*Treatment	7.704	2	3.852	17.333	< 0.001
Time*Class*Treatment	1.630	4	0.407	1.833	> 0.05
Error	4.000	18	0.222		

behaviour in terms of microhabitat selection as compared to the effect observed due to forced tail autotomy. However, further studies are necessary to determine the maximum length of tail that may be amputated without producing behavioural responses in lizard microhabitat selection. Lin and Ji (2005) found that locomotor performance in *Takydromus septentrionalis*, a very long-tailed, grass runner lacertid, was almost unaffected by tail loss until at least more than 71% of the tail length was experimentally removed. The effects of tail autotomy on lizard survival and behaviour are well documented (for reviews see: Arnold, 1984, 1988; Maginnis, 2006; Bateman and Fleming 2009, Clause and Capaldi, 2006). Although tail autotomy is a common phenomenon in lizards, the highest rates have been associated to increased exposure to inefficient predation (Medel et al., 1998, Bateman and Fleming, 2011). Significantly, Bateman and Fleming (2011) showed that the frequency of regenerated tails in brown anoles was dependent of the behaviour of both the predator and the lizard. They provided empirical support for the hypothesis that predator efficiency, and not necessarily the number of predators, is the mechanism through which selection may act to retain tail autotomy as a defensive trait. The proportion of regenerated tails of adult *P. bocagei* species varied from 54.55% to 80.95% in different localities from coastal N Portugal (Carretero, unpublished). In the study area, the rate was 65%, hence falling within commonly found interval. Thus, the proximity of houses in the study areas likely favouring the presence of domestic cats as inefficient predator (according to Bateman and Fleming, 2011) did not severely altered inefficient predation pressure.

Microhabitat studies in lacertids have demonstrated behavioural changes in individuals as a consequence of tail autotomy (Martín and Salvador, 1992, 1993). Tailless lizards may become more cryptic, use different substrates, and shift to different environments. Thus, autotomy is expected to have relevant consequences on individual fitness (Arnold 1988; McConnachie and Whiting, 2003) in terms of costs associated with locomotion, tail regeneration, and, as shown here, on microhabitat selection. However, our study demonstrate that collecting a small piece of tail (<1 cm) does not result on short-term effects on microhabitat selection by *P. bocagei*, while forced caudal autotomy occurring at the extreme base of the tail may greatly reduce fitness of lizards.

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