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Departamento de Biología de Organismos y Sistemas
Programa de Doctorado en Biogeociencias

Conflictos funcionales y de asignación de recursos en los procesos de regeneración: consecuencias de la autotomía en lacértidos (Squamata: Lacertidae)

Functional and trade-off conflicts in regenerating processes: consequences of autotomy in lacertids (Squamata: Lacertidae)

TESIS DOCTORAL

Irene Fernández Rodríguez

Director: Dr. Florentino Braña Vigil
Codirector: Dr. Alfredo Fernández-Ojanguren García-Comas

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RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Conflictos funcionales y de asignación de recursos en los procesos de regeneración: consecuencias de la autotomía en lacértidos (Squamata: Lacertidae)	Inglés: Functional and trade-off conflicts in regenerating processes: consequences of autotomy in lacertids (Squamata: Lacertidae)

2.- Autor	
Nombre: Irene Fernández Rodríguez	DNI/Pasaporte/NIE:
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RESUMEN (en español)

Algunos de los factores que más influyen en los ciclos de vida de los organismos son la fenología de ciertos eventos clave y la cantidad de recursos destinados a ellos. La inversión en el desarrollo de un rasgo a menudo implica una menor cantidad de recursos disponibles para otros rasgos, desencadenando un conflicto de asignación de recursos o *trade-off*. Los *trade-offs* son muy comunes y tienen consecuencias tanto a nivel fisiológico como evolutivo, constituyendo incluso la base de varias teorías sobre evolución de ciclos de vida.

La autotomía es la pérdida voluntaria de una parte del cuerpo, como un acto reflejo cuando el animal se siente amenazado. Ocurre tanto en invertebrados como en vertebrados y su beneficio más común es permitir el escape de depredadores. La autotomía caudal es particularmente frecuente en reptiles, especialmente en saurios, en los que su uso como estrategia antidepredación está realmente extendido. Sin embargo, en muchas especies la cola juega un papel importante en la locomoción o en la acumulación de reservas, de manera que su pérdida puede tener consecuencias negativas a corto plazo. La regeneración tras la autotomía permite reemplazar las partes perdidas y restaurar sus funcionalidades asociadas, de manera que aumente la probabilidad de supervivencia y reproducción. Sin embargo, implica una gran demanda de energía y materiales que puede interferir con otros procesos con alta demanda, como el crecimiento o la reproducción.

El balance entre los costes y beneficios de la autotomía y de la regeneración moldea su evolución y su presencia o ausencia en los animales. Por consiguiente, el estudio de este balance y de sus implicaciones fisiológicas, funcionales y ecológicas, es un enfoque apropiado para entender la evolución de estos dos fenómenos. En este contexto, esta Tesis Doctoral aborda el estudio de los efectos de la autotomía y la regeneración sobre varios aspectos de los ciclos de vida y funciones vitales de los saurios, utilizando como especies modelo a los lacértidos *Podarcis muralis* y *P. bocagei*. En particular, se estudiaron los efectos de la autotomía caudal en la locomoción y el comportamiento antidepredación de *P. muralis*, y en la termorregulación e hidrorregulación de *P. bocagei.* Asimismo, se estudiaron los costes de la regeneración caudal sobre el crecimiento corporal de individuos juveniles y sobre la inversión reproductora de hembras adultas en *P. muralis*.

Nuestros resultados demostraron que la autotomía caudal perjudica algunos aspectos del rendimiento locomotor de las lagartijas adultas, principalmente reduciendo la efectividad de los saltos intercalados en secuencias de carrera rápida. Esto afectará al desempeño de actividades rutinarias que dependen de la movilidad y dificultará el escape ante nuevos ataques de depredadores, de manera que probablemente se enfrenten a un mayor riesgo de depredación. Sin embargo, las lagartijas sin cola frecuentemente recurren a comportamientos compensatorios que reducen la probabilidad de detección y aumentan la probabilidad de supervivencia inmediata, aunque a costa de reducir el rendimiento de otras actividades relevantes, como la obtención de alimento. La pérdida de la cola no parece afectar a otros aspectos comportamentales y fisiológicos, como el termopreferendo o las tasas de deshidratación.

La regeneración caudal permite restaurar la capacidad de locomoción y reduce los costes de la autotomía, aumentando la probabilidad de supervivencia. Sin embargo, el valor antidepredador de las colas, expresado por la intensidad y duración de sus movimientos tras la autotomía, se recupera sólo parcialmente tras la regeneración. La inversión en regeneración provoca una disminución en la tasa de crecimiento corporal de los juveniles, pero sólo cuando disponen de poco alimento, siendo la disponibilidad de alimento un factor determinante de la severidad de los costes de la regeneración. Asimismo, nuestros resultados sugieren que en juveniles la regeneración podría priorizarse incluso a costa del crecimiento corporal, para restaurar las funcionalidades perdidas lo antes posible y aumentar así la probabilidad de supervivencia. Por último, la inversión en regeneración provoca una reducción de la inversión reproductora de las hembras adultas, especialmente cuando ésta ocurre a la vez que la vitelogénesis realizada bajo una estrategia *income breeding.* Cuando la regeneración ocurre meses antes de la vitelogénesis, durante la fase de acumulación de reservas para la formación de la primera puesta anual bajo una estrategia *capital breeding,* los costes de la regeneración son relevantes pero menores que en el primer caso. La correlación negativa entre inversión en regeneración caudal y en desarrollo de la puesta evidencia un *trade-off* entre estos dos procesos.

RESUMEN (en Inglés)

Some of the main determinant of organisms' life histories are the timing of development of certain key events and the amount of resources allocated to them. Since resources are always limited in lifetime, the investment in the development of one trait will imply fewer resources available for other traits, causing a conflict of resource allocation (also known as allocation trade-off). Trade-offs are very common in organisms and have both physiological and evolutionary consequences, even constituting the base of several life history theories.

Autotomy is the voluntary loss of a body part as a reflex response when the animal is threatened. It is present both in vertebrates and invertebrates, and its most common benefit is facilitating the escape from predators. Among vertebrates, caudal autotomy is particularly common in reptiles and very specifically in lizards, among which it is widely used as an antipredator mechanism. However, the tail plays important roles in locomotion, social signalling and accumulation of reserves in many lizard species, so that its loss may have short-term negative consequences. Regeneration after autotomy allows the replacement of the lost pats and the restoration of their functionalities, which increases the probability of survival and reproduction. However, regeneration implies a great demand of energy and materials, so it may interfere with other highly demanding processes like growth or reproduction.

The balance between the costs and benefits of autotomy and regeneration shapes their evolution and is determinant for their presence or absence in animals. The study of this balance and its physiological, functional and ecological implications is an appropriate approach to understand the evolution of autotomy and regeneration. In this context, this PhD Thesis addresses the study of the effects of autotomy and regeneration on various aspects of the life cycles and whole-organism functions of lizards, in a scheme that can surely be extended to other animals, using the lacertids *Podarcis muralis* and *P. bocagei* as model species. In particular, we studied the effects of caudal autotomy on the locomotor performance and antipredator behaviour of *P. muralis*, and on the thermal preferences and hydroregulation of *P. bocagei*. Besides, we studied the effects of tail regeneration on juveniles' body growth rates and adult females' reproductive investment in *P. muralis*.

Our results showed that caudal autotomy impairs some aspects of adult wall lizards' locomotor performance, mainly by reducing the effectiveness of jumping dynamics interspersed in fast running sequences. This locomotor impairment will probably affect routine activities that depend on mobility and will difficult the escape from new predator attacks, so that lizards will probably face a higher predation risk. However, tailless lizards frequently resort to compensatory behaviours that reduce the probability of detection and increase the probability of immediate survival, but at the cost of reducing the performance of other important activities like foraging or mating. Tail loss apparently does not affect other behavioural and physiological aspects, like the thermal preference or the dehydration rates.

Regeneration allows the restoration of locomotor capacities, increasing the probability of survival and the efficiency of autotomy, by decreasing its associated costs. However, the antipredator value of tails, as expressed by the intensity and duration of their movements immediately after the autotomy, is only partially retrieved after regeneration. The investment in regeneration provokes a decrease in juveniles' body growth rates when there is a shortage of food, but not when food is supplied "ad libitum", being, therefore, the availability of food a determining factor for the severity of the costs of regeneration on growth. Moreover, our results suggest that caudal regeneration in juveniles could be prioritized even at the expenses of body growth, to restore the lost functionality as soon as possible and increase survivorship. Finally, the investment in tail regeneration provokes a reduction of the reproductive investment of adult females, especially when it occurs concurrently with vitellogenesis performed under an income breeding strategy. When regeneration occurs some months before vitellogenesis, during the phase of accumulation of reserves that are essential for the development of the first annual clutch under a capital breeding strategy, the costs of regeneration are relevant but lower than in the first case. The investment in tail regeneration is negatively correlated with the investment in clutch development, which evidences a trade-off between these two processes.

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A mis padres

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«Averigua de la mejor manera que puedas qué es lo que más quieres hacer en ciencia. Obedece esa pasión mientras dure. Aliméntala con el conocimiento que la mente necesita para crecer. (...) La decisión y el trabajo arduo basado en la pasión duradera nunca te abandonarán». Ese es, según Edward O. Wilson, uno de los principios que debe guiar a los jóvenes científicos en su elección del camino a seguir, y yo me siento muy afortunada por haber tenido el privilegio de ser y hacer durante estos años lo que siempre quise. Durante este tiempo he compartido muchas cosas con mucha gente sin la que probablemente este proyecto no hubiese salido adelante, y sin la que no sería la persona que soy hoy.

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“Their amazing powers of survival and regeneration following almost every imaginable mutilation pose questions that are difficult to resist trying to answer.” Goss, 1969 (*Principles of Regeneration*).

“Sus increíbles capacidades de supervivencia y regeneración tras casi cualquier mutilación imaginable plantean preguntas a las que es difícil resistirse a tratar de dar respuesta”.

Cover photos: *Podarcis muralis*, *Ambystoma mexicanum* and *Marthasterias glacialis*

I. GENERAL INTRODUCTION

General Introduction

Two thousand and four hundred years ago Aristotle noticed and stated that: "The tails of lizards and of serpents, if they be cut off, will grow again" (Aristotle, History of Animals, Book II, chapter 17, 508b 4-7). Among the great diversity of animals' life histories and adaptations, the ability to shed and regenerate body parts has intrigued scientists during centuries. These two abilities have arisen in many animal taxa from invertebrates to vertebrates, and its evolution within animals' life histories depends on many factors. The consequences of autotomy and regeneration may vary among different species, but also intra-specifically among different populations, sexes, life-stages or environmental conditions. Therefore, the study of the balance between the costs and benefits of autotomy and regeneration, including the analysis of their physiological and ecological implications, is an appropriate way to understand the evolution of autotomy and regeneration across different animal lineages.

Life histories and trade-offs

Some of the main determinant of life histories are the timing of development of certain key events and the amount of resources allocated to them. In other words: since resources are always limited in lifetime, decisions on when and where allocate them will shape individuals' life histories (Roff 1992; van der Meer 2019). In this scenario, the investment in the development of one trait will imply fewer resources available for other traits, causing a conflict of resource allocation (also known as allocation trade-offs; Stearns 1992). Trade-offs are very common in organisms and have both physiological and evolutionary consequences, even constituting the base of several life history theories (Stearns 1992). Allocation conflicts vary depending on the limiting factor: they may arise when devoting time to one activity implies the abandon of other activities, when the development of one functional trait constrains the development or performance of another one (functional trade-off), or when the limiting factor is the energy and resources available for the development of several traits (allocation trade-offs). Some classical examples of allocation trade-offs in animals are the investment in current vs. future reproduction, somatic growth vs. reproduction (which implies, in organism with indeterminate growth, the decision of when reaching sexual maturity), egg size vs. clutch size, etc (Elliott 1994; Roff 2002).

Van Noordwijk & de Jong (1986) illustrated the concept of trade-offs through the Y-model of resources allocation, with different situations in which two or more traits require resources from the same supply (Figure 1.1). In the Y-model diagram, an overall energy source diverges into two branches that represent two competing traits, expressing the conflict of allocation: a greater investment on trait A implies a poorer development of trait B, as both traits are negatively correlated (Fig. 1.1.A). Sometimes the trade-off may occur even when there is a positive correlation between traits, which often occurs when there is a great resource acquisition (i.e., there are enough resources to allow the development of both traits, Figure 1.1B), or when there is a high variability among individuals in the acquisition of resources (van Noordwicjk & de Jong 1986). In such regard, according to the metaphor “big house, big car” from Reznick *et al.* (2020), those individuals with high resource availability would be able to invest much in both traits (“big car and big house”), while those with few resources would invest little in both traits (“small car and small house”). In such case, it could be expected a positive correlation between traits at the inter-individual level, but it does not imply a positive correlation at the intra-individual level when there is a high variability among individuals. However, the model is frequently more complex, as it is common that more than two traits compete for resources from the same supply, leading to a hierarchical model of successive allocations, in a way that certain current investments can compete and incur in trade-offs with future investments (Figure 1.1C).

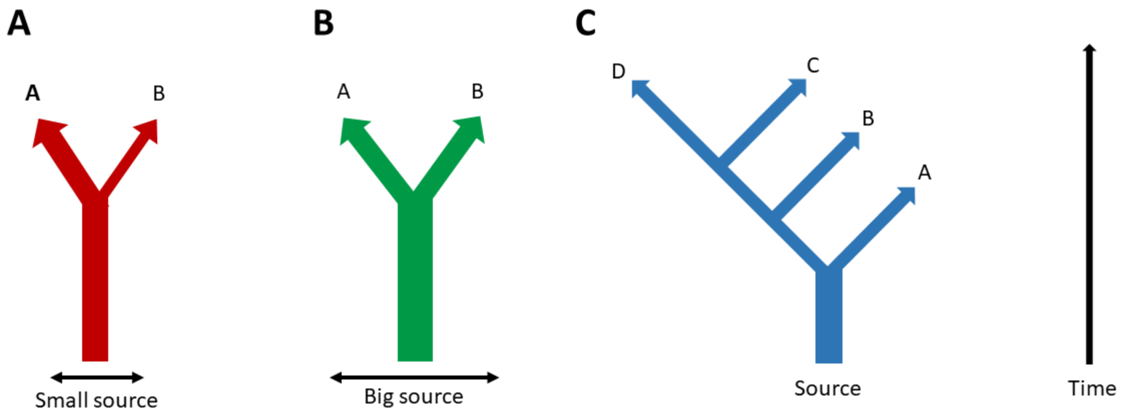

Figure 1.1. Y-model of resource allocation proposed by Van Noordwijk & de Jong (1986). **A**, with a small source, there are not enough resources for the development of two competing traits, so the investment in trait A implies less development of trait B, being both traits negatively correlated. **B**, if the source is big, there are enough resources for the development of both traits, and it may happen that there is no negative correlation between them. **C**, hierarchized allocation of resources to different traits (A-D) whose development is staggered in time (A, B, C, D).

Autotomy and regeneration of body parts, despite their potential benefits, may cause several functional and allocation trade-offs that may shape the presence or absence of these two adaptations in the animals (Maginnis 2006). Therefore, the study of the costs associated to these two abilities will shed light on their evolution.

Autotomy: origin, mechanics and consequences

Autotomy literally means "self-amputation", and it was firstly defined by Fredericq (1892) as the voluntary and/or programmed (i.e., not traumatic) loss of a body part as a reflex response when the animal is threatened. According to Emberts *et al.* (2019), autotomy has evolved independently at least nine times in different animal lineages, both in invertebrates and vertebrates: cnidarians, annelids, molluscs (gastropods, bivalves and cephalopods), platyhelminths, nematodes, phoronids, arthropods (arachnids, decapods and insects), echinoderms, hemichordates and vertebrates (amphibians, reptiles and mammals). Autotomy usually occurs through predefined fracture planes (also called "autotomy septa"), which facilitate the breakage and minimize the injury costs by reducing fluid loss, diminishing the probability of infections and accelerating wound healing (Maginnis 2006). Surviving sublethal predation is the most common benefit of autotomy (Fleming *et al.* 2007; Emberts *et al.* 2019) but, for some animals, autotomy also includes other benefits like solving molting complications (Maginnis 2008), eliminating toxins from a certain body part (Moore *et al.* 1989) or surviving physical damages caused by abiotic factors (Wulff 2006).

Among vertebrates, caudal autotomy is particularly common in reptiles and very specifically in lizards, among which it is really widespread, being present in 13 out of 20 saurian families (Clause & Capaldi 2006). Tail autotomy in lizards occurs trough preformed areas of weakness present in the postpygal vertebrae, called fracture planes, which are much more frequently intravertebral (e.g., lacertids, skinks, geckos, cordylids or teiids) than intervertebral (e.g., some agamids). Intravertebral fracture planes cross each vertebra transversely, passing through its centrum and neural arch; however, there is some variability among species on the exact position of the fracture plane within the vertebra: it can be near the middle or close to the anterior end (Bellairs & Bryant 1985). On the other hand, intervertebral autotomy presents no obvious caudal modifications, as it occurs between vertebrae. Intravertebral autotomy is the most common mechanism in lizards and seems to be the ancestral condition, while intervertebral autotomy apparently represents a re-evolution of the ability to shed the tail in certain groups that had lost intravertebral autotomy (Arnold 1988).

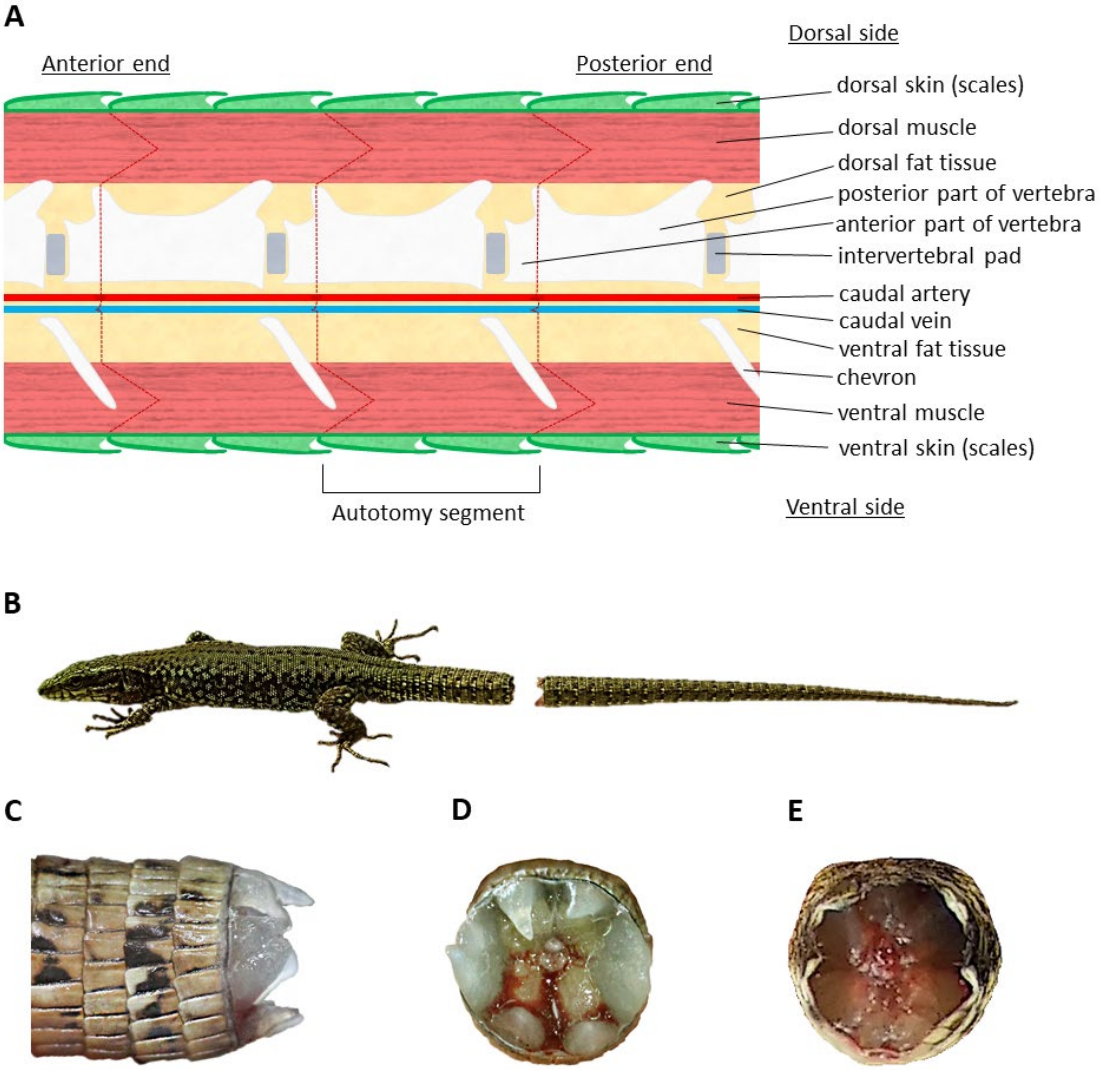

Figure 1.2. A, Schematic representation of a longitudinal section of an adult lizard's tail (midcaudal region), showing the autotomy septum (in dashed red lines) passing through the muscle, fat, vertebra and caudal vessels. Based on the semmidiagramatic longitudinal section through midcaudal region of adult *Zootoca vivipara* from Sheppard & Bellairs (1972) in Bellairs & Bryant (1985). **B**, Adult male wall lizard (*Podarcis muralis*) just after tail autotomy. **C**, **D**, lateral and transversal views of a detached tail of an adult male *P. muralis* immediately after autotomy. **E**, Transversal view of the tail stub of an adult male *P. bocagei* immediately after tail autotomy.

The characteristics and mechanisms of caudal autotomy in lizards, and particularly in lacertids, has been widely reviewed by Bellairs & Bryant (1985). The vertebral column in the postpygal region is surrounded by four bands of fat tissue, the caudal artery and vein (located ventrally), four bands of caudal muscles and an external layer of skin, including the epidermic scales (Figure 1.2). The fat and muscle bands are divided by septa of connective tissue (fracture planes), forming the autotomy segments (Sheppard & Bellairs 1972). Each autotomy segment is comprised of a segment of skin, a myomere (i.e., muscle segment), a fragment of fat tissue, the posterior part of one vertebra and the anterior part of the next vertebra (Figure 1.2). The fragment of skin of each autotomy segment has two scale rows in lacertid lizards, being frequently the anterior shorter than the posterior one, and the split occurs through the scale hinges in front of a short row (Moffat & Bellairs 1964). Grasping or fixing the tail of a lizard triggers its shedding at the point where it is grasped; tail autotomy begins with a strong, sudden lateral flexion whose strain is concentrated at a single fracture plane (in contrast to normal flexion of the tail, whose strain extends along several vertebrae). After this flexion, the skin breaks in the convex side and the muscles split, which triggers the separation of the remaining caudal tissues. Then there is another strong dorsoventral flexion (with a rotatory effect) that causes the rupture of the remaining muscles (Sheppard & Bellairs 1972). After the complete shedding of the tail, the skin surrounding the proximal edge contracts and the remaining posterior projections of the caudal muscles immediately adhere to the central area of the stump, “closing” and protecting the injury (Figure 1.2E). The caudal artery and vein are torn with autotomy, as they are not divided by fracture planes, but they usually present sphincters and valves (respectively) at the level of each fracture plane, which prevents from important blood loss after autotomy. These adaptations considerably minimize the costs of the injury (Bellairs & Bryant 1985).

Tail autotomy is frequently used as an antipredator strategy in lizards and has a great incidence in natural populations of some species, in which more than a half of the adult population have undergone caudal autotomy at least once in life (Arnold 1988; Downes & Shine 2001; Pafilis *et al.* 2008 for lacertid lizards). Caudal autotomy allows the lizard to break away from the predator when seized from the tail and provides an effective distraction of the predator, as the tail performs vigorous complex movements and flips immediately after detachment (Arnold 1988; Fernández-Rodríguez & Braña 2020). These movements make the tail more attractive to predators, diverting their attention to the tail while the lizard moves away, thus facilitating the escape (Dial & Fitzpatrick 1983). However, the tail holds important roles in locomotion, social signalling and accumulation of reserves in many lizard species, so that its loss may have short-term negative consequences, inducing behavioural alterations and decreasing the efficiency of other functions like foraging, mating or patrolling.

Regeneration: significance, process and implications

Regeneration is the partial or complete restoration of a body part lost through traumatic or voluntary injury (Maginnis 2006) and allows the replacement of lost parts that were not vital but increased the probability of survival or the capacity of reproductive investment (Goss 1969). We could say that regeneration shares some characteristics with the processes of asexual reproduction and embryogenesis, but it has a distinct developmental basis and different evolutionary fate (Bely 1999). In such regard, Bely & Nyberg (2010) proposed the following three aspects that distinguish regeneration from other developmental processes: i) it triggers on the wounded and multicellular stub of an unpredictable injury that mutilates a body part; ii) it requires specific developmental features, like regeneration specific gene expression; and iii) it has a distinct phylogenetic distribution.

Regeneration likely originated in early animals with the development of multicellularity (Bely & Nyberg 2010), possibly as a secondary effect of the continuous access to developmental programmes, that could be activated whenever a body part was amputated (epiphenomenon hypothesis, Goss 1992; Mani & Tlusty 2021). Nowadays the capacity to regenerate body parts varies considerably across animals. For instance, all basal metazoan lineages (placozoans, poriferans, ctenophores and cnidarians), together with some lophotrochozoans and non-craniate deuterostomes, are able to regenerate all body parts, while such complete regeneration capacity is absent in ecdysozoans and in craniates (for a review, see Bely & Nyberg 2010). Although some clades in almost all animal phyla are able to regenerate at least some structures (Bely & Nyberg 2010), the capacity of regeneration seems to be related to the model of embryonic development of the lost parts (Galis *et al.* 2003). For instance, limb regeneration is possible when it is developed as a semiautonomous module and is not involved in interactions with transient structures, like in amphibians and lungfishes. On the contrary, it is not possible when the limb develops early and requires inductive interactions with transient structures that will not be present anymore after such embryonic stage, as it happens in amniotes, teleost fishes and chondrichthyans (Galis *et al.* 2003).

In lizards, complete tail regeneration takes around three months in most species (Bellairs & Bryant 1985) and is an epimorphic process (i.e., implies cell proliferation and formation of a blastema, Goss 1969). Regeneration occurs in three phases: i) wound covering and healing, ii) cell dedifferentiation and blastema formation and iii) cell proliferation and tail formation (Bryant *et al.* 2002; Gilbert *et al.* 2013). The two first phases, that have a rapid development in the initial stages of the regeneration process, are considered as a latent period, as there is no evident tail growth or elongation during that time. After tail detachment, the remaining muscle and skin tissues contract to enclose the injury; these tissues will dry and clot to form a scab that will protect the injury during the phases of wound healing and blastema formation (McLean & Vickaryous 2011). During these first days there is an important cell migration and dedifferentiation beneath the scab to form the blastema, which is a mass of dedifferentiated cells derived from pre-existing stump tissue (Bellairs & Bryant 1985; Lozito & Tuan 2016). The injury is protected by the scab during this period, until the spinal cord elongates to form an ependymal tube that infiltrates the blastema and approaches to the wound epithelium (Figure 1.3A), and the epidermis of the blastema covers the surface of the injury, forming an apical cap (Gilbert *et al.* 2015). The distal one-half of the remaining fragment of autotomized vertebra is ablated by the action of osteoclasts and fused with the scab (Bellairs & Bryant 1985), which is then released, approximately a week after autotomy. The ependyma induces tail re-growth and the formation of a cartilage tube, which encloses the ependymal tube and attaches to the terminal end of the remains of the ablated vertebra (Figure 1.3; Bryant *et al.* 2002). Effective elongation begins 10-15 days after autotomy, which marks the end of the latent period (McLean & Vickaryous 2011; Fernández-Rodríguez & Braña 2020). At this point, the external aspect of the regenerated portion is dark purplish and has a thin epidermis without scales (Figure 1.4); skin keratinization, scales formation and pigmentation will not begin until approximately 3 weeks after autotomy (Bellairs & Bryant 1985).

The regenerated tail is not a perfect replica of the original one; the main difference between them is the skeleton axis, which in the regenerated tail is an unsegmented axis of cartilage instead of articulated vertebrae (Figure 1.3B; Hughes & New 1959). This difference has strong functional implications, since the continuous cartilage axis not only limits the motility and flexibility of the regenerated fragment, but also prevents the tail to be autotomized through the regenerated portion, as the cartilage tube lacks fracture planes. Therefore, new autotomy events will only be possible through the proximal portion of intact tail. In addition to these differences, muscle bands of the regenerated tail are irregular, variable in number and attach unevenly to each other and to the cartilage tube. On the contrary, the muscles of the intact tail form regular quadrants that attach to the vertebral column and hold a role both in the mobility of each particular vertebra and in the fracture in a specific site during autotomy (Fischer *et al.* 2012).

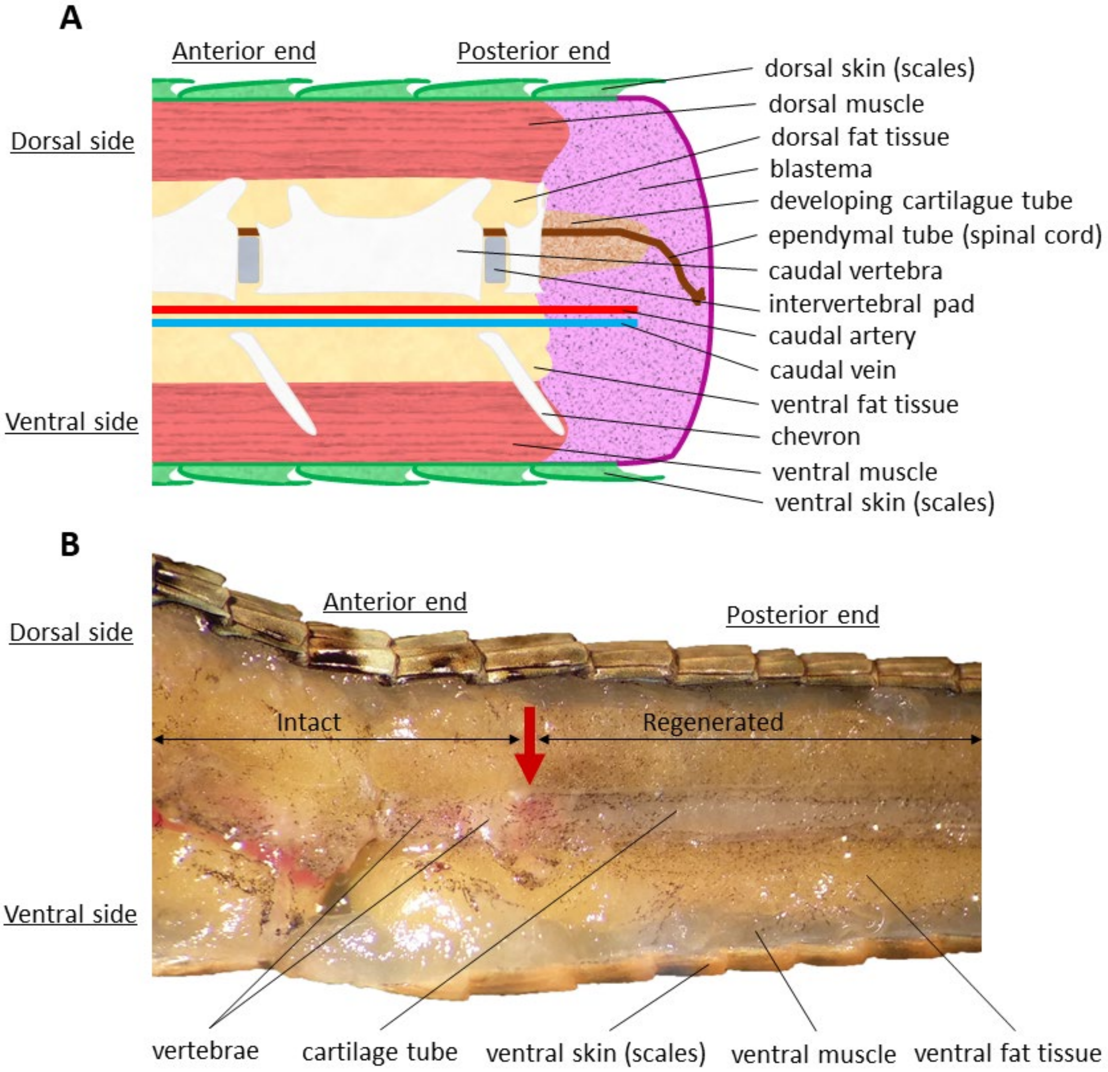

Figure 1.3. A, Schematic representation of a longitudinal section of an adult lizard's tail (midcaudal region) 2-3 weeks after autotomy, showing the blastema shortly after scab release. Based on the semmidiagramatic longitudinal section of adult *Zootoca vivipara* from Bellairs & Bryant (1985). **B**, Longitudinal section of an adult wall lizard's tail (*Podarcis muralis*), showing the intact (left) and regenerated (right) portions. The point where the cartilage tube attaches to the remains of the ablated vertebra is marked with a red arrow.

Moreover, the regenerated tail is richer in fat than the original one (Vitt *et al.* 1977), and the scales are smaller and frequently more homogeneous in shape than in the intact tail (Gilbert *et al.* 2015). In addition, the regenerated tail does not usually reach the same length as the intact tail (Zamora-Camacho *et al.* 2016) and present a limited mobility and flexibility (Fernández-Rodríguez & Braña 2020). Despite these morphological differences, regeneration allows the restoration of some functionalities lost with tail autotomy, providing several benefits that increase lizards' probability of survival and fitness (Bateman & Fleming 2009). However, regeneration implies a great demand of energy and materials, and this may interfere with other highly demanding processes like growth or reproduction, with potential consequences for survival and lifetime fitness (Maginnis 2006).

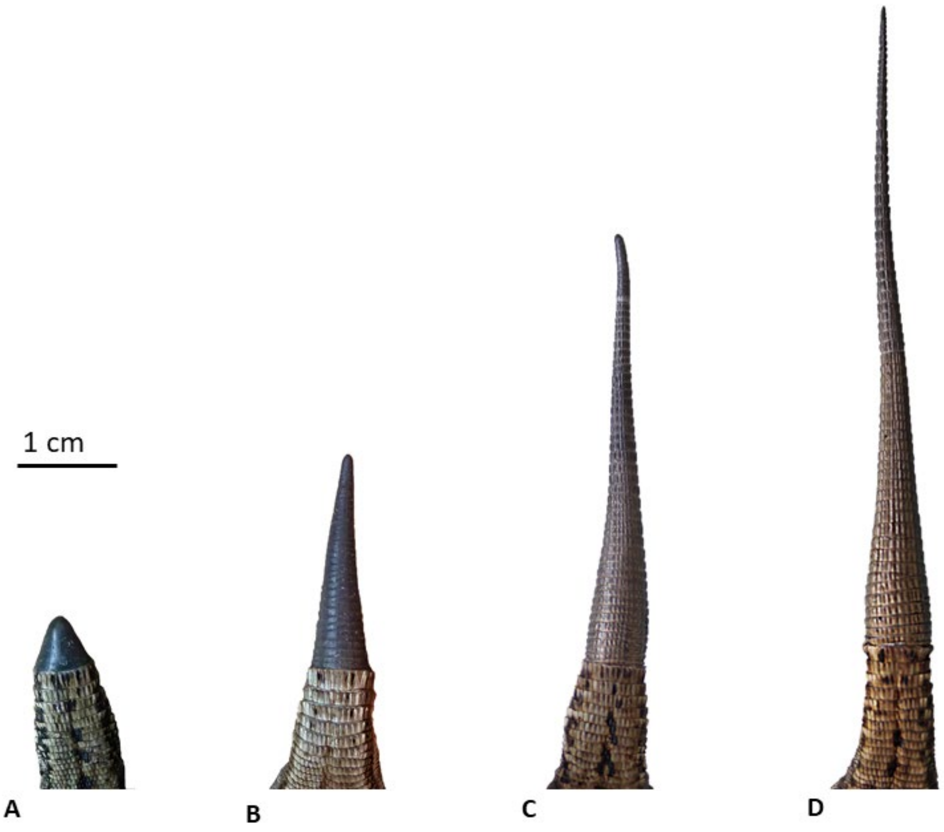

Figure 1.4. Tails of *Podarcis muralis* in different regeneration stages. **A**, Beginning of the elongation phase. **B**, Elongation phase and initial differentiation of scales. **C**, Regenerated tail with differentiated scales. **D**, Complete regenerated tail.

The lacertid lizard *Podarcis muralis* as a model

This thesis was focused on the common wall lizard, *Podarcis muralis* (Laurenti, 1768) as the model organism, as it was very abundant in the study area, easy to maintain in the laboratory and there was already much information about the biology of the species. The common wall lizard is a small lacertid (Reptilia: Lacertidae) that inhabits rocky habitats of both natural and urban areas from South Europe, from 0 to 2400 m of elevation (Salvador 2014). Adult males range from 4.8 to 6.8 cm of snout-vent-length (SVL), and adult females from 4.85 to 6.91 cm of SVL (Salvador 2014); snout-to-vent length at birth ranges from 2.26 to 2.83 cm of SVL (for newborns of our experimental incubations studied in Chapter 4) and hatchling females are slightly larger than males (Braña & Ji 2000) (Figure 1.5A-C). Males are more robust than females and their head is larger and wider, which confer them a greater bite force and allow them to consume larger preys (Braña 1996; 2003).

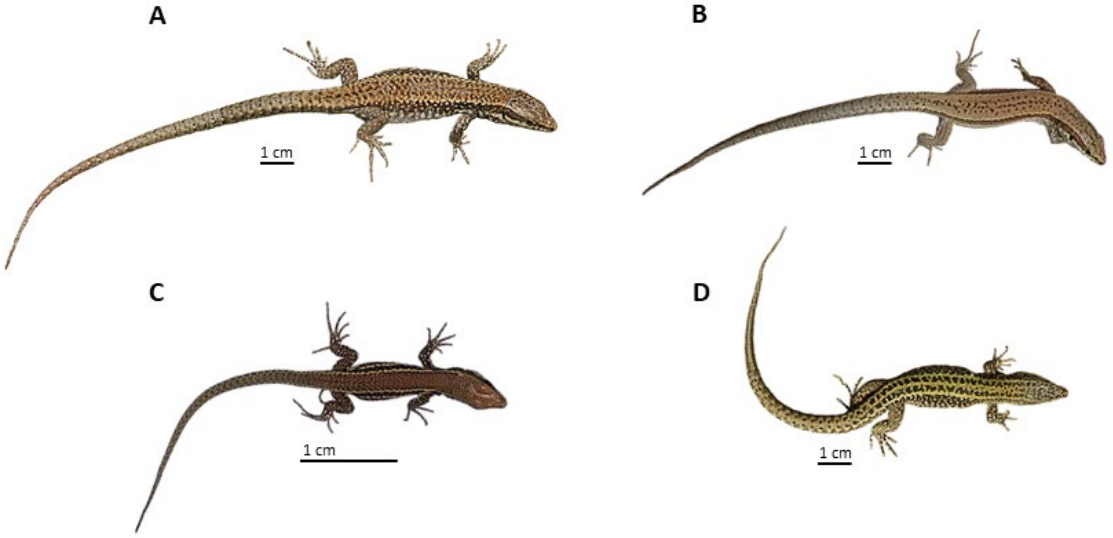

Figure 1.5. A-E, *Podarcis muralis*. **A**, Adult male with intact tail. **B**, Adult female with intact tail. **C**, Hatchling. **D**, Adult male *P. bocagei* with intact tail.

Wall lizards are active from March-April to October in Asturias (our study area) with a bimodal activity on sunny days during summer, being active during the morning and late afternoon and hiding during the central hours of the day, when temperatures reach the daily maximum (Braña 1991). The brumation period is relatively short in this species, ranging from October-November to March. They usually reach sexual maturity at the age of 2 years (Braña 1984) and multiple paternity within the same clutch is very frequent (Oppliger *et al.* 2007). Males' spermatogenesis begins at the end of summer (August-September) and stops at the spermiogenesis phase, at the end of the activity period (October-November) at temperatures below 21 °C (Joly & Saint-Girons 1975). In spring males emerge earlier from winter brumation than females, in order to complete the spermatogenesis (spermiogenesis) before females are active and initiate ovulation (Saint Girons & Duguy 1970; Braña 1983). Reproduction occurs from the end of April to the beginning of July and females lays 2-3 clutches per breeding season (i.e., they are multivoltine) with an interval of approximately one month between clutches (Saint Girons & Duguy 1970; Braña 1984; Ji & Braña 2000). The first clutch is larger (both in number of eggs and in mean egg size) than the other two (Ji & Braña 2000) and its vitellogenesis is based on the fat body storages acquired in the previous summer and autumn that are hardly used during winter brumation (under a capital breeding strategy), while the second and third clutches mainly depend on the immediate food intake (thus, under an income breeding strategy) (Braña *et al.* 1992). Survival until hatching is very high at incubation temperatures from 24 to 29 °C and decreases abruptly at temperatures from 32 to 35 °C. The optimal temperature for incubation is 29 °C, since at this temperature the incubation time is minimized (around 30 days) without damaging the embryos; temperatures above 29 °C have negative effects on development, leading to phenotypes with deformities (Ji & Braña 1999; Braña & Ji 2000; Van Damme *et al.* 1992).

Wall lizards are active-search hunters and larger individuals have access to a greater variety and size of preys. Their diet is based mainly on arthropods, mostly arachnids (Arachnida), flies (Diptera), earwings (Dermaptera), homopterans (Homoptera) and ants (Formicidae) (Braña 1981; 1984; García-Fernández *et al.* 1989; Herrel *et al.* 2001). Several species of visual hunters have been reported as predators of *Podarcis muralis*, like lizards (*Lacerta schreiberi*), raptor birds both Falconiformes (*Buteo buteo*, *Falco tinnunculus*) and Strigiformes (*Athene noctua*), and mammals (*Felis silvestris*, *Vulpes vulpes*, *Genetta genetta*, *Crodidura* spp., *Martes martes* and *Mustela nivalis*) (Braña 1984; Veiga 1985). Juvenile wall lizards are also potential prey for some arthropods, such as the insect *Mantis religiosa* and the arachnid *Nuctenea umbratical* (García *et al.* 1998). All these predators are generalist, with the exception of *Coronella austriaca*, *C. girondica* and young individuals of *Vipera seoanei*, that are saurophagous specialists (Braña 1984; Braña *et al.* 1987).

Mean cloacal temperatures of active individuals of *P. muralis*, both in the field and in the laboratory, were around 34 °C and their preferred thermal range is 32–36 °C (Braña 1991; 1993). Besides, pregnant females select lower temperatures than males (Braña 1993; Monasterio *et al.* 2009). Wall lizards usually performs intermittent movements with frequent stops to evaluate the surroundings; during thermoregulation and exploration they perform short and slow movements, while they run rapidly during fights against conspecifics or escapes from predators (Braña 2003). They usually remain motionless to prevent detection, especially pregnant females, which rely more on crypsis, allowing a closer approach of potential predators and remaining close to the refuge (Braña 1993). Refuges are used to avoid predation (Amo *et al.* 2003), but they are able to recognize chemical cues of snake predators like *C. austriaca* and avoid crevices with snake odour (Amo *et al.* 2005).

Unlike the other chapters of this thesis, studies included in chapter 5 were done with the Bocage’s wall lizard, *P. bocagei* (López Seoane, 1884) (Figure 1.5D), as the study were carried out during an international stay in the research center CIBIO-InBIO, in northern Portugal, were there is no presence of *P. muralis*, while the closely related Bocage´s lizard is abundant and occupies similar spaces. *Podarcis bocagei* is phylogenetically close to *P. muralis* (Harris & Sá-Sousa 2002) and has similar ecological requirements (Galán 1986). It is an endemism of the northwestern Iberian Peninsula, where it inhabits humid rocky and bushy temperate-Mediterranean areas, from 0 to 1900 m of elevation (Galán 2014). It is similar to *P. muralis* in size, although slightly smaller: adult males range from 43.3 to 64.9 cm SVL and females from 41 to 61.9 cm SVL (Galán 1986). The period of activity is longer than for *P. muralis*, as *P. bocagei* occurs in areas with a temperate climate that allows activity from February-March to November, or even an almost continuous activity during all the year in some areas where temperatures do not drop below 10 °C (Galán 1995; Galán 2014). As in *P. muralis,* reproduction occurs from April to July and females lay 2–3 clutches (Galán 1997; 2014).

The preferred range of temperatures of this species is 29.4–32.0 °C (Sannolo *et al.* 2018). It is an active-search hunter with a very similar dietary composition to *P. muralis* (Galán 2014). The saurophagous snakes *C. austriaca*, *C. girondica*, *V. seoanei*, *V. latastei* and *Rhinechis scalaris* are common predators of *P. bocagei* (Galán 2014; Brito 2004). Other visual predators reported for this species are the coleopter *Carabus (Mesocarabus) macrocephalus*, the common kestrel (*Falco tinnunculus*) and the domestic cat (*Felis silvestris catus*) (Galán 1999; 2014; Alarcos & Flechoso 2012).

OBJECTIVES

Caudal autotomy and regeneration are particularly frequent in lizards, in which they report several benefits but also some costs. The main advantage of caudal autotomy in lizards is facilitating the escape from a predator, but many other aspects may be affected by tail loss and its posterior regeneration, like locomotor performance, activity patterns, behaviour, thermoregulation, hydroregulation, somatic growth or reproduction. The intensity of these effects may vary among sexes, life stages or environmental conditions, and lizards may have developed some mechanisms to counter the negative effects of autotomy and regeneration. Under this framework, the general objectives of this thesis are to study the effects of caudal autotomy and regeneration on several aspects of lizards' life histories, as well as the strategies developed to minimize those costs. Consequently, the following specific objectives were defined for the achievement of the aforementioned general objectives:

1) To study the costs of caudal autotomy in several functional aspects of lizard's biology, whose modification is likely to influence behaviour and performance in ecological functions with potential relevance to fitness. In relation to this objective, the following aspects have been studied: locomotor performance (**Chapter 1**), antipredator behaviour (**Chapter 2**), thermoregulation and hydroregulation (**Chapter 3**).

2) To evaluate the costs of caudal regeneration and to study, within the framework of allocation trade-offs, how they affect the two main compartments where growth is assigned at different life stages. In this regard we have studied the structural body growth in pre-reproductive juveniles (**Chapter 4**) and the reproductive investment (egg size, clutch size, clutch mass and clutch frequency) in adult females (**Chapter 5**).

References

Alarcos, G. & Flechoso, F. (2012). Nota sobre depredación de reptiles por gatos y gallinas. *Boletín de la Asociación Herpetológica Española*, 23(2): 74–75.

Amo, L.; López, P. & Martín, J. (2003). Risk level and thermal costs affect the choice of escape strategy and refuge use in the wall lizard, *Podarcis muralis*. *Copeia*, 2003(4): 899–905.

Amo, L.; López, P. & Martín, J. (2005). Chemical assessment of predation risk in the wall lizard, *Podarcis muralis*, is influenced by time exposed to chemical cues of ambush snakes. *Herpetological Journal*, 15: 21–25.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Bateman, P.W. & Fleming, A. (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. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bely, A.E. & Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170.

Bely, A.E. (1999). Decoupling of fission and regenerative capabilities in an asexual oligochaete. *Hydrobiologia*, 406: 243–251.

Braña, F. & Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *Journal of Experimental Zoology,* 286: 422–433.

Braña, F. (1981). Sobre la alimentación de *Podarcis muralis* (Reptilia: Lacertidae) en Asturias: espectro taxonómico y tamaño de las presas. *Resúmenes de Comunicaciones de la V Reunión Bienal de la Real Sociedad Española de Historia Natural*. Oviedo: 179.

Braña, F. (1983). La reproducción en los Saurios de Asturias (Reptilia: Squamata): ciclos gonadales, fecundidad y modalidades reproductoras. *Revista de Biología de la Universidad de Oviedo*, 1: 29–50.

Braña, F. (1984). *Biogeografía, biología y estructura de nichos de la taxocenosis de saurios de Asturias.* PhD Thesis, University of Oviedo, Spain.

Braña, F. (1991). Summer activity patterns and thermoregulation in the wall lizard, *Podarcis muralis*. *The Herpetological Journal*, 1: 544–549.

Braña, F. (1993). Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos*, 66(2): 216–222.

Braña, F. (1996). Sexual dimorphism in lacertid lizards: male head increase vs female abdomen increase? *Oikos*, 75: 511–523.

Braña, F. (2003). Morphological correlates of burst speed and field movement patterns: the behavioral adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society*, 80(1): 135–146.

Braña, F.; Bea, A. & Saint-Girons, H. (1987). Composición de la dieta y ciclos de alimentación en *Vipera seoanei* Lataste, 1879. Variaciones en relación con la edad y el ciclo reproductor. *Munibe (Ciencias Naturales)*, 40: 19–27.

Braña, F.; González, F. & Barahona, A. (1992). Relationship between ovarian and fat body weights during vitellogenesis for three species of lacertid lizards. *Journal of Herpetology*, 26: 515–518.

Brito, J.C. (2004). Feeding ecology of *Vipera latastei* in Northern Portugal: ontogenetic shifts, prey size and seasonal variations. *Herpetological Journal*, 14: 13–19.

Bryant, S.V.; Endo, T. & Gardiner, D.M. (2002). Vertebrate limb regeneration and the origin of limb stem cells. *International Journal of Developmental Biology*, 46: 887–896.

Clause, A.R. & Capaldi, E.A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology Part A Comparative Experimental Biology*, 305: 965–973.

Dial, B.E. & Fitzpatrick, L.C. (1983). Lizard tail autotomy: Function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science,* 219: 391–393.

Downes, S. & Shine, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology,* 82: 1293–1303.

Elliott, J.M. (1994). *Quantitative Ecology and the Brown Trout*. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Emberts, Z.; Escalante, I. & Bateman, P.W. (2019). The ecology and evolution of autotomy. *Biological Reviews*, 94(6): 1881–1896.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15(6): 511–521.

Fischer, R.E.; Geiger, L.A.; Stroik, L.K.; Hutchins, E.D.; George, R.M.; Denardo, D.F.; Kusumi, K.; Rawls, J.A. & Wilson-Rawls, J. (2012). A histological comparison of the original and regenerated tail in the green anole, *Anolis carolinensis*. *The Anatomical Record*, 295: 1609–1619.

Fleming, P.A.; Muller, D. & Bateman, P.W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews*, 82: 481–510.

Fredericq, L. (1892). Nouvelles recherches sur l'autotomie chez le crab. *Archives de Biologie*, 12: 169–197.

Galán, P. (1986). Morfología y distribución del género *Podarcis* Wagler, 1830 (Sauria, Lacertidae) en el noroeste de la Península Ibérica. *Revista Española de Herpetología*, 1: 85–142.

Galán, P. (1995). Ciclos de actividad de Podarcis bocagei en el noroeste ibérico. *Revista Española de Herpetología*, 9: 37–47.

Galán, P. (1997). Reproductive ecology of the lacertid lizard *Podarcis bocagei*. *Ecography*, 20: 197–209.

Galán, P. (1999). Demography and population dynamics of the lacertid lizard *Podarcis bocagei* in Northwest Spain. *Journal of Zoology*, 249: 203–218.

Galán, P. (2014). *Podarcis bocagei*. In: Salvador, A. (Ed.), *Fauna Ibérica.* Vol. 10, Reptiles. 2nd edition. Museo Nacional de Ciencias Naturales CSIC, Madrid, Spain, pp. 503–527.

Galis, F.; Wagner, G.P. & Jockusch, E.L. (2003) Why is limb regeneration possible in amphibians but not in reptiles, birds, and mammals? *Evolution & Development*, 5: 208–220.

García, J.J.; Gosá, A. & Alcalde, J.T. (1998). Artrópodos depredando lagartijas del género *Podarcis*. *Boletín de la Asociación Herpetológica Española*, 9: 27–28.

García-Fernández, J.; Martín-Vallejo, J. & Pérez-Mellado, V. (1989). Dieta comparada de dos especies del género Podarcis Wagler, 1830 en la Sierra de Guadarrama (Sist. Central). *Resúmenes del IV Congreso Nacional de Herpetología*, Madrid: 45.

Gilbert, E.A.; Payne, S.L.; Vickaryous, M.K. (2013). The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological & Biochemical Zoology*, 86: 631–44.

Gilbert, E.A.B.; Delorme, S.L. & Vickaryous, M.K. (2015). The regeneration blastema of lizards: an amniote model for the study of appendage replacement. *Regeneration*, 2(2): 45–53.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Goss, R.J. (1992). The evolution of regeneration: Adaptive or inherent? *Journal of Theoretical Biology*, 159: 241–260.

Harris, D.J. & Sá-Sousa, P. (2002). Molecular phylogenetics of Iberian wall lizards (*Podarcis*): Is *Podarcis hispanica* a species complex? *Molecular Phylogenetics and Evolution*, 23(1): 75–81.

Herrel, A.; Van Damme, R.; Vanhooydonck, B. & De Vree, F. (2001). The implications of bite performance for diet in two species of lacertid lizards. *Canadian Journal of Zoology*, 79(4): 662–670.

Hughes, A. & New, D. (1959). Tail regeneration in the geckonid lizard, *Sphaeridactylus. Journal of Embryology and experimental Morphology,* 7: 281–302.

Ji, X. & Braña, F. (1999). The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). *Comparative Biochemistry and Physiology, Part A*, 124: 205–213.

Ji, X. & Braña, F. (2000). Among clutch variation in reproductive output and egg size in the wall lizard (*Podarcis muralis*) from a lowland population of Northern Spain. *Journal of Herpetology*, 34(1): 54–60.

Joly, J. & Saint-Girons, H. (1975). Influence de la temperature sur la vitesse de la spermatogenèse, la durée de l'activité spermatogénétique et l'évolution des caracteres sexuels secondaires du lèzard des murailles, *Lacerta muralis* L. (Reptilia, Lacertidae). *Archives d'Anatomie Microscopique et de Morphologie Expérimentale*, 64(4): 317–336.

Lozito, T.P. & Tuan, R.S. (2016). Lizard tail skeletal regeneration combines aspects of fracture healing and blastema-based regeneration. *Development*, 143(16): 2946–2957.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Maginnis, T.L. (2008). Autotomy in a stick insect (Insecta: Phasmida): predation versus molting. *Florida Entomologist*, 91: 126–127.

Mani, S. & Tlusty, T. (2021). A comprehensive survey of developmental programs reveals a dearth of tree-like lineage graphs and ubiquitous regeneration. *BMC Biology*, 19(1): 111.

McLean, K.E. & Vickaryous, M.K. (2011). A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius. BMC Developmental Biology*, 11: 50.

Moffat, L.A. & Bellairs, A. (1964). The regenerative capacity of the tail in embryonic and post-natal lizards (*Lacerta vivipara* Jacquin). *Journal of Embryology and Experimental Morphology*, 12: 769–786.

Monasterio, C.; Salvador, A.; Iraeta, P. & Díaz, J.A. (2009). The effects of thermal biology and refuge availability on the restricted distribution of an alpine lizard. *Journal of Biogeography*, 36 (9): 1673–1684.

Moore, A.; Tabashnik, B.E. & Stark, J.D. (1989). Leg autotomy: A novel mechanism of protection against insecticide poisoning in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Enthomology*, 82(5): 1295–1298.

Oppliger, A.; Degen, L.; Bouteiller-Reuter, C. & John-Alder, H.B. (2007). Promiscuity and high level of multiple paternity in common wall lizards (*Podarcis muralis*): data from microsatellite markers. *Amphibia-Reptilia*, 28(2): 301–303.

Pafilis, P.; Pérez-Mellado, V. & Valakos, E. (2008). Postautotomy tail activity in the Balearic lizard, *Podarcis lilfordi*. *Naturwissenschaften*, 95: 217–221.

Reznick, D.N.; Nunney, L. & Tessier, A. (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution*, 15: 421–425.

Roff, D.A. (1992). *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York.

Roff, D.A. (2002). *Life History Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Saint Girons, H. & Duguy, R. (1970). Le cycle sexuel de *Lacerta muralis* L. en plaine et en montagne. *Bulletin du Muséum national d'histoire naturelle*, 42: 609–625.

Salvador, A. (2014). *Podarcis muralis*. In: Salvador, A. (Ed.), *Fauna Ibérica.* Vol. 10, Reptiles. 2nd edition. Museo Nacional de Ciencias Naturales CSIC, Madrid, Spain, pp. 576–589.

Sannolo, M.; Barroso, F.M. & Carretero, A.M. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, 126: 58–64.

Sheppard, L. & Bellairs, A. (1972). The mechanism of autotomy in *Lacerta*. *British Journal of Herpetology*, 4: 267–286.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford University Press, London.

Van Damme, R.; Bauwens, D.; Braña, F. & Verheyen, R.F. (1992). Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica*, 48(2): 220–228.

van der Meer, J. (2019). Metabolic theories in ecology: the dynamic energy budget theory and the metabolic theory of ecology. *Encyclopedia of Ecology (Second Edition)*, 3: 463–471.

van Noordwicjk, A.J. & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, 128(1): 137–142.

Veiga, J.P. (1985). *Ecología de las rapaces de un ecosistema mediterráneo de montaña. Aproximación a su estructura comunitaria*. PhD Thesis, Complutense University of Madrid, Spain.

Vitt, L.J.; Congdon, J.D. & Dickson, N.A. (1977). Adaptive strategies and energetics of tail autotomy in lizards. *Ecology,* 58: 326–337.

Wulff, J.L. (2006). Resistance vs. recovery: morphological strategies of coral reef sponges. *Functional Ecology*, 20: 699–708.

Zamora-Camacho, F.J.; Rubiño-Hispán, M.V.; Reguera, S. & Moreno-Rueda, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid *Psammodromus algirus*. *Herpetological Journal*, 26: 213–218.

“Autotomy can be regarded as a compromise adaptation, often involving sacrifice, which a lizard cannot easily afford but which is preferable to the alternative of certain death”. Bellairs & Bryant, 1985 (In: Gans & Billet eds., *Biology of Reptilia*, vol. 15).

“La autotomía puede considerarse como un compromiso adaptativo, a menudo implicando un sacrificio que el lagarto no puede asumir sin dificultad, pero que es preferible a la alternativa de una muerte segura”.

Cover photo: *Podarcis muralis*

II. Chapter 1

The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy

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The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy

Abstract

Autotomy has evolved independently several times in different animal lineages. It frequently involves immediate functional costs, so regeneration evolved in many instances to restore the functionality of that body part. Caudal autotomy is a widespread antipredator strategy in lizards, although it may affect energy storage, locomotion dynamics, or survival in future encounters with predators. Here we assessed the effect of tail loss on the locomotor performance of wall lizards (*Podarcis muralis*), as well as the recovery of locomotor functionality of lizards with regenerated tails, and the movement dynamics of shed tails that were either intact or having regenerated portions. Tail loss had no effect on locomotion over unhindered spaces, possibly due to compensation between a negative effect on the stride of front limbs, and a positive effect of losing mass and friction force. We found a clear negative impact of tail loss on locomotion in spaces with interspersed obstacles, in which tailed lizards jumped larger distances when leaving the obstacles. Besides, lizards that used the tail to push off the ground were able to approach the obstacles from further, so that the tail seemed to be useful when used during jumping. Regeneration fully restores lizard's locomotor capacities, but tail antipredator value, as indicated by the intensity of post-autotomic movements, is only partially retrieved. From these results we propose that, together with the recovery of post-autotomy antipredator capacities, the restoration of the organismal locomotor performance may have been an important, yet frequently neglected factor in the evolution of lizard's regeneration ability.

Keywords: autotomy, locomotor performance, regeneration, tail, wall lizard

Introduction

Many animals, both vertebrates and invertebrates, are able to self-induce the shed of an expendable appendage as a reflex response to threats. This phenomenon, called "autotomy" (Fredericq 1892), does not represent the accidental loss of a body part, but an innate response integrated in the behaviour and physiology of the animal (Bely & Nyberg 2010) that has evolved independently several times in different lineages (Fleming *et al.* 2007; Emberts *et al.* 2019). Autotomy may have evolved since it provides benefits, such as avoiding predation (Cooper *et al.* 2004), solving molting complications (Maginnis 2006), eliminating toxins from a certain body part (Moore *et al.* 1989), or allowing survival after physical damages (Wulff 2006). However, autotomy frequently involves immediate functional costs, as it represents the loss of a useful appendage (such as the tail of a lizard or the arm of a starfish) which may be important, for instance, for feeding, locomotion or reproduction (for a review, see Bateman & Fleming 2009). Besides, the loss of a body part often involves social costs, which may affect the social status (Fox & Rostker 1982) or habitat selection (Fox *et al.* 1981) and, as a result, it might decrease survivorship (Fox & McCoy 2000). Consequently, autotomy is often followed by some behavioural changes aiming to compensate the functional losses, such as modifications of the locomotor dynamics and the escape strategies (Dial & Fitzpatrick 1981; Clause & Capaldi 2006), changes in feeding behaviour (Ramsay *et al.* 2001) or in activity time and patterns (Díaz-Guisado *et al.* 2006; Barrios *et al.* 2008). However, behavioural adjustments are often costly and fail to fully restore the impaired function, and subsequently regeneration often evolved to recover lost parts that are not vital but increase survivorship and fitness (Goss 1969; Lin *et al.* 2017). In turn, regeneration implies an energy cost, thereby involving allocation trade-offs that may negatively affect somatic growth (Ballinger & Tinkle 1979), reproductive investment (Bellairs & Bryant 1985; Barrios *et al.* 2008) or immune function (Argaez *et al.* 2018), likely having physiological and evolutionary consequences (Stearns 1992).

The post-anal tail is a characteristic structure of chordates (and particularly of vertebrates) that assumes important functions (Kardong 2014); for example, lizards' tails often act as a counterbalance influencing the distribution of body weight (Arnold 1988) and providing stability during climbing (Jusufi *et al.* 2008; Medger *et al.* 2008), so it is an essential element for jumping dynamics and efficient landings (Gillis *et al.* 2009; Gillis *et al.* 2013). Even so, in many species of lizards, more than a half of the adult population have mutilated or regenerated tails, so they have undergone caudal autotomy at least once (e.g., Downes & Shine 2001; Cooper *et al.* 2004; this study). According to Arnold (1988), the benefits of caudal autotomy regarding predation avoidance are associated to two different escape strategies: i) breaking away from a predator when the lizard is seized by the tail (in this case the smallest possible fragment of the tail is shed), and ii) breaking the tail as a distraction to escape from the predator prior to capture (which in most cases implies basal autotomy). Vigorous post-autotomy movements, along with bright ventral colours (Castilla *et al.* 1999), make the tail more visible, setting the attention of predators and thus facilitating escape (Dial & Fitzpatrick 1983). Basal autotomy, although implies a less economical detachment, offers a large fragment to distract the predator, therefore increasing the probability of escape (Bellairs & Bryant 1985).

Locomotion is a function of widespread use for many kinds of activities in a variety of ecologically relevant contexts (feeding, escape from predators, mating, social interactions, etc; see, e.g., Huey & Pianka 1981; Webb 1986; Robson & Miles 2000; Husak *et al.* 2006), and is accordingly closely related to fitness (Garland & Losos 1994;

Miles 2004; Bauwens *et al.* 1995). The value of autotomy and regeneration relies on the balance between the costs and benefits of losing a body part (Arnold 1988). Given that locomotor performance may be important for survival under predatory pressure (e.g., Jayne & Bennett 1990; Braña 2003), it is probable that some mechanisms have evolved to counter the functional costs associated to caudal autotomy. Therefore, assessing the magnitude of the costs of tail loss and underlying possible mechanisms that evolved to minimize its consequences is essential to reach an accurate understanding of the evolutionary scenarios in which autotomy evolved.

Considering the value of caudal autotomy as a generalized antipredator strategy in lizards, the main aim of this study was to assess the effect of tail loss on locomotor performance of wall lizards (*Podarcis muralis*), in order to understand the functional costs of tail loss, together with the potential of the regeneration to achieve the functional recovery of lost capacities. On the other hand, since the antipredatory effectiveness of caudal autotomy depends on the ability of the tail to attract and maintain the predator's attention, we have furthermore examined the movement dynamics of autotomized tails in order to assess their function as a predator distraction. At this respect, we have compared tails having signs of previous regeneration with apparently intact tails. Finally, we propose some scenarios in which autotomy and regeneration may have evolved in lacertid lizards.

Material and methods

Laboratory trials and measurements

Adult individuals of *Podarcis muralis* (i.e., larger than 4.8 cm in snout-vent-length, see Salvador 2014) were captured by noose from May to July of 2017 (N = 67; 36 males and 31 females), in several close localities of central Asturias (northern Spain). Autotomy is frequent in this population, as 65.54% of sighted adults in our study area had mutilated or regenerated tails (N = 148). Lizards were transported to the Zoology laboratory (University of Oviedo), measured for snout-vent-length (SVL) and tail length (TL) to the nearest 0.01 cm, and weighed to the nearest 0.001 g. Lizards were kept in terrariums with 35 W lamps, supplied *ad libitum* with water containing supplementary vitamins and calcium, and fed daily with mealworms, grasshoppers or crickets. These procedures were ethically reviewed and approved by the Principality of Asturias Regional Government and the University of Oviedo.

A before-after design was used to test the differences between tailed and tailless individuals. Lizards were divided into a control (tailed) group (N = 32, 17 males and 15 females; mean ± SD SVL: 5.99 ± 0.41 cm; tail length: 8.03 ± 1.88 cm; body weight: 4.49 ± 1.23 g) and an experimental (hereafter tailless) group (N = 35, 19 males and 16 females; mean ± SD SVL: 5.97 ± 0.48 cm; tail length: 7.98 ± 1.51 cm; body weight: 4.44 ± 1.00 g). Groups were homogeneous with respect to body length (SVL), tail length and mass (ANOVA, $p > 0.5$ in all cases). Because animals live in a tridimensional habitat (vertical walls and stone clusters in the case of wall lizards) and spatial complexity often interferes with maximal locomotor performance (Braña 2003), lizards were subjected to two different locomotor trials (with 5 minutes resting time between them), one on a smooth corridor without obstacles and the other one with two obstacles of 3.2 cm high located at 20 cm and 47 cm respectively from the beginning of the track. First, all the lizards of both the control and experimental group performed these two locomotor trials (“before” trials), having long tails (intact or completely regenerated). After this, we induced caudal autotomy (i.e., the self-detachment of the tail) to the experimental group (hereafter tailless) by grasping each lizard firmly from the base of the tail, between thumb and index fingers. To avoid behavioural biases during the locomotor trials after autotomy, lizards of the control group were handed and manipulated in a similar way than were experimental individuals but preventing tail loss. After that, all the lizards were allowed to rest for two days so that they could recover from the physical trauma and (for the tailless lizards) get used to move in their new tailless condition; then we repeated the same two locomotor trials again for all the individuals from both the tailed and the tailless groups (“after” trials). The shed tails were measured (length and width) and weighed, and its condition (totally intact or with regenerated portions) was registered. Autotomized individuals were left a tail stub of almost 1 cm long (mean ± SD tail stub: 0.87 ± 0.16 cm), and the detached piece of tail represented a mean of 1.34 times SVL and 91% of the complete tail. After autotomy, the detached tail moves vigorously to attract the attention of the predator: both duration of movement and distance travelled by the detached tails in each of the successive movements were measured, recording sequential positions every time the tail made a perceptible movement, until the tail stopped moving.

Before conducting all the locomotor tests and before inducing tail autotomy, lizards were placed in an incubator at 35 °C for 30 minutes to set body temperature close to the optimal temperature for maximum locomotor performance (Braña & Ji 2000). Locomotor trials were conducted inside a room with controlled temperature at 30 °C, corresponding to a frequent environmental temperature for the studied population. Lizards were placed for running in a racetrack of 1.2 m long and 4.5 cm wide, with a transparent sidewall and provided with a coarse surface to ensure a proper traction, and the race was recorded with a video camera (PANASONIC Lumix DMC-TZ10), filming at constant 30 frames s^{-1}. Videos of the races performed on smooth, unhindered substrates, were examined for maximum speed (calculated with the fastest 4 consecutive frames), average speed during the whole race (calculated without considering the frames in which the animal did not move) and number of stops during the race (considering a stop as at least three consecutive frames without moving). The videos of the races on the track with obstacles were examined for the total race time, distance from which the animal jumps to the first obstacle (pre-obstacle distance), distance covered by the animal when jumping to leave the first obstacle (post-obstacle distance), and total time stopped over the obstacle. Behavioural observations were made on whether lizards use or not the tail to push off the ground before jumping to the first obstacle (tail push), on whether they approach to the obstacle jumping or running, on how they pass over the obstacle (jumping the obstacle/running over it), on how they get off the obstacle (falling from it or jumping) and on whether they stop over the obstacle or not.

Statistical analysis

A Kolmogorov-Smirnov test was used to check the assumption of normality in the locomotor variables studied, and the variables that did not show normal distribution were $\log_{10}$-transformed in order to achieve normality. Linear regressions were conducted to dilucidate whether the locomotor variables measured before inducing caudal autotomy were dependent on tail length and SVL. To evaluate the effect of tail loss on locomotion, a repeated measures ANOVA (with tailed group and sex as inter-subject factors) was carried out with the locomotor performance variables (SVL was not included as the covariate for any of the locomotor variables studied, as size was not significantly related to any of the performance traits). One way ANOVA with tail push (i.e., whether lizards use or not the tail to push off the ground when jumping to the obstacle) as the categorical predictor variable was done to test its influence on the total running time in obstacle races and on the pre-obstacles distance (only with variables measured before inducing autotomy, to evaluate all the individuals with tail). A Pearson's Chi-square test was carried out to check for differences between tailed and tailless individuals in the qualitative locomotor variables. Some individuals captured in the field had complete regenerated portions of the tail, while others had entire intact tails ($N_{intact} = 24$; $N_{regenerated} = 43$); the influence of tail condition (i.e., intact or completely regenerated) on locomotor performance was evaluated for all the individuals before inducing autotomy to the experimental group, by a one way ANOVA with the locomotor variables measured (in the "before" trials) and the tail condition as factor. Besides, a two-way ANOVA was done with the locomotor performance variables from the trials after autotomy, considering tail condition and tail group as categorical predictor variables, to search for possible effects of previous autotomy experiences on locomotor performance.

Linear regressions were used to test whether the length and the mass of the shed tails were related to the movement performance of autotomized tails. Tail robustness was calculated as the residuals from the regression of tail mass on tail length. Before doing parametric analyses, a Kolmogorov-Smirnov test was used to check the assumption of normality in the post-autotomy variables. Two out of 35 detached tails barely moved (less than 2 seconds), so they were considered as outliers and were not included in the analysis. Since the total number of movements was quite different among tails, we analysed only the first 20 movements for all the tails that reached that number (25 out of a total of 33 detached tails), grouping movements in successive intervals of 5 each one. A repeated measures ANOVA was done to test possible differences in movement dynamics (in successive intervals) between regenerated and intact tails. Two-way ANOVA was used to examine differences in time of movement and distance covered by tails after autotomy between tails with regenerated portions and intact ones, and between sexes. Intact tails were slightly longer than regenerated ones, so two-way ANCOVA including tail length as the covariate was also done. Two-way ANCOVA was done for distance moved by the tail, with time of tail movement as the covariate and tail condition and sex as the factors. Due to the presence of the hemipenes at the base of the tail, males have greater robustness and some morphological specializations in that region; for example, the first complete fracture plane occurs in the 6th–7th vertebra in males, while in females occurs in the 5th–6th vertebra (Barbadillo *et al.* 1995; Barbadillo & Bauwens 1997). For these reasons, we tested for possible differences between males and females in the performance of the detached tails, including sex as factor in the previous two-way ANOVAs and ANCOVAs. For all the analyses carried out in this study, significant level was set at $p < 0.05$.

Results

Effect of tail loss on locomotor performance

The main locomotor performance traits for all lizards before and after autotomy are summarized in Table 2.1. Tailless and tailed individuals did not show significant differences in maximum speed, average speed, or number of stops in races on smooth, unhindered substrates, nor in pre-obstacles distance and stop time over the obstacle in obstacle races. On the contrary, tailless individuals performed the obstacle race in a longer time than the tailed ones (Repeated measures ANOVA: $F_{1,63} = 10.581$, $p = 0.002$) (Figure 2.1A) and jumped a shorter distance when getting off the obstacle (Repeated measures ANOVA: $F_{1,63} = 6.4201$, $p = 0.014$) (Figure 2.1B). Sex of lizards was never a significant factor on the measured locomotor variables (data not shown).

Tailed lizards tend to jump more frequently than the tailless ones, rather than run when approaching the obstacle ($X^2_1 = 5.558$, $p = 0.018$) or fall when leaving it ($X^2_1 = 6.777$, $p = 0.009$) (Figure 2.2), but there were no differences between tailed and tailless individuals regarding how they overcome the obstacles (i.e., if they jump the obstacle or they run over it; $X^2_1 = 2.251$, $p = 0.134$) or whether they stop over the or not ($X^2_1 = 0.239$, $p = 0.625$). Besides, among tailed lizards, those that used the tail to push off the ground jumped to the obstacles from a longer distance (pre-obstacles distance, $F_{1,65} = 9.170$, $p = 0.004$) and performed the races in a shorter time ($F_{1,65} = 4.525$, $p = 0.037$) than those that did not use the tail. Tail length positively influenced the pre-obstacle distance (total length measured before inducing autotomy; $R^2 = 0.056$, $p = 0.053$), but not maximum speed ($R^2 = 0.002$, $p = 0.728$), average speed in the straight race ($R^2 = 0.001$, $p = 0.822$), running time in obstacles race ($R^2 = 0.024$, $p = 0.207$) or post-obstacle distance ($R^2 = 0.005$, $p = 0.587$). There were no differences in the locomotor performance after autotomy between tailless and tailed individuals depending on their previous tail condition (i.e., if they had intact or previously regenerated tails) (two-way ANOVA for max. speed and average speed in straight race, running time in obstacles race, pre-obstacle distance and post-obstacle distance; $p > 0.300$ in all cases for the interaction between tail group and tail condition).

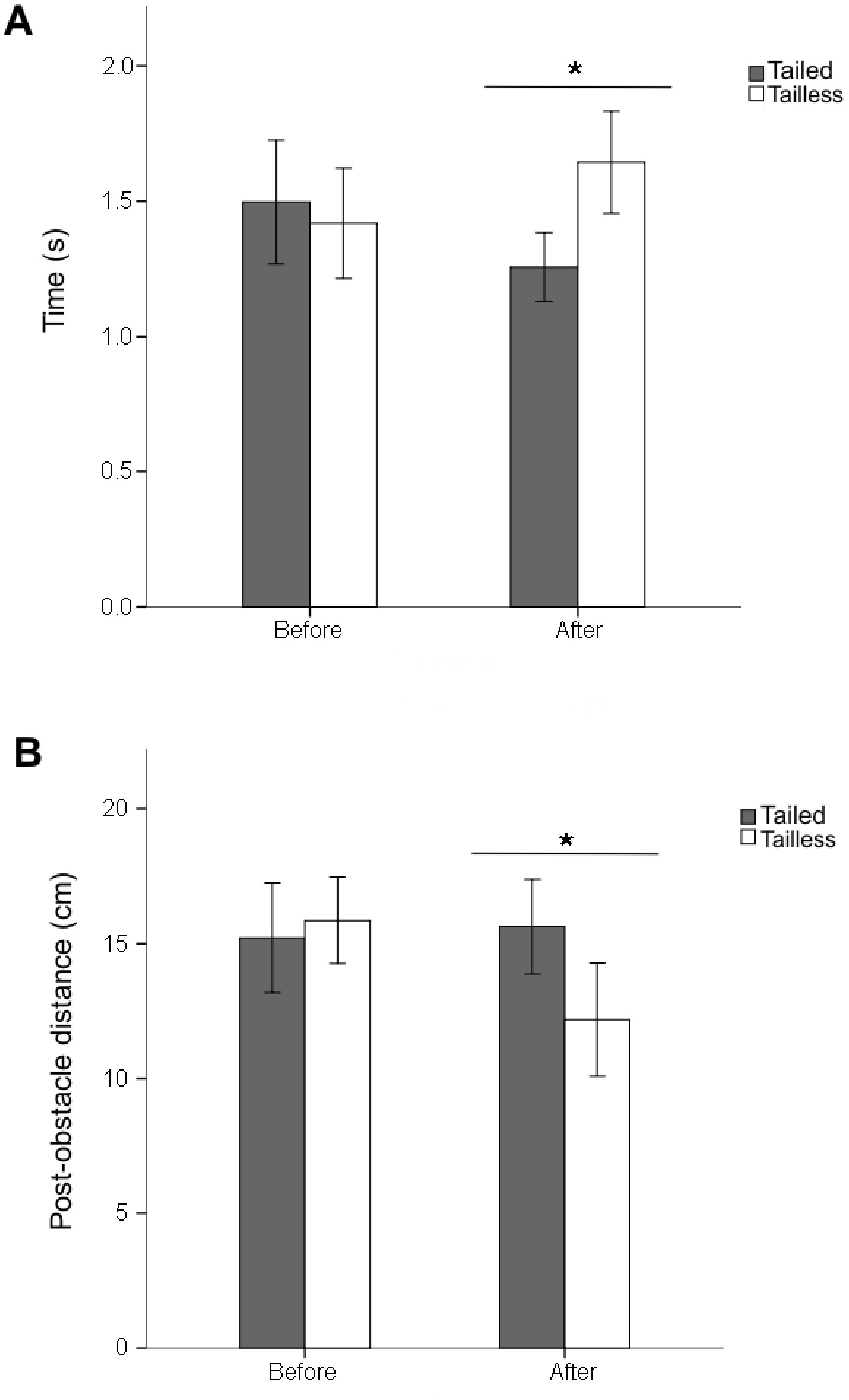

Figure 2.1. Locomotor performance of control (tailed, grey bars; N = 32) and experimental (tailless, white bars; N = 35) lizards, before and after inducing caudal autotomy to the experimental group. **A**, Time of running for races with obstacles. **B**, Mean post-obstacle distance reached by tailed and tailless lizards. Values are means ± SE; * $p < 0.05$.

Table 2.1. Descriptive statistics from the main variables measured in the locomotor performance trials (with and without obstacles) done before and after inducing caudal autotomy to the experimental (tailless) group. Repeated measures ANOVA were carried out with tail group as the categorical predictor variable in all cases. $N_{tailed} = 32$; $N_{tailless} = 35$. Values are means ± SE.

		Before		**After**		**ANOVA**	
		Tailed	**Tailless**	**Tailed**	**Tailless**	**F**	***P***
Without obstacles	**Max speed (cm/s)**	1.31 ± 0.06	1.32 ± 0.05	1.44 ± 0.06	1.39 ± 0.05	0.390	0.535
	Average speed (cm/s)	0.92 ± 0.06	0.87 ± 0.04	0.90 ± 0.04	0.90 ±0.04	0.455	0.502
With obstacles	**Time (s)**	1.50 ± 0.12	1.42 ± 0.10	1.26 ± 0.06	1.64 ± 0.09	10.581	0.002**
	Pre-obst. dist. (cm)	5.30 ± 0.79	5.49 ± 0.73	5.20 ± 0.84	4.09 ± 0.83	0.554	0.460
	Post-obst. dist. (cm)	15.22 ± 1.02	15.87 ± 0.80	15.64 ± 0.88	12.19 ± 1.05	6.420	0.014*

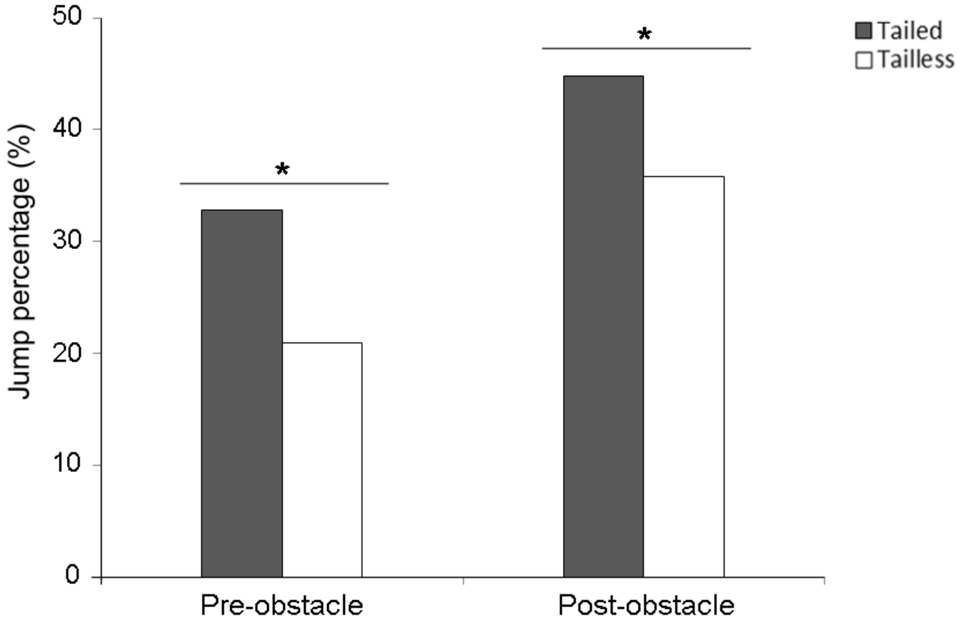

Figure 2.2. Behaviour of tailed (grey bars; N = 32) and tailless (white bars; N = 35) lizards when approaching an obstacle ("pre-obstacle") and when leaving it ("post-obstacle"). Values are percentage of individuals that jump instead of running towards the obstacle or falling from it. * $p < 0.05$.

Functionality of regenerated tails

Individuals with previously completely regenerated tails did not show differences when compared to individuals with entire intact tails in the locomotor variables measured before inducing tail loss to the experimental group (one factor ANOVA, max. speed in straight race: $F_{1,65} = 0.128$, $p = 0.722$; average speed in straight race: $F_{1,65} = 0.010$, $p = 0.921$; running time in obstacles race: $F_{1,65} = 0.712$, $p = 0.402$; pre-obstacle distance: $F_{1,65} = 0.031$, $p = 0.862$; post-obstacle distance: $F_{1,65} = 1.304$, $p = 0.258$). Autotomized tails moved at a non-linear decreasing rate until they stopped moving, dropping substantially during the first movements and then decreasing slowly until definitive stop. Intensity of movement in the initial 20 intervals (i.e., accumulated distance moved) was significantly higher for intact tails than for the regenerated ones (repeated measures ANOVA, $F_{1,23} =$

9.317; $p = 0.006$; Figure 2.3). Intact tails were slightly longer than the regenerated ones (intact TL: 8.38 ± 0.98 cm; regenerated TL: 7.28 ± 1.17 cm; ANOVA, $F_{1,23} = 7.581$; $p = 0.017$), so we analysed tail movement intensity using tail length as the covariate, resulting again that intact tails moved further than the regenerated ones (repeated measures ANOVA with TL as the covariate, $F_{1,23} = 4.739$; $p = 0.041$). Total time of movement and total distance travelled by shed tails were lower for tails having regenerated portions than for the intact ones (Two way ANOVA for time of movement: $F_{1,31} = 5.875$, $p = 0.022$; distance travelled: $F_{1,31} = 12.863$, $p = 0.001$), but such differences were mainly due to differences in tail length, and disappeared when including it as covariate (Two way ANCOVA with tail length as the covariate for time of movement: $F_{1,31} = 0.214$, $p = 0.648$; distance travelled: $F_{1,31} = 1.292$, $p = 0.265$).

Distance moved by intact tails was higher than for regenerated ones (Two-way ANCOVA with tail movement time as the covariate; $F_{1,31} = 6.132$, $p = 0.020$). Total movement time of autotomized tails depended on tail length ($R^2 = 0.286$, $p = 0.001$), but not on tail robustness (tail mass residuals: $R^2 = 0.023$, $p = 0.397$), and the same happened for the total distance travelled by the tail until definitive stop (tail length: $R^2 = 0.414$, $p < 0.001$; tail mass residuals: $R^2 = 0.016$, $p = 0.479$). Females' autotomized tails moved during significantly more time than those of males (Two-way ANOVA; $F_{1,31} = 4.380$, $p = 0.045$; $N = 33$; mean ± SD males: 2.48 ± 0.73 s; mean ± SD females: 3.27 ± 1.36 s), but no significant between sex differences were found in the total distance covered by their tails before stopping (Two-way ANOVA; $F_{1,31} = 1.222$, $p = 0.352$).

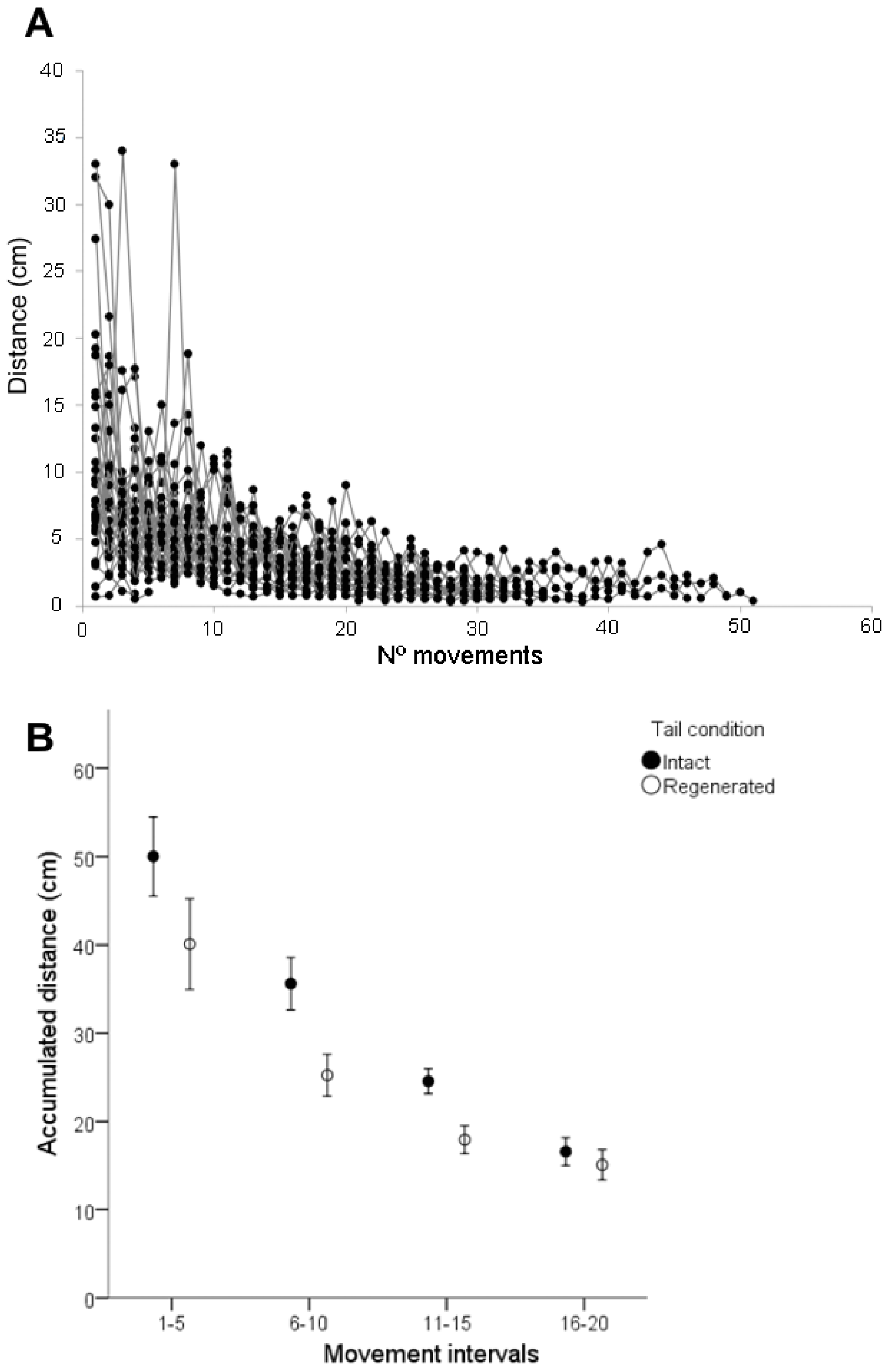

Figure 2.3. A, Distance moved by each tail in successive movements after autotomy (N = 33). **B**, Accumulated distance moved by intact (black, N = 12) and regenerated (white, N = 13) tails in successive movements after autotomy, grouped in four intervals, for tails that performed at least 20 movements. Values on B are means ± SE of the total distance moved in each interval.

Discussion

Effect of tail loss on locomotor performance

It is well known the role of the post-anal tail in the locomotion of terrestrial vertebrates, providing stability during running and climbing, and influencing jumping dynamics (Arnold 1988; Jusufi *et al.* 2008). Consequently, tail loss can be expected to affect the performance of ecologically relevant tasks, such as pursuit and capture of prey, territorial patrolling or escape from predators, that depend on mobility and are closely related to fitness (e.g., Garland & Losos 1994; Bauwens *et al.* 1995; Braña 2003; Miles 2004; Husak 2006). Several studies have been conducted to test the effect of tail loss on locomotion in different lizard species, and their results have shown considerable variation: in most studies, lizards experienced a reduction in maximum burst speed after autotomy (Daniels 1985; Chapple *et al.* 2004; Fleming *et al.* 2009), while in some others there was apparently no effect (Medger *et al.* 2008; Gillis *et al.* 2009) and even in a few cases tailless lizards exhibited an increase in maximum sprint speed (Brown *et al.* 1995; Ekner-Grzyb *et al.* 2013). Apart from methodological differences among these studies, changes in sprint speed after caudal autotomy vary among the different lizard families, each having different morphological and behavioural specificities: it usually decreases in skinks and iguanids, while there is a larger variation in lacertids and geckos (McElroy & Bergmann 2013). Such variability may be partially explained by differences in body shape (Bergmann & Irschick 2012) and in the biomechanics of locomotion, including the function of the tail and limbs (McElroy & Bergmann 2013).

Our study on wall lizards showed that autotomy has no effect on locomotor performance over unhindered spaces but has a clear negative impact on locomotion in spaces with obstacles, which represents a more realistic approach to locomotion in natural conditions. Our results suggested two possible causal mechanisms for the higher locomotor performance of tailed lizards in habitats with higher structural complexity: i) non mutilated lizards that used the tail to push off the ground were able to approach the obstacle from a larger distance, and ii) tailed individuals jumped a larger distance when leaving the obstacles. Besides, tailed lizards were more prone to jump when approaching the obstacle and when leaving it, rather than run towards it or fall from it, while mutilated individuals tended to run/fall more frequently than the tailed ones.

It is well known that the tail may be important for several aspects of lizards' locomotion, such as impulse and balance (Gillis & Higham 2016), and it seems to play a key role in physical interactions with the substrate, which may also influence trajectory and body posture (Gillis *et al.* 2013) and stability while climbing (Jusufi *et al.* 2008). As a consequence, tail loss decreases escape speed of the Cape dwarf gecko *Lygodactylus capensis* over a leaning surface (Medger *et al.* 2008) and has a destabilizing effect in *Anolis carolinensis* during running (Hsieh 2016). Thereby, caudal autotomy affects physiology and biomechanics, and therefore locomotor performance and behaviour, which will finally affect the fitness of the individual (Gillis & Higham 2016). In our study, tail removal in fact had biomechanical and also behavioural effects on jumping dynamics. For example, when leaving the obstacle, 68.57% of tailless individuals (i.e., 24 out of a total of 35) jumped rather than fell from it, in contrast with the 93.75% of tailed lizards (i.e., 30 out of 32). This difference seems to be the main explanation for the distance reached when leaving the obstacle, which was significantly larger for tailed lizards. In addition, locomotor biomechanics may be affected after tail loss, producing a reduction in jumping and climbing effectiveness. Many cursorial lizards raise their tail during running, acting like a counterpoise to the head and body, and thus the body weight is mainly concentrated on the hind limbs. The loss of a long and heavy tail displaces to the front the body mass centre (Snyder 1949; Arnold 1984), which results in more weight being transferred to the fore limbs, as it has been shown for *Podarcis sicula* or *A. carolinensis* (Arnold 1984; Gillis *et al.* 2013). According to the biomechanical models proposed by Ballinger *et al.* (1979) and Punzo (1982), we assume that such displacement of the body mass centre to the fore limbs after tail loss would decrease efficiency of propulsive force in the lizards we tested, affecting their jumping distance and total race time, which may result in a decrease of locomotor effectiveness.

On the contrary, we did not find clear negative effects of tail loss on locomotor performance of lizards when tested in open, obstacle-free spaces. As said before, tail loss may have different effects on locomotion depending on lizards' morphology and the role of their tails, either increasing or reducing locomotor performance. Tail often acts as a counterbalance for lateral bendings of the spinal cord, which influence stride length and frequency. Sprint speed is mainly determined by stride length and frequency (see Braña 2003, for wall lizards), and the lack of tail may have a negative effect on those kinematic parameters, thus decreasing sprint speed (Martín & Avery 1998; Cromie & Chapple 2012). On the other hand, long and heavy tails are often dragged during the race, increasing friction force and total body mass, thus acting as a mechanical impairment, and in such case caudal autotomy should imply a positive effect on burst speed (Arnold 1997; Willey *et al.* 2004). The absence of effect of caudal autotomy on velocity over a smooth horizontal surface found in our experiments could be the result of compensation between the negative effect of tail loss on stride length of front limbs, and the positive effect of losing mass and tail friction force after autotomy, as suggested by Medger *et al.* (2008).

Functionality of regenerated tails

Regeneration evolved to minimize the negative effects of the loss of a valuable appendage (Goss 1969), so the regenerated appendage should restore at least partially the functional role of the lost appendage (Clause & Capaldi 2006), thus providing some benefits to the animal (Arnold 1988). We found that the functionality of fully regenerated tails was equivalent to that of the intact ones in terms of locomotor performance, and that tailless animals that had suffered previous autotomy events did not exhibit better locomotor performance than the tailless ones without previous experience. Lin *et al.* (2017) found that lizards with regenerated tails had similar survivorship than those with intact tails but higher than tailless individuals, so that regeneration seemed to restore the functionality of the tails. Brown *et al.* (1995) working on a population of *P. muralis* introduced in Ohio (United States) found that individuals with regenerated tails were slower than those with intact tails. Besides, they found that tailless individuals that had previously experienced caudal autotomy, ran faster than tailless individuals that had intact tails just before the experimentally induced caudal autotomy, attributing these findings to learning effects and previous experience. On the contrary, our results suggest that lizards suffer temporary effects on locomotor performance after autotomy, regardless of their previous experience, which could be compensated by behavioural changes, as proposed by Dial and Fitzpatrick (1981), Downes and Shine (2001), or Chapple and Swain (2002). These differences between studies may be due to methodological differences and also maybe to the fact that intact and previously regenerated tails have large size differences in the sample studied by Brown *et al.* (1995), unlike in ours.

Caudal autotomy in lizards can be considered as a defensive response to attempted predation (Arnold 1988), and its success will depend on the ability to distract the predator until the lizard manages to escape, which depends largely on tail colour and mobility

(Castilla *et al.* 1999; Cooper *et al.* 2004; Kuriyama *et al.* 2016). Post-autotomy performance of mutilated tails exhibited a significant positive correlation with its length in our experiments. Studies carried out with *Lampropholis* sp. and *Trachylepis maculilabris* showed that the longer the shed tail, the longer the distance it covered after autotomy, but in contrast to our results, those studies did not show any correlation between tail length and movement time (Cooper & Smith 2009; Cromie & Chapple 2012). Our results evidenced a longer duration of tail movement after autotomy in females and, in contrast, a larger distance travelled by males' tails in relation to the total time of movement. Males have more robust base tail to accommodate hemipenes (Barbadillo *et al.* 1995), and this could allow more energetic initial movements of the detached tail, which might constrain their total duration. A long tail probably increases the probability of performing flips when bending as a consequence of the violent movements that immediately follows autotomy. Complex movements and flips of the autotomized tail are common in species that live in an exposed habitat because they involve unpredictable trajectories that increase the probability of distracting the predator (Higham & Russell 2010). Our results show that movements of shed tails after autotomy were stronger at the beginning, and their strength (measured as the mean distance travelled in each of the first 20 movements immediately after autotomy) experienced an exponential decrease with time. A forceful initial response may ensure predator's distraction (Bellairs & Bryant 1985) and could be in many cases decisive for the success of the escape response, and therefore movements during the first seconds after autotomy would be under a strong selective pressure. In our study, shed tails with regenerated portions performed weaker movements and during a shorter time than intact tails, but these differences were mainly explained by tail length, which was slightly longer for intact tails. Moreover, intensity of the first movements was significantly stronger for intact tails than for the regenerated ones, even after correction for differences in length. These findings may be explained by some structural differences between intact and regenerated tails. Skeleton of regenerated portions of the tails consists of a continuous, unsegmented cartilage axis, rather than a succession of articulated vertebrae (Hughes & New 1959). Besides, arrangement of regenerated muscle bundles is less regular than in intact tails, and they are not attached to the cartilage tube (Bellairs & Bryant 1985). In addition, according to Higham *et al.* (2013), muscles of intact tails were more resistant to fatigue. As a consequence of these different anatomic and physiological features, intensity of the initial movements, which is crucial for the escape success and determines the antipredator value of autotomy, would be diminished in regenerated tails.

In conclusion, our results evidenced that tail loss impairs locomotor performance, and that tail regeneration fully restores locomotor capacities. But, given that regenerated tails exhibit clearly diminished intensity of movement after autotomy, even after removing the effect of its different size, it seems likely that the antipredator post-autotomy value of tails is only partially retrieved after regeneration. These findings shed light on the possible adaptive scenarios in which autotomy and regeneration could have evolved, suggesting that the restoration of the antipredator post-autotomy function (i.e., another tail shed to increase the survival probability in future encounters with predators) has not been the only selective pressure that led to the evolution of regenerative abilities in lacertid lizards. The restoration of the efficiency of locomotion, a function of widespread use for many kind of activities in a variety of ecologically relevant contexts and that is accordingly closely related to fitness, may have been equally important in the evolution of lizard's regeneration ability.

References

Argaez, V.; Solano-Zavaleta, I. & Zúñiga-Vega, J.J. (2018). Another potential cost of tail autotomy: tail loss may result in high ectoparasite loads in *Sceloporus* lizards. *Amphibia-Reptilia*, 39(2): 191–202.

Arnold, E.N. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History*, 18: 127–169.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Arnold, E.N. (1997). Interrelationships and evolution of the east Asian grass lizards, *Takydromus* (Squamata, Lacertidae). *Zoological Journal of the Linnean Society*, 119: 267–296.

Ballinger, R.E.; Nietfeldt, J.W. & Krupa, J.J. (1979). An experimental analysis of the role of the tail in attaining high running speed in *Cnemidophorus sexlineatus* (Reptilia: Squamata: Lacertilia). *Herpetologica*, 35: 114–116.

Ballinger, R.E. & Tinkle, D.W. (1979). On the cost of tail regeneration to body growth in lizards. *Journal of Herpetology*, 13: 375–375.

Barbadillo, L.J. & Bauwens, D. (1997). Sexual dimorphism of tail length in lacertid lizards: Test of a morphological constraint hypothesis. *Journal of Zoology*, 242: 473–482.

Barbadillo, L.J.; Bauwens, D.; Barahona, F. & Sánchez-Herráiz, M.J. (1995). Sexual differences in caudal morphology and its relation to tail autotomy in lacertid lizards. *Journal of Zoology*, 236: 83–93.

Barrios, J.V.; Gaymer, C.F.; Vásquez, J.A. & Brokordt, K.B. (2008). Effect of the degree of autotomy on feeding, growth, and reproductive capacity in the multi-armed sea star *Heliaster helianthus. Journal of Experimental Marine Biology and Ecology*, 361: 21–27.

Bateman, P.W. & Fleming, A. (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.

Bauwens, D.; Garland, T.; Castilla, A.M. & Van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioural covariation. *Evolution*, 49(5): 848–863.

Bellairs, A. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bely, A.E. & Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170.

Bergmann, P.J. & Irschick, D.J. (2012). Vertebral evolution and diversification in squamate reptiles. *Evolution*, 66: 1044–1058.

Braña, F. (2003). Morphological correlates of burst speed and field movement patterns: the behavioural adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society*, 80(1): 135–146.

Braña, F. & Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *Journal of Experimental Zoology*, 286: 422–433.

Brown, R.M.; Taylor, D.H. & Gist, D.H. (1995). Effect of caudal autotomy on locomotor performance of wall lizards (*Podarcis muralis*). *Journal of Herpetology*, 29: 98–105.

Castilla, A.; Gosá, A.; Galán, P. & Pérez-Mellado, V. (1999). Green tails in lizards of the genus *Podarcis*: do they influence the intensity of predation? *Herpetologica*, 55(4): 530–537.

Chapple, D.G.; McCoull, C.J. & Swain, R. (2004). Effect of Tail Loss on Sprint Speed and Growth in Newborn Skinks, *Niveoscincus metallicus*. *Journal of Herpetology*, 38(1): 137–140.

Chapple, D.G. & Swain, R. (2002). Effect of caudal autotomy on locomotor performance in a viviparous skink, *Niveoscincus metallicus*. *Functional Ecology*, 16: 817–825.

Clause, A.R. & Capaldi, E.A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology*, 305A: 965–973.

Cooper, W.E.; Pérez-Mellado, V. & Vitt, L.J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoological Society of London*, 262: 243–255.

Cooper, W.E. & Smith, C.S. (2009). Costs and economy of autotomy for tail movement and running speed in the skink *Trachylepis maculilabris*. *Canadian Journal of Zoology*, 87: 400–406.

Cromie, G.L. & Chapple, D.G. (2012). Impact of tail loss on the behaviour and locomotor performance of two sympatric *Lampropholis* skink species. *PLoS ONE*, 7(4): e34732.

Daniels, C.B. (1985). The effect of tail autotomy on the exercise capacity of the water skink, *Sphenomorphus quoyii. Copeia*, 1985: 1074–1077.

Dial, B.E. & Fitzpatrick, L.C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia*, 51: 310–317.

Dial, B.E. & Fitzpatrick, L.C. (1983). Lizard tail autotomy: Function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science*, 219: 391–393.

Díaz-Guisado, D.; Gaymer, C.F.; Brokordt, K.B. & Lawrence, J.M. (2006). Autotomy reduces feeding, energy storage and growth of the sea star *Stichaster striatus*. *Journal of Experimental Marine Biology and Ecology*, 338: 73–88.

Downes, S. & Shine, R. (2001). Why does tail loss increase a lizard’s later vulnerability to snake predators? *Ecology*, 82: 1293–1303.

Ekner-Grzyb, A.; Sajkowska, Z.; Dudek, K.; Gawalek, M.; Skórka, P. & Tryjanowski, P. (2013). Locomotor performance of sand lizards (*Lacerta agilis*): effects of predatory pressure and parasite load. *Acta Ethologica*, 16(3): 173–179.

Emberts, Z.; Escalante, I. & Bateman, P.W. (2019). The ecology and evolution of autotomy. *Biological Reviews*, 94(6): 1881–1896.

Fleming, P.A.; Muller, D. & Bateman, P.W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews*, 82: 481–510.

Fleming, P.A.; Verburgt, L.; Scantlebury, M.; Medger, K. & Bateman, P.W. (2009). Jettisoning ballast or fuel? Caudal autotomy and locomotory energetics of the Cape dwarf gecko, *Lygodactylus capensis* (Gekkonidae). *Physiological and Biochemical Zoology*, 82: 756–765.

Fox, S.F. & McCoy, K.J. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia*, 122: 327–334.

Fox, S.F.; Rose, E. & Myers, R. (1981). Dominance and the acquisition of superior home ranges in the lizard *Uta stansburiana*. *Ecology*, 62: 888–893.

Fox, S.F. & Rostker, M.A. (1982). Social costs of tail loss in *Uta stansburiana*. *Science*, 218: 692–693.

Fredericq, L. (1892). Nouvelles recherches sur l'autotomie chez le crab. *Archives de Biologie*, 12: 169–197.

Garland, T. & Losos, J.B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P.C. & Reilly, S.M. (Eds.), *Ecological morphology.* University of Chicago Press, Chicago, pp. 240–302.

Gillis, G.B.; Bonvini, L.A. & Irschick, D.J. (2009). Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis. Journal of Experimental Biology*, 212: 604–609.

Gillis, G.B. & Higham, T.E. (2016). Consequences of lost endings: caudal autotomy as a lens for focusing attention on tail function during locomotion. *Journal of Experimental Biology*, 219: 2416–2422.

Gillis, G.B.; Kuo, C.-Y. & Irschick, D.J. (2013). The impact of tail loss on stability during jumping in green anoles (*Anolis carolinensis*). *Physiological and Biochemical Zoology*, 86: 680–689.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Higham, T.E.; Lipsett, K.R.; Syme, D.A. & Russell, A.P. (2013). Controlled chaos: three-dimensional kinematics, fiber histochemistry, and muscle contractile dynamics of autotomized lizard tails. *Physiological and Biochemical Zoology*, 86(6): 611–630.

Higham, T.E. & Russell, A.P. (2010). Flip, flop and fly: modulated motor control and highly variable movement patterns of autotomized gecko tails. *Biology Letters*, 6: 70–73.

Hsieh, S.-T.T. (2016). Tail loss and narrow surfaces decrease locomotor stability in the arboreal green anole lizard (*Anolis carolinensis*). *Journal of Experimental Biology*, 219: 364–373.

Huey, R.B. & Pianka, E.R. (1981). Ecological consequences of foraging mode. *Ecology*, 62: 991–999.

Hughes, A. & New, D. (1959). Tail regeneration in the geckonid lizard, *Sphaeridactylus. Journal of Embryology and experimental Morphology*, 7: 281–302.

Husak, J.F. (2006). Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology*, 20: 174-179.

Husak, J.F.; Fox, S.F.; Lovern, M.B. & Van Den Bussche, R.A. (2006). Faster lizards sire more offspring, Sexual selection on whole-animal performance. *Evolution*, 60(10): 2122–2130.

Jayne, B.C. & Bennett, A.F. (1990). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution*, 44: 1204–1229.

Jusufi, A.; Goldman, D.I.; Revzen, S. & Full, R.J. (2008). Active tails enhance arboreal acrobatics in geckos. *Proceedings of the National Academy of Sciences of the United States of America*, 105: 4215–4219.

Kardong, K. (2014). *Vertebrates, Comparative Anatomy, Function, Evolution.* 7th edition. McGraw-Hill Education.

Kuriyama, T.; Morimoto, G.; Miyaji, K. & Hasegawa, M. (2016). Cellular basis of anti-predator adaptation in a lizard with autotomizable blue tail against specific predators with different color visión. *Journal of Zoology*, 300: 89–98.

Lin, J.-W.; Chen, Y.-R.; Wang, Y.-H.; Hung, K.-C. & Lin, S.-M. (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(1847): 20162538.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals, a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Martín, J. & Avery, R.A. (1998). Effects of tail loss on the movement patterns of the lizard *Psammodromus algirus*. *Functional Ecology*, 12: 794–802.

McElroy, E.J. & Bergmann, P.J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology*, 86: 669–679.

Medger, K.; Verburgt, L. & Bateman, P.W. (2008). The influence of tail autotomy on the escape response of the Cape Dwarf Gecko, *Lygodactylus capensis*. *Ethology*, 114: 42–52.

Miles, D.B. (2004). The race goes to the swift, fitness consequences of variation in sprint performance in juvenile lizards. *Evolutionary Ecology Research*, 6: 63–75.

Moore, A.; Tabashnik, B.E. & Stark, J.D. (1989). Leg autotomy, A novel mechanism of protection against insecticide poisoning in diamondback moth (Lepidoptera, Plutellidae). *Journal of Economic Enthomology*, 82(5): 1295–1298.

Punzo, F. (1982). Tail autotomy and running speed in the lizards *Cophosaurus texanus* and *Uma nonata*. *Journal of Herpetology*, 16: 329–331.

Ramsay, K.; Kaiser, M.J. & Richardson, C.A. (2001). Invest in arms, behavioural and energetic implications of multiple autotomy in starfish (*Asterias rubens*). *Behavioral Ecology and Sociobiology*, 50: 360–365.

Robson, M.A. & Miles, D.B. (2000). Locomotor performance and dominance in male Tree Lizards, *Urosautrus ornatus*. *Functional Ecology*, 14: 338–344.

Salvador, A. (2014). *Podarcis muralis*. In: Salvador, A. (Ed.), *Fauna Ibérica.* Vol. 10, Reptiles. 2nd edition. Museo Nacional de Ciencias Naturales CSIC, Madrid, Spain, pp. 576–589.

Snyder, R.C. (1949). Bipedal locomotion of the lizard *Basiliscus basiliscus. Copeia*, 1949: 129–137.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford University Press, London.

Webb, P.W. (1986). Locomotion and predator-prey relationships. In: Feder, M.E. & Lauder, G.V. (eds.), *Predator-prey relationships. Perspectives and approaches* *from the study of lower vertebrates*. University of Chicago Press, Chicago, pp. 24–41.

Willey, J.S.; Biknevicius, A.R.; Reilly, S.M. & Earls, K.D. (2004). The tale of the tail, limb function and locomotor mechanics in *Alligator mississippiensis*. *Journal of Experimental Biology*, 207: 553–563.

Wulff, J.L. (2006). Resistance vs. recovery, morphological strategies of coral reef sponges. *Functional Ecology*, 20: 699–708.

“On the many artifices evolved to thwart would-be predators, few are so extreme as tail autotomy” Bellairs & Bryant, 1985 (In: Gans & Billet eds., *Biology of Reptilia*, vol. 15).

“De los muchos artificios desarrollados para frustrar a posibles depredadores, pocos son tan extremos como la autotomía”.

Cover photo: *Coronella austriaca*

III. Chapter 2

Behavioural patterns in the early-stage antipredator response change after tail autotomy in adult wall lizards

Under Review in *Journal of Experimental Zoology A: Ecological and Integrative Physiology*: Fernández-Rodríguez, Irene & Braña, Florentino. Behavioural patterns in the early-stage antipredator response change after tail autotomy in adult wall lizards.

Behavioural patterns in the early-stage antipredator response change after tail autotomy in adult wall lizards

Abstract

Autotomy is a very drastic antipredator defence consisting of the voluntary shedding of a body part to escape from the predators. The loss of a body part may impair locomotion, feeding or mating, so animals may face a higher predation risk shortly after autotomy. Thus, until regeneration is completed, prey may adjust their behaviour to reduce predation risk, and these could involve secondary costs. We assessed the effect of tail loss on the antipredator behaviour of wall lizards (*Podarcis muralis*), comparing the behaviour of tailed and tailless individuals exposed to a predatory snake (*Coronella austriaca*) scent, under controlled experimental conditions. Tailless lizards spent significantly more time performing behaviours with antipredatory significance (e.g., moving slowly), whereas tailed individuals performed exploratory walking for significantly more time. Moreover, tailless lizards spent more time basking, which probably increases the effectiveness of their cryptic design and decreases detection by predators. Lizards intensified the tongue flick rates when exposed to a pungent control or snake scents, as compared to their response to a neutral control. Besides, both tailed and tailless lizards intensified some aspects of their antipredator behaviour (walking slowly and avoiding refuge use) when exposed to snake scent, which indicates discrimination of the smell of predatory snakes. Lizards decreased refuge use when exposed to predator scents, probably because the refuges are evaluated as unsafe due to a high concentration of snake scents. To conclude, our experiments showed that, after losing their tails, wall lizards modify their behaviour in a way that likely minimizes predation risk.

Keywords: predator recognition, olfactory cues, predator avoidance, *Podarcis muralis*, *Coronella austriaca*.

Introduction

The evolution of animal morphology and behaviour is partially driven by predation, which implies a strong selecting pressure, so that prey have evolved diverse antipredator strategies to avoid being killed (Abrams 2000; Johnson & Belk 2020). Prey's defensive strategies can be categorized into primary and secondary defences (Greene 1988; Lind & Cresswell 2005; Langerhans 2007). Primary defences reduce the probability of detection and identification by a predator (e.g., cryptic designs, immobility or anachoresis; Caro 2005; Michelangeli & Wong 2014), while secondary defences diminish the probability of capture and death once the attack has been initiated (e.g., armours, spines, toxins, mimicry, or elusiveness signalling; Ruxton *et al.* 2018). Among the secondary defences, aimed at disrupting attacks and allowing the escape from the predator, autotomy is a particularly drastic one, as the threatened animal suffers the voluntary detachment of a body part to wriggle out of the predator, and to provide a distraction that enables the escape success (Maginnis 2006). Autotomy is relatively common among vertebrates and can affect different peripheral expendable parts of the body, such as the limbs, the skin or the tail (Higham *et al.* 2013). In particular, caudal autotomy to avoid predation is very common and taxonomically widespread in lizards (Bellairs & Bryant 1985; Greene 1988; Bateman & Fleming 2009), to such an extent that in many natural populations more than 50 % of adult animals have mutilated or regenerated tails (Downes & Shine 2001; Chapple *et al.* 2002; Fernández-Rodríguez & Braña 2020, for the herein studied population of *Podarcis muralis*).

Despite the benefits of autotomy to avoid predation, the loss of a body part can entail several immediate consequences, such as decreased locomotor performance, degradation of social status or restriction in communication between conspecifics, which may negatively affect daily activities, like habitat selection, foraging, moving, mating, or facing new predator encounters (see the reviews of Maginnis 2006; Bateman & Fleming 2009; Lawrence 2010; Emberts *et al.* 2019). Because of these major drawbacks, autotomy is frequently followed by regeneration of the lost parts, which restores partially or completely their functionality (Lin *et al.* 2017; Fernández-Rodríguez & Braña 2020). However, regeneration takes time and, until it is complete, animals suffer from locomotor impairment and lack the possibility of using the lost parts as a distraction in new encounters with predators, so they might face an increased risk of predation (Fox & McCoy 2000; Lin *et al.* 2017). Thus, to cope with these costs and reduce predation risk after autotomy, animals have frequently evolved the ability to adopt behavioural adjustments, such as changes in activity time, space use, foraging or escape tactics (Fox *et al.* 1981; Ramsay *et al.* 2001; Bateman & Fleming 2011). Some of the behavioural modifications may, in turn, imply secondary costs, so there could be a trade-off between avoiding predation and performing other fitness-related activities. For instance, increasing foraging time to fulfil the extra energetic requirements for regeneration may increase predation risk (Fox 1978; Dial & Fitzpatrick 1981), and increasing anachoresis (i.e., staying longer in shelters) may imply a decrease on activity time, mating and feeding opportunities, even leading to a decrease in body condition (Martín 2001).

The adaptive value of autotomy relies on the balance between its costs and benefits (Arnold 1988), so it is necessary to identify the behavioural changes associated with the loss of a part of the body, and to evaluate the potential costs derived from such injury. The functional costs of tail loss have been widely studied in lizards (for a review, see Bateman & Fleming 2009), which are very suitable models to study the behavioural adjustments undergone to reduce the risk of predation after autotomy (Wilson 1992; Martín & Salvador 1993; Salvador *et al.* 1995; Michelangeli *et al.* 2020). Within this framework, the aims of this study are to assess the effect of tail loss on the antipredator behaviour of wall lizards (*P. muralis*), comparing the activity and behaviour of tailed and tailless individuals in the laboratory when exposed to olfactory cues from the smooth snake (*Coronella austriaca*), a specialized predator whose diet is mainly composed by small reptiles. Most squamate reptiles strongly rely on chemical cues to develop social, reproductive, exploratory, predatory and antipredator behaviours, for which they have evolved complex vomeronasal chemoreception systems (Schwenk 1995; Cooper 1997). Specifically, several studies have shown that lacertid lizards are able to detect predators from their chemical cues and deposits (Thoen *et al.* 1986; Van Damme & Quick 2001; see, for *P. muralis*, Amo *et al.* 2004a; 2005), so we have carried out laboratory tests in order to assess the possible differences in behaviour and activity of tailed and tailless lizards exposed to olfactory signals from predators.

Material and methods

Laboratory experiments

The smooth snake (*Coronella austriaca* Laurenti, 1768) is a small constrictor snake (Reptilia: Colubridae) of 50–60 cm length that inhabits rocky and shrubland areas of south Europe, from 0 to 1700 m of elevation (Galán 2014). The smooth snake feeds mostly on lacertid lizards, like *Podarcis muralis*, although it incorporates an increasing number of small mammals into its diet as it grows (Rugiero *et al.* 1995; Reading & Jofré 2013).

Thirty-three adult individuals of wall lizards ($N_{females}$ = 17; N_{males} = 16) were captured by noose in rural areas of Asturias (northern Spain) with presence of smooth snakes, so the lizards included in our experiment likely have had some previous contact with this specialist predator in its natural environment. The experiments were carried out in September 2018, once the reproductive period is over, thus reducing behavioural biases due to sex and the reproductive condition of the individuals. The animals were transported to the laboratory of Zoology (University of Oviedo), housed in terraria (50 L x 37 W x 25 H, cm) exposed to a natural photoperiod (approximately 12h light and 12h darkness) and provided with water and food *ad libitum* (crickets, mealworms and cockroaches). Animals were randomly housed in groups of three lizards each, composed of either two females and one male, or two males and one female. Lamps of 35 W suspended 20 cm above the cages allowed lizards to thermoregulate within their thermal preferred range. Both males and females were measured for SVL and divided into a control ($N_{females} = 8$; $N_{males} = 8$; mean ± SD SVL: 5.95 ± 0.41 cm) and an experimental group ($N_{females} = 9$; $N_{males} = 8$; mean ± SD SVL: 6.08 ± 0.47 cm). Tail autotomy was induced to the experimental group by holding the animals firmly from the base of the tail for 2-5 seconds, until they released it autonomously, leaving a tail stub of 0.96 ± 0.17 cm (mean ± SD). To minimize behavioural biases due to handling when inducing autotomy, lizards of the control group were manipulated in a similar way to the experimental individuals but avoiding tail detachment. Animals were allowed to rest for 2 days after manipulation, so that tailless lizards could recover from the physical trauma and acclimate to moving and performing their routine activities in their new tailless condition. Each lizard was subjected on three consecutive days to behavioural tests conducted in three structurally homogeneous environments, only differing in olfactory cues, namely: one environment impregnated with a neutral odour (distilled water), another with a predator odour (*C. austriaca*) and a third with an intense odour with no specific biological significance (cologne); each individual did only one trial per day and the order of the trials for each individual was randomized. The trials consisted in leaving the lizards in an open opaque-walled terrarium (62 L x 43 W x 40 cm H), with a 100 W lamp suspended in the middle of the arena to give them the opportunity to thermoregulate, and the floor covered with absorbent paper impregnated with the specific odours. A hollow brick was placed in the middle of the terrarium under the heat source, which could be used either as a substrate for thermoregulation or as a shelter. Lizards were allowed to move freely in the test arena for 15 minutes and, during that time, behaviours with possible meaning in antipredator defence were recorded and timed. Before starting the trials, lizards were placed in an incubator at 25 °C for 30 min, so that they could reach a body temperature close to the lower limit measured in active animals in the field. This was aimed to guarantee that they were active from the beginning of the trial, but close to the lower limit of the preferred thermal range of the species, which is near 32 °C (Braña 1991; 1993; Bauwens *et al.* 1995), so that lizards will probably have the urge to thermoregulate.

For the neutral control trial, the bottom of the terrarium (brick and paper) was sprayed with distilled water. The trial with smooth snake scent aimed to simulate the presence of a specific predator in order to evaluate the possible differences between tailed and tailless lizards in their response to specific predator cues. To impregnate the brick and the substrate of the terrarium with the snake's scent, an adult smooth snake was placed in the terrarium, letting it move freely for 20 hours and removing it just before starting the trial. For this procedure, we used two adult smooth snakes (female's total length: 64 cm; male's total length: 55 cm) that were housed in a terrarium (116 L x 52 W x 41 H, cm) located in a different area than the terraria of lizards, so that lizards were visually and olfactory isolated from the snakes. During the experiment, the snakes were fed approximately once per week with fresh or thawed detached lizard tails. In order to determine if lizards recognize the scent of the snake or just react to an intense odour, we tested, as an additional control, the behaviour of the lizards when exposed to a pungent odour without a specific biological meaning (cologne), according to the usual experimental protocol for olfactory recognition tests in reptiles (e.g., Dial & Schwenk 1996; Van Damme & Quick 2001). For the pungency control we used the commercial cologne Deliplus “Brisa”, from Maverick laboratories S.L.U,. After each trial the terraria and the bricks were cleaned and disinfected with alcohol and diluted bleach, and then rinsed with water to reduce all scents.

All the trials were recorded with a videocamera (Sony HDR-CX210E) mounted above the experimental terrarium and, based on previous studies on lizard behaviour (Thoen *et al.* 1986; Van Damme *et al.* 1990; Amo *et al.* 2004a; Ortega *et al.* 2018), six types of behavioural responses were recognized and timed in the videorecordings (played back with the software Windows Media Player), with a total time of 15 minutes for each lizard in each of the three trials. We have considered the following behavioural responses:

- **Slow moving**: slow and intermittent motion, with frequent stops of 1–2 seconds and scattered or jerky movements (Avery 1993; Braña 2003), likely aiming to evaluate the surrounding area or trying to minimize detectability by possible predators (Kramer & McLaughlin 2001).
- **Normal moving**: Rather fast and continuous movement, often with some changes in pace and direction, interpreted as mainly exploratory.
- **Motionless**: the lizard does not perform any displacement for at least 5 seconds and neither adopts the typical thermoregulatory postures (see “basking”).
- **Hide in the refuge**: enter and remain inside the refuge (hollow brick) for at least 5 seconds.
- **Basking**: the lizard is located under the heat source, immobile and adopting the characteristic postures of thigmothermic and/or heliothermic thermoregulation (see, e.g., Bradshaw & Main 1968; Muth 1977).

- **Tongue flick**: number of times that the lizard protruded and rapidly retracted the tongue, as a measure of exploratory activity supposedly aimed at capturing chemosensory information.

Other behavioural patterns that have been described as part of the lizard's antidepredatory repertoire, such as “foot shake” or “tail waving” (e.g., Font *et al.* 2012), have been infrequent or unclear in our recordings and have not been considered.

Statistical analyses

All the variables met the assumptions of normality and homogeneity of variances (Kolmogorov-Smirnov and Levene tests, $p > 0.05$ in all cases) , except for the variables “refuge” in the trials with snake scent (Kolmogorov-Smirnov test: $Z = 1.935$, $p = 0.001$) and “motionless” in the control trials (Kolmogorov-Smirnov test: $Z = 1.444$, $p = 0.030$). Thus, the variables “refuge” and “motionless” were log10 transformed to achieve normality. A one-way ANOVA with SVL as the dependent variable and tail group as factor was done to check that the control and the experimental groups were homogeneous in terms of size (SVL). To test possible differences in the behaviour of tailed and tailless individuals in the three trials (neutral control, cologne scent and snake scent), a two-way repeated measures multivariate analysis of variance (MANOVA) was done with all the behavioural variables measured (i.e., total time moving slowly, walking normally, motionless, hiding in the refuge and basking) as the dependent variables, and type of trial, tail group and sex as factors; post hoc comparisons between the pairs of trials were done with Tukey tests.

Table 3.1. Statistical results of the two-way repeated measures MANOVA carried out with the time spent by lizards performing five characteristic behaviours. Type of trial (neutral control, pungent control and snake scent), tail group (tailed or tailless) and sex (male or female) were included as categorical inter-subject factors.

	Behavioural variables	**F-value**	**p-value**
Trial	**Basking**	0.101	0.888
	Normal moving	0.024	0.964
	Slow moving	29.530	**< 0.001**
	Motionless	0.122	0.873
	Refuge	7.024	**0.005**
Tail group	**Basking**	5.921	**0.021**
	Normal moving	8.223	**0.008**
	Slow moving	5.022	**0.033**
	Motionless	0.299	0.589
	Refuge	3.236	0.082
Sex	**Basking**	0.493	0.488
	Normal moving	1.364	0.252
	Slow moving	0.661	0.423
	Motionless	3.690	0.065
	Refuge	0.781	0.384
Trial*Tail group	**Basking**	1.280	0.286
	Normal moving	0.321	0.696
	Slow moving	2.508	0.110
	Motionless	0.070	0.923
	Refuge	0.093	0.859
Trial*Sex	**Basking**	0.211	0.791
	Normal moving	1.915	0.163
	Slow moving	1.227	0.291
	Motionless	0.643	0.520
	Refuge	0.929	0.390
Tail group*Sex	**Basking**	0.183	0.672
	Normal moving	1.565	0.221
	Slow moving	1.920	0.176
	Motionless	0.268	0.609
	Refuge	0.520	0.448

The frequency of tongue movements was analysed independently, as it was measured as a rate (number per unit of time), while the other variables express the time spent on certain behaviours which are mutually exclusive, unlike the movements of the tongue, which were always performed simultaneously with some other behavioural pattern. Therefore, possible differences in the rate of tongue flicks between tailed and tailless lizards and between the different olfactory stimuli were tested by a repeated measures ANOVA with tail group and sex as inter-subject factors. The assumption of sphericity was checked with a Mauchly's test. Post hoc comparisons to identify particular between-pair differences among the three trial types were done with Tukey tests when the overall analyses indicated significant differences. Significant level was set at 0.05 for all the analyses done in this study. Statistical analyses were performed in SPSS (version 20).

Results

Tailed and tailless lizards did not differ in SVL (one-way ANOVA: $F_{1,31} = 0.807$, $p = 0.376$). Results of the two-way repeated measures MANOVA carried out on variables that indicate the time spent in developing characteristic behaviours are summarised in Table 3.1 and Figure 3.1. There are several behavioural changes associated with tail condition: tailless individuals spent more time performing stereotyped slow moving and basking than tailed lizards, whereas tailed lizards spent more time walking normally than tailless ones. No differences were found between tailed and tailless lizards in the time spent performing other behaviours, and sex was not a significant factor for any of the variables included in the analysis.

Regarding the behavioural patterns in the three different trials (neutral control, pungent control, snake scent), lizards spent significantly more time walking slowly during the trial with snake odour than during the neutral and cologne control tests (post-hoc comparisons, neutral control vs. cologne: $p = 0.323$, neutral control vs. snake: $p < 0.001$, cologne vs. snake: $p < 0.001$). Besides, lizards spent significantly less time inside the refuge during the trial with snake scent than in the neutral and pungent control trials (post-hoc comparisons: neutral control vs. cologne: $p = 0.250$, neutral control vs. snake: $p = 0.034$, cologne vs. snake: $p = 0.001$). No differences were found between the different types of trials with respect to the time spent performing any other behaviour (Table 3.1; $p > 0.05$ in all cases). No significant interactions were found between type of trial and sex or tail group in any of the behavioural variables (Table 3.1).

The assumption of sphericity was met in the repeated measures ANOVA with tongue flicks as dependent variable (Mauchly's test: $W = 0.899$, $p = 0.224$). Lizards did not exhibit differences in tongue flick rate according to their tail group (tailed/tailless) or their sex (Repeated measures ANOVA, $p > 0.05$ in both cases; Figure 3.2) but performed significantly more flicks per minute when confronted with snake than in the neutral control trial (Repeated measures ANOVA: $F_{2,28} = 3.753$, $p = 0.036$; post-hoc comparisons: neutral control vs. cologne: $p = 0.159$, neutral control vs. snake: $p = 0.010$, cologne vs. snake: $p = 0.509$).

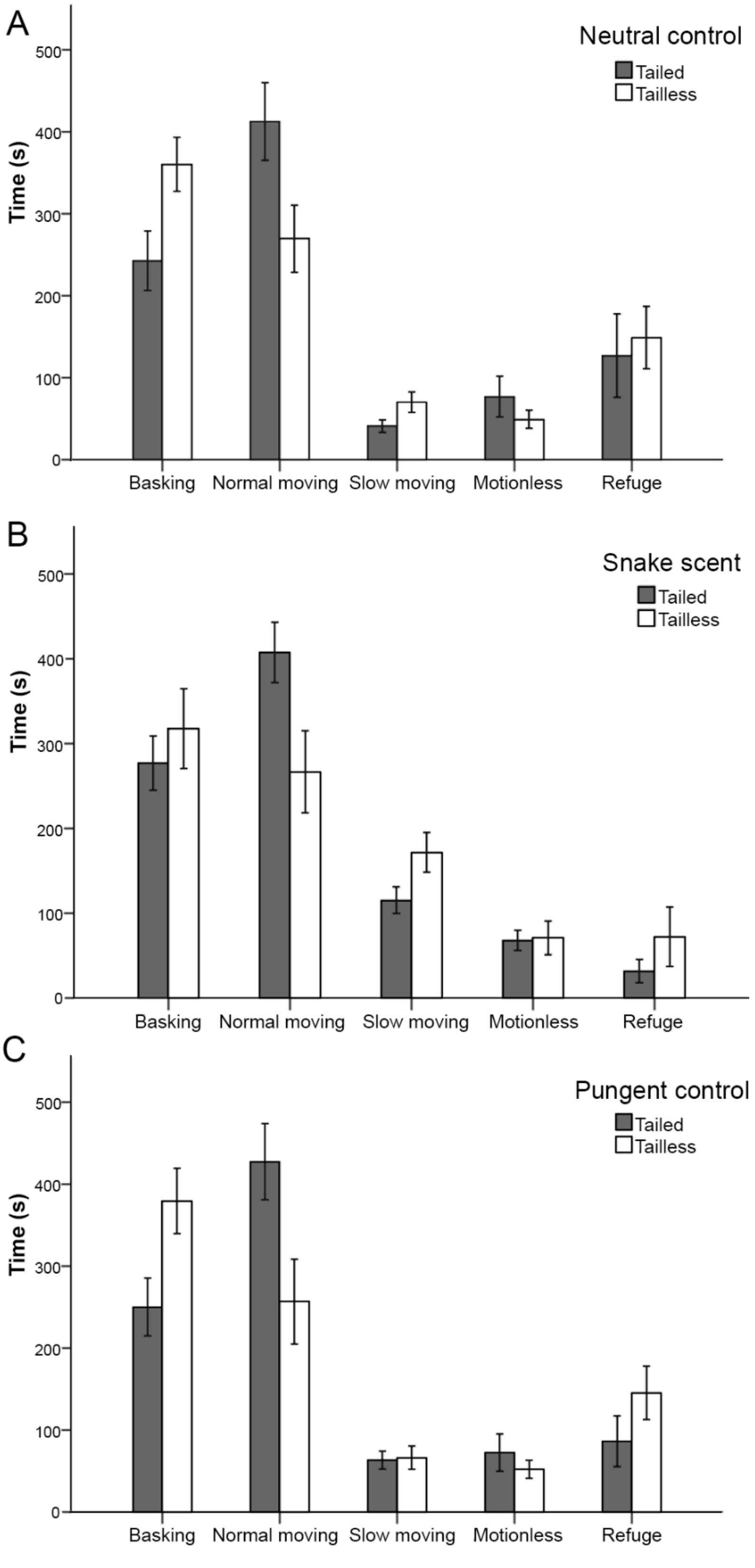

Figure 3.1. Behavioural responses of tailed (grey) and tailless (white) lizards, in three trials conducted using different olfactory stimuli: **A**, a neutral control (distilled water); **B**, a predator snake (*Coronella austriaca*) scent; **C**, a pungent odour (cologne). Values are mean (± SE) total time spent by the lizards in each activity during the trial.

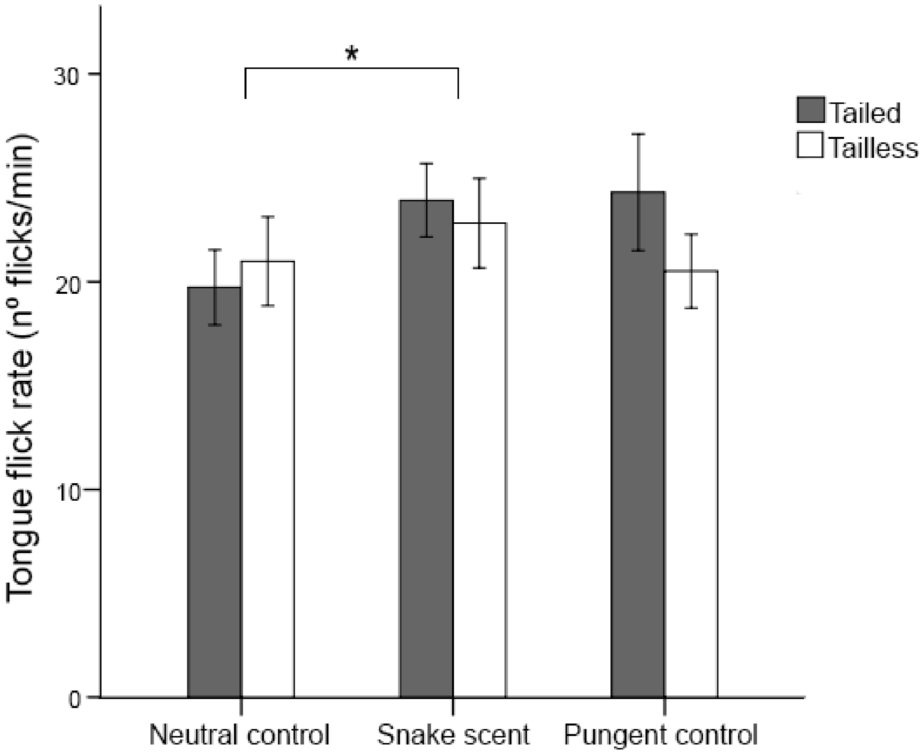

Figure 3.2. Tongue flick rate per minute of tailed (grey) and tailless (white) lizards in the three trials in which the environment is impregnated with different smells: a neutral control (distilled water), a predator snake (*Coronella austriaca*) scent and a pungent control (cologne). Values are means ± SE; * $p < 0.05$.

Discussion

Tail autotomy in lizards is an effective, yet drastic antipredator response once the predator has initiated a pursuit (Arnold 1988; Ruxton *et al.* 2018). But lacking the tail often constrains locomotor performance (Medger *et al.* 2008; Fernández-Rodríguez & Braña 2020), which is essential for a number of ecologically relevant tasks that are closely related to fitness, such as feeding, territory patrolling or mating (Garland & Losos 1994; Braña 2003; Husak 2006). In addition, the probability of avoiding predation, either by fleeing or by shedding again the tail, decreases after autotomy (Fox & McCoy 2000), so lizards are expected to exhibit some behavioural adjustments to minimize the costs of lacking the tail, at least until regeneration is completed. According to this prediction, and regardless of the presence of predator scents, tailless lizards in our experiment spent significantly more time than tailed ones performing behaviours with probable antipredator significance, such as stereotyped slow moving, and tended to minimize behaviours that imply greater exposure and increase detectability, such as exploratory walking. Previous studies showed that walking slowly and performing slow and jerky movements, together with tail waving and foot shaking, are general antipredator behaviours in lacertid lizards (Thoen *et al.* 1986; Mencía *et al.* 2016; Ortega *et al.* 2018; see Font *et al.* 2012 for *Podarcis muralis*), and probably reduce the likelihood of detection by predators or favour the ability to respond to their attacks (Labra & Niemeyer 2004). Besides, remaining motionless while basking surely enhances the cryptic value of the dorsal design, thus reducing the probability of detection and, on the other hand, decreases the stimulus for attack by predators, which are frequently triggered by sudden movements of the prey (see, for predatory snakes, Shine & Sun 2003). Moreover, the greater tendency of tailless lizards to remain immobile is consistent with the pattern observed in wall lizards in other situations of locomotor impairment, such as the burden of pregnancy (Braña 1993). On the other hand, basking is known to be costly in terms of exposure to visual predators (Alford & Lutterschmidt 2012), specially depending on the microhabitats selected for thermoregulation, which may change after tail loss (Martín & Salvador 1993), but these potential shifts in microhabitat use after tail loss and its relation to thermoregulation, are beyond the aims and design of this study. Another explanation for the higher time devoted by tailless lizards to thermoregulation would be to optimize their physiological functions to accelerate healing and regeneration.

The present study shows anti-predatory behavioural changes in tailless wall lizards, a species on which we had shown in a previous study that suffers a significant decrease in locomotor ability after the loss of the tail (Fernández Rodríguez & Braña 2020). Other lizards, like the iguanian *Sceloporus virgatus*, the lacertid *Psammodromus algirus* and the skinks *Lampropholis delicata*, *L. guichenoti* and *Scincella lateralis*, also present alterations in their antipredator behaviour and a locomotor impairment after tail loss (Formanowicz *et al.* 1990; Martín & Avery 1998; Downes & Shine 2001; Cromie & Chapple 2012; Michelangeli *et al.* 2020). On the other hand, the geckos *Amalosia lesueurii* and *Teraroscincus scincus*, and the skink *Pseudocordylus melanotus*, do not suffer from locomotor impairment after tail loss and neither do alter their antipredator behaviour after tail loss (McConachie & Whiting 2003; Kelehear & Webb 2006; Lu *et al.* 2010). This supports the idea that such behavioural adjustments are adopted to minimize vulnerability after tail loss, at least partially because of the consecutive reduction in locomotor capacity, but further studies would be necessary to specifically address this issue.

Lizards use information mediated by chemical signals for feeding, social and sexual communication or predator detection, and tongue flicking contributes to the chemosensory perception mechanism associated to the vomeronasal organ (Cooper 1994), so that the frequency of tongue movements outside the mouth can be reliably interpreted as an indicator of variations in exploratory and information gathering behaviours (Gove 1979; Cooper & Burghardt 1990). In our experimental tests, the behavioural responses related to chemoreception were not affected by tail loss, since both tailed and tailless lizards exhibited similar patterns of tongue flicking during the three trials. Regardless of their tail condition, wall lizards increased tongue flick rates in environments impregnated with intense olfactory signals (either colony or snake scent), thus indicating an intensification of the exploratory pattern, but this does not provide evidence that they specifically recognize the predator. However, although the tongue movements are almost equally intensified by the exposure to the smell of snakes and to a pungent odour, which is not significant from the perspective of the set of lizards' ecological interactions, other behavioural responses observed in our trials indicate that lizards were able to discriminate the smell of predatory snakes. For example, both tailed and tailless lizards intensified certain patterns of antipredator behaviour (e.g., moving slowly) when exposed to snake scent, as compared to the patterns shown in the neutral and the pungent control trials. Indeed, the differentiated response to snake chemical cues does not imply the recognition of *C. austriaca* as a lizard-specific predator, but some studies conducted on the antipredatory responses of lacertid lizards frequently showed that lizards are able to discriminate between the chemical cues of lizard-predatory snakes and those of non-saurophogous snakes (e.g., Van Damme & Quick 2001; Mencía *et al.* 2016; Ortega *et al.* 2018; see, for *P. muralis*, Amo *et al.* 2004a; Durand *et al.* 2012). Therefore, given that the lizards used in our experiment were adults collected in the field in a locality where they coexist with smooth snakes, it is plausible to propose that their recognition of the snake's scent may have been species-specific.

Wall lizards in our experiments spent significantly less time inside the refuge in the predator-scented environment, which indicates that they did not resort to anachoresis when exposed to predator chemical cues. The avoidance of shelters could occur for two different reasons: one immediate, based on the olfactory information provided by the refuge itself; and the other general, based on an a priori consideration of the safety of a shelter as protection against a specific type of predator. Regarding the first aspect, we have verified that the smooth snakes placed in the terrarium often spent a long time sheltering inside the holes of the bricks, so those shelters surely had a high concentration of snake chemical cues, and this is a possible reason why they were generally avoided by the lizards. Secondly, since the smooth snake is an ambush foraging predator that frequently lurks from crevices and holes between stones (Amo *et al.* 2004c), the brick holes could be evaluated as unsafe shelters by lizards (Amo *et al.* 2004b; 2005; Durand *et al.* 2012), especially when the environment is saturated with the snake scent, indicating a nearby presence of the predator. It is worth remembering that the lizards used in this study were adults caught in the field, so that they had possibly had previous contact with predatory snakes, which makes it difficult to conclude whether these behaviours are innate or learned, although some previous studies have suggested a limited role of experience in the expression of anti-snake behaviour in wall lizards (Durand *et al.* 2012).

To conclude, our study gives evidence of how lizards resort to behavioural modifications after tail loss, which likely minimizes the higher risk of predation until the lost capacities are restored after the completion of regeneration. Increasing wariness may contribute to minimize detection by the predators and diminish predation risk, which lizards achieve by decreasing activity levels (Martín & Salvador 1995; Downes & Shine 2001; Michelangeli *et al.* 2020), changing the microhabitat use (Fox *et al.* 1981) or modifying the escape tactics (Dial & Fitzpatrick 1981; Cooper 2003; 2007; Fleming *et al.* 2007). According to Brodie *et al.* (1991), defensive mechanisms can be divided into predator-avoidance (reducing the probability of detection and encounter with the predator) and antipredator strategies (reducing the probability of capture and death after detection). Lizards' tail autotomy is clearly an antipredator mechanism that occurs once the attack has been triggered, while the behavioural modifications displayed in the presence of olfactory signals are early-stage predator-avoidance mechanisms. Tailless lizards not only have reduced locomotor capacities (Fernández-Rodríguez & Braña 2020), but nor can they resort again to autotomy to avoid predation, so their antipredator defences are diminished until regeneration is completed. Our study gives evidence of an exacerbation of lizards' predator-avoidance mechanisms when their antipredator mechanisms are diminished as a consequence of tail loss. These kinds of behavioural changes have also been observed in other animal taxa that undergo autotomy, such as starfishes, crabs, damselflies or grasshoppers (see the reviews of Maginnis 2006; Fleming *et al.* 2007; Emberts *et al.* 2019). However, these anti-predatory responses come at a cost, since they imply an allocation of time and energy and thus may incur trade-offs with other functions directly related to self-maintenance and fitness (Lind & Cresswell 2005), and even so, animals modify their behaviour after autotomy, as shown by this study, suggesting that the benefits of wariness outcome its costs.

References

Abrams, P.A. (2000). The evolution of predator-prey interactions: Theory and evidence. *Annual Review of Ecology and Systematics*, 31: 79–105.

Alford, J.G. & Lutterschmidt, W.I. (2012). Modelling energetic and theoretical costs of thermoregulatory strategy. *Journal of Biological Dynamics*, 6: 63–79.

Amo, L.; López, P. & Martín, J. (2004a). Chemosensory recognition and behavioral responses of wall lizards, *Podarcis muralis*, to scents of snakes that pose different risks of predation. *Copeia*, 2004(3): 691–696.

Amo, L.; López, P. & Martín, J. (2004b). Wall Lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. *Animal Behaviour*, 67: 647–653.

Amo, L.; López, P. & Martín, J. (2004c). Chemosensory recognition of its lizard prey by the ambush smooth snake, *Coronella austriaca*. *Journal of Herpetology*, 38: 451–454.

Amo, L.; López, P. & Martín, J. (2005). Chemical assessment of predation risk in the wall lizard, *Podarcis muralis*, is influenced by time exposed to chemical cues of ambush snakes. *Herpetological Journal*, 15: 21–25.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Avery, R.A. (1993). Experimental analysis of lizard pause-travel movement: pause increases probability of prey capture. *Amphibia-Reptilia*, 14: 423–427.

Bateman, P.W. & Fleming, P.A. (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.

Bateman, P.W. & Fleming, P.A. (2011). Failure to launch? The influence of limb autotomy on the escape behavior of a semiaquatic grasshopper *Paroxya atlantica* (Acrididae). *Behavioral Ecology*, 22(4): 763–768.

Bauwens, D.; Garland, T.; Castilla, A.M. & van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution*, 49(5): 848–863.

Bellairs, A. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bradshaw, S.D. & Main, A.R. (1968). Behavioural attitudes and regulation of temperature in *Amphibolurus* lizards. *Journal of Zoology*, 154: 193–221.

Braña, F. (1991). Summer activity patterns and thermoregulation in the wall lizard, *Podarcis muralis*. *The Herpetological Journal*, 1: 544–549.

Braña, F. (1993). Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos*, 66(2): 216–222.

Braña, F. (2003). Morphological correlates of burst speed and field movement patterns: the behavioral adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society*, 80(1): 135–146.

Brodie, E.D. Jr.; Formanowicz, D.R. Jr. & Brodie, E.D. III (1991). Predator avoidance and antipredator mechanisms: Distinct pathways to survival. *Ethology, Ecology and Evolution*, 3: 73–77.

Caro, T.M. (2005). *Antipredator defenses in birds and mammals*. University of Chicago Press, USA.

Chapple, D.G.; McCoull, C.J. & Swain, R. (2002). Changes in reproductive investment following caudal autotomy in viviparous skinks (*Niveoscincus metallicus*): lipid depletion or energetic diversion? *Journal of Herpetology*, 36: 480–486.

Cooper, W.E. Jr. & Burghardt, G.M. (1990). A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. *Journal of Chemical Ecology*, 16: 45–65.

Cooper, W.E. (1994). Chemical discrimination by tongue-flicking in lizards: A review with hypotheses on its origin and its ecological and phylogenetic relationships. *Journal of Chemical Ecology*, 20: 439–487.

Cooper, W.E. (1997). Correlated evolution of prey chemical discrimination with foraging, lingual morphology and vomeronasal chemoreceptor abundance in lizards. *Behavioral Ecology and Sociobiology*, 41: 257–265.

Cooper, W.E. (2003). Shifted balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (*Holbrookia propinqua*). *Behavioral Ecology and Sociobiology*, 54: 179–187.

Cooper, W.E. Jr. (2007). Compensatory changes in escape and refuge use following autotomy in the lizard *Sceloporus virgatus*. *Canadian Journal of Zoology*, 85: 99–107.

Cromie, G.L. & Chapple, D.G. (2012). Impact of tail loss on the behaviour and locomotor performance of two sympatric *Lampropholis* skink species. *PLoS ONE*, 7(4): e34732.

Dial, B.E. & Fitzpatrick, L.C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia*, 51: 310–317.

Dial, B.E. & Schwenk, K. (1996). Olfaction and predator detection in *Coleonyx brevis* (Squamata: Eublepharidae), with comments on the functional significance of buccal pulsing in geckos. *Journal of Experimental Zoology*, 276: 415–424.

Downes, S. & Shine, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology*, 82: 1293–1303.

Durand, J.; Legrand, A.; Tort, M.; Thiney, A.; Michniewicz, R.J.; Coulon, A. & Aubret, F. (2012). Effects of geographic isolation on anti-snakes responses in the wall lizard, *Podarcis muralis*. *Amphibia-Reptilia*, 33: 199–206.

Emberts, Z.; Escalante, I. & Bateman, P.W. (2019). The ecology and evolution of autotomy. *Biological Reviews*, 94: 1881–1896.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15: 511–521.

Fleming, P.A.; Muller, D.L. & Bateman, P.W. (2007). Leave it all behind: an evolutionary and taxonomic perspective of autotomy in invertebrates. *Biological Reviews*, 82: 481–510.

Font, E.; Carazo, P.; Pérez i de Lanuza, G. & Kramer, M. (2012). Predator-elicited foot shakes in wall lizards (*Podarcis muralis*): evidence for a pursuit-deterrent function. *Journal of Comparative Psychology*, 126: 87–96.

Formanowicz, D.R.J.; Brodie, E.D.J. & Bradley, P.J. (1990). Behavioural compensation for tail loss in the ground skink*, Scincella lateralis*. *Animal Behaviour*, 40: 782–784.

Fox, S.F. & McCoy, K.J. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia,* 122: 327–334.

Fox, S.F. (1978). Natural selection on behavioral phenotypes of the lizard *Uta stansburiana*. *Ecology*, 59: 834–847.

Fox, S.F.; Rose, E. & Myers, R. (1981). Dominance and the acquisition of superior home ranges in the lizard *Uta stansburiana*. *Ecology*, 62: 888–893.

Galán, P. (2014). *Coronella austriaca*. In: Salvador, A. (Ed.), *Fauna Ibérica.* Vol. 10, Reptiles. 2nd edition. Museo Nacional de Ciencias Naturales CSIC, Madrid, Spain, pp. 681–706.

Garland, T. & Losos, J.B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P.C. & Reilly, S.M. (Eds.), *Ecological morphology.* University of Chicago Press, USA, pp. 240–302.

Gove, D. (1979). A comparative study of snake and lizard tongue-flicking with an evolutionary hypothesis. *Zeitschrift für Tierpsychologie*, 51: 58.

Greene, H.W. (1988). Antipredator mechanisms in reptiles. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 1–152.

Higham, T.E.; Russell, A.P. & Zani, P.A. (2013). Integrative biology of tail autotomy in lizards. *Physiological and Biochemical Zoology*, 86: 603–610.

Husak, J.F. (2006). Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology*, 20: 174–179.

Johnson, J.B. & Belk, M.C. (2020). Predators as agents of selection and diversification. *Diversity*, 12: 415.

Kelehear, C. & Webb, J.K. (2006). Effects of tail autotomy on anti-predator behavior and locomotor performance in a nocturnal gecko. *Copeia*, 2006: 803–809.

Kramer, D.L. & McLaughlin, R.L. (2001). The behavioral ecology of intermittent locomotion. *American Zoologist*, 41: 137–153.

Labra, A. & Niemeyer, H.M. (2004). Variability in the assessment of snake predation risk by *Liolaemus* lizards. *Ethology*, 110: 649–662.

Langerhans R.B. (2007). Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. In: Elewa, A.M.T. (Ed.), *Predation in Organisms*. Springer, pp. 177–220.

Lawrence, J.M. (2010). Energetic costs of loss and regeneration of arms in stellate Echinoderms. *Integrative and Comparative Biology*, 50(4): 506–514.

Lin, J.-W.; Chen, Y.-R.; Wang, Y.-H.; Hung, K.-C. & Lin, S.-M. (2017). Tail regeneration after autotomy revives survival: a case from a long-term monitored lizard population under avian predation. *Proceedings Royal Society B*, 284: 20162538.

Lind, J. & Cresswell, W. (2005). Determining the fitness consequences of antipredation behavior. *Behavioral Ecology*, 16: 945–956.

Lu, H.; Ding, G.; Ding, P. & Ji, X. (2010). Tail autotomy plays no important role in influencing locomotor performance and anti-predator behavior in a cursorial gecko. *Ethology*, 116: 627–634.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Martín, J. (2001). When hiding from predators is costly: Optimization of refuge use in lizards. *Etologia*, 9: 9–13.

Martín, J. & Avery, R.A. (1998). Effects of tail loss on the movement patterns of the lizard *Psammodromus algirus*. *Functional Ecology*, 12: 794–802.

Martín, J. & Salvador, A. (1993). Thermoregulatory behaviour of rock lizards in response to tail loss. *Behaviour*, 124: 123–126.

Martín, J. & Salvador, A. (1995). Effects of tail loss on activity patterns of rock-lizards, *Lacerta monticola*. *Copeia*, 1995(4): 984–988.

McConnachie, S. & Whiting, M.J. (2003). Costs associated with tail autotomy in an ambush foraging lizard, *Cordylus melanotus melanotus*. *African Zoology*, 38(1): 57–65.

Medger, K.; Verburgt, L. & Bateman, P.W. (2008). The influence of tail autotomy on the escape response of the Cape Dwarf Gecko, *Lygodactylus capensis*. *Ethology*, 114: 42–52.

Mencía, A.; Ortega, Z. & Pérez-Mellado, V. (2016). Chemical discrimination of sympatric snakes by the mountain lizard *Iberolacerta galani* (Squamata: Lacertidae). *The Herpetological Journal*, 26: 149–155.

Michelangeli, M. & Wong, B.B.M. (2014). A recent predatory encounter influences subsequent male signalling behaviour in a desert-dwelling fish. *Behavioral Ecology*, 25: 928e932.

Michelangeli, M.; Melki-Wegner, B.; Laskowski, K.; Wong, B.B.M. & Chapple, D.G. (2020). Impacts of caudal autotomy on personality. *Animal Behaviour*, 162: 67–78.

Muth, A. (1977). Body temperature and associated postures of the zebra-tailed lizard, *Calissaurus draconoides*. *Copeia,* 1977: 122–125.

Ortega, Z.; Mencía, A. & Pérez-Mellado, V. (2018). Antipredatory behaviour of a mountain lizard towards the chemical cues of its predatory snakes. *Behaviour*, 155(10–12): 817–840.

Ramsay, K.; Kaiser, M.J. & Richardson, C.A. (2001). Invest in arms: behavioural and energetic implications of multiple autotomy in starfish (*Asterias rubens*). *Behavioral Ecology and Sociobiology*, 50: 360–365.

Reading, C.J. & Jofré, G.M. (2013). Diet composition changes correlated with body size in the Smooth snake, *Coronella austriaca*, inhabiting lowland heath in southern England. *Amphibia-Reptilia*, 34: 463–470.

Rugiero, L.; Capula, M.; Filippi, E. & Luiselli, L. (1995). Food habits of Mediterranean populations of the smooth snake (*Coronella austriaca*). *Herpetological Journal*, 5: 316–318.

Ruxton, G.D.; Allen, W.L.; Sherratt, T.N. & Speed, M.P. (2018). *Avoiding attack: The evolutionary ecology of crypsis, aposematism and mimicry.* 2nd edition. Oxford University Press, Oxford.

Salvador, A.; Martín, J. & López, P. (1995). Tail loss reduces home range size and access to females in male lizards, *Psammodromus algirus*. *Behavioral Ecology*, 6: 382–387.

Schwenk, K. (1995). Of tongues and noses: chemoreception in lizards and snakes. *Trends in Ecology and Evolution*, 10: 7–12.

Shine, R. & Sun, L.-X. (2003). Attack strategy of an ambush predator: which attributes of the prey trigger a pit-viper's strike? *Functional Ecology*, 17: 340–348.

Thoen, C.; Bauwens, D. & Verheyen, R.F. (1986). Chemoreceptive and behavioural responses of the common lizard *Lacerta vivipara* to snake chemical deposits. *Animal Behaviour*, 34: 1805–1813.

Van Damme, R. & Quick, K. (2001). Use of predator chemical cues by three species of lacertid lizards (*Lacerta bedriagae, Podarcis tiliguerta, Podarcis sicula*). *Journal of Herpetology*, 35: 27–36.

Van Damme, R.; Bauwens, D.; Vanderstighelen, D. & Verheyen, R.F. (1990). Responses of the lizard *Lacerta vivipara* to predator chemical cues: the effects of temperature. *Animal Behavior*, 40: 298–305.

Wilson, B.S. (1992). Tail injuries increase the risk of mortality in free-living lizards (*Uta stansburiana*). *Oecologia*, 92: 145–52.

“Evolution is a two-way street. For each advance there may be an opposite, if not equal, loss. (…) A decision is a choice, and choices are made by the process of elimination”. Goss, 1969 (*Principles of Regeneration*).

“La evolución es una carretera de doble sentido. Por cada avance puede haber una pérdida opuesta, si no igual. (...) Una decisión es una elección, y las elecciones se hacen mediante el proceso de eliminación”.

Cover photo: *Podarcis bocagei*

IV. Chapter 3

An integrative analysis of the short-term effects of tail autotomy on thermoregulation and dehydration rates in wall lizards

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An integrative analysis of the short-term effects of tail autotomy on thermoregulation and dehydration rates in wall lizards

Abstract

Maintaining body temperature is essential for the optimal performance of physiological functions. Ectotherms depend on external heat sources to thermoregulate. However, thermoregulation may be constrained by body condition and hydration state. Autotomy (i.e., the voluntary shed of a body part) evolved in various animal lineages and allowed surviving certain events (such as predator attacks), but it may affect body condition and volume/surface ratios, increase dehydration and constrain thermoregulation. In the framework of a general analysis of the evolution of autotomy, here we assessed the effects of tail loss on the thermal preferences and evaporative water loss rates (EWL) in the lizard *Podarcis bocagei*, integrating the thermal and hydric factors. We did not observe shifts in the thermal preferences of experimentally autotomized lizards when compared to the controls, which contradicted the hypothesis that they would raise preferred temperature to increase metabolic rates and accelerate regeneration. Evaporative water loss rates were also similar for tailed and tailless individuals, suggesting negligible increase of water loss through the injury and no specific ecophysiological responses after autotomy. Therefore, the changes observed in autotomized lizards in the field are to be considered primarily behavioural, rather than physiological, and thermoregulation could be secondarily affected by behavioural compensations for an increased predation risk after autotomy. Functional studies are necessary to understand how lizards' interaction with the environment is altered after autotomy, and further studies including different dehydration levels would be useful to fully understand the effect of water shortage on lizards' performance after caudal autotomy.

Keywords: autotomy; *Podarcis bocagei*; ecophysiology; thermoregulation; dehydration; evaporative water loss rates (EWL)

Introduction

Most physiological functions of animals (e.g., locomotion, digestion, osmoregulation) are strongly affected by body temperature (Hillman *et al.* 2009), so that their performance is maximized within an optimal range of temperatures (Huey & Stevenson 1979). External heat sources are essential for the thermoregulation of ectothermic animals, in order to adjust their body temperature to an optimal thermal range. Shuttling heliotherms, such as many lizards, do so by adjusting the frequency and duration of basking events and selecting thermally optimal microhabitats (Huey 1982; Angilletta 2009). However, thermoregulatory behaviour may be costly in terms of predation exposure and time budgets (Verwaijen & Van Damme 2007; Herczeg *et al.* 2006). Thermoregulation is constrained by climate and the physical environment (Carrascal *et al.* 1992; Aguado & Braña 2014; Sannolo *et al.* 2019), but also depends on organismal traits such as reproductive condition (Braña 1993; Rodríguez-Díaz *et al.* 2010), feeding state (Brown & Griffin 2003; Gilbert & Miles 2016), colour (Clusella-Trullas *et al.* 2009), metabolic and cardiovascular rates (Seebacher & Franklin 2005; Brown & Au 2009), body size (Stevenson 1985; Carrascal *et al.* 1992), or hydration state (Sannolo & Carretero 2019; Rozen-Rechels *et al.* 2021). In particular, dehydrated ectotherms select lower temperatures, spend more time hidden, use shaded microhabitats, decrease activity and therefore exhibit a poorer thermoregulation, which will ultimately have repercussions on their survival and fitness (Crowley 1987; Ryan *et al.* 2016; Sannolo & Carretero 2019). Consequently, a physiological trade-off between thermoregulation (i.e., maintaining an optimal body temperature) and hydration (i.e., avoiding water loss) may be expected (Angilletta 2017).

Autotomy, meaning “self-detachment”, is an adaptation present in both vertebrate and invertebrate groups that implies the self-induced loss of a body part as a strategy that allows escape from predators, solve molting complications, survive physical damages or eliminate toxins (Maginnis 2006). In many lizards, tail autotomy is a widespread antipredator strategy (McConnachie & Whiting 2003). In the lizard groups with most efficient mechanisms, autotomy occurs along predefined fracture planes within the vertebrae and surrounding tissues and is usually followed by the regeneration of the lost tail, which will be constituted by an unsegmented cartilaginous axis instead of osseous vertebrae (Bellairs & Bryant 1985; Arnold 1988). Tail loss can constrain locomotion, shift activity patterns and microhabitat use, affect feeding capacity and immune system, or decrease social status (Fox *et al.* 1981; Fox & Rostker 1982; Ramsay *et al.* 2001; Barrios *et al.* 2008; Kuo *et al.* 2013; Fernández-Rodríguez & Braña 2020). More importantly, recently autotomized lizards become more vulnerable to predation (Fox & McCoy 2000). Hence, the evolution of regeneration allowed the restoration of the lost functionality (Goss 1969; Lin *et al.* 2017; Fernández-Rodríguez & Braña 2020), but this requires time and a strong energy input that may compromise other highly demanding processes, such as reproduction or growth (Barrios *et al.* 2008; Bateman & Fleming 2009).

Determining the costs and benefits of autotomy and regeneration is essential for a proper understanding of the evolution of both biological functions. Among the potential costs of tail loss in lizards (see the review of Bateman & Fleming 2009 for more details), the possible effects on thermoregulation have been less studied and remain rather unclear. As mentioned above, thermoregulation is necessary for performance of other functions while is affected by the animal’s condition (e.g., injuries, mutilations, or morphological changes). Overall lizard morphology, and likely the surface-to-volume ratio, change after tail loss, which could alter the heat exchange patterns (Chapple & Swain 2004). Besides, after tail loss some lizards increase their standard metabolic rate during regeneration up to 36% (Naya *et al.* 2007), which is probably associated to shifts in the protein metabolism (Sagonas *et al.* 2017). Since the regenerated tail restores the lost capacities after autotomy (e.g., sprint speed or jumping dynamics, see Zamora-Camacho *et al.* 2016 and Fernández-Rodríguez & Braña 2020), lizards could be expected to increase their body temperature after tail loss, in order to raise their metabolic rate and accelerate regeneration.

On the other hand, the loss of the tail can represent up to 19 % decrease of lizards' body mass in some species (e.g., *Zootoca vivipara*, Herczeg *et al.* 2004), with a substantial decrease of fat storage (Chapple & Swain 2002) and loss of fluids contained in the tail (like blood). To date, dehydration has been neglected as a possible immediate consequence of tail loss, although it could account for the shifts in lizards' activity and behaviour observed in the field after tail autotomy, especially considering that hydration state may constrain thermoregulation and microhabitat selection (Ryan *et al.* 2016; Sannolo *et al.* 2018; Sannolo & Carretero 2019; Rozen-Rechels *et al.* 2021).

Under this framework, we aimed to assess the short-term effects of tail loss on the thermal and hydric physiology using Bocage's wall lizards (*Podarcis bocagei*) as a model organism, by investigating the thermal preferences and the evaporative water loss rates (EWL) of tailed and tailless lizards. We hypothesize that after tail loss lizards may either increase their preferred temperature to increase regeneration rate or select lower temperatures to avoid an increase of the dehydration rates. As such, we designed two experiments to disentangle thermal and hydric factors. First, we recorded the temperature selected by lizards in a thermal gradient where water was provided *ad libitum*, so that thermoregulation was unrestricted by hydration state. Second, we measured the evaporative water loss at the preferred temperature of this species. With this design we were able to integrate thermoregulation and water balance in relation to tail autotomy under an ecophysiological framework in order to understand how the interaction of ectotherms with the thermal and hydric environment (Sannolo & Carretero 2019) is modified by autotomy.

Material and methods

Forty-five adult males of *Podarcis bocagei* (i.e., longer than 46 mm snout-vent-length) with complete tails (original or fully regenerated) were captured by noosing in July 2020, from Vila do Conde (41.33º N, 8.67º W) municipality (NW Portugal). Animals were transported to the laboratory and kept in individual terraria with food (*Tenebrio molitor* larvae) and water supplied *ad libitum*. Animals were kept in the natural photoperiod, supplied by natural light from a window, with supplemental heating provided daily by 150 W infrared reflector bulbs. Lizards were weighed in a precision balance (Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany) to the nearest 0.0001 g and their snout-vent-length (SVL), tail length (TL) and tail width (TW) were measured with digital callipers to the nearest 0.01 cm. Reproduction in this species occurs from April to July (Carretero *et al.* 2006), thus these experiments were conducted at the end of the reproductive season, when most of the lizards (including those participating in the experiments) had finished breeding.

Lizards were split into a control (tailed, N = 22) and experimental (tailless, N = 23) group. Every animal from each group underwent three trials of two consecutive experiments: first the preferred temperature (T_{pref}) experiment, followed by the desiccation (EWL) rates experiment in the following day. Using a before-after design, the two experiments were performed three times for each individual. The order of the individual was randomly selected to ensure a mix of animals from both groups tested simultaneously and to provide the necessary resting time between experiments (T_{pref} and EWL rates) and between trials.

The first trial of the two experiments (T_{pref} and EWL) was performed a week after capture, before inducing caudal autotomy to the experimental group (i.e., all the lizards were tailed, so these were the control or baseline measurements). Afterwards, tail loss was induced in the experimental group by grasping the animals from the base of the tail, until the lizards self-detached it (according to the methodologies used by Fernández-Rodríguez & Braña 2020), leaving a tail stub (TS) of around 1 cm (mean ± SD: 1.23 ± 0.25 cm). There was no remaining regenerated tissue in the tail stub of the autotomized lizards (experimental group), and all those belonging to the control (tailed) group had completely intact tails (i.e., without regenerated portions). We decided not to include regenerated lizards in the control group because one of our hypotheses was that regenerated tails might behave differently regarding dehydration. As such, we maintained a completely homogeneous control group in order to compare autotomized lizards (experimental group) to completely intact individuals. However, such design implies that some lizards of the experimental group would lose the tail for the first time while others would autotomize it for the second time, which could have an effect on how they face the effects after autotomy. Therefore, to confirm the robustness of our results and conclusions, we repeated the analyses to test the thermal and hydric variables measured pooling tailed and tailless lizards but using only the lizards from the experimental group and considering their initial tail condition (intact or regenerated) at the moment of capture. These results are included in the Suppl. Material of Chapter 3 and showed that intact and previously regenerated lizards in our experiment responded equally (for dehydration rates and preferred temperatures) after tail autotomy, regardless of their previous tail condition.

Immediately following autotomy, lizards were weighed and measured for TS and the tails were weighed and measured for TL and TW. After one day of rest, both the experimental and control groups underwent a second trial of experiments (T_{pref} and EWL), to evaluate the immediate effects of tail loss. We also aimed to test the possible shifts on temperature selection and EWL during the first stages of regeneration, so all the lizards performed the third trial once the tailless ones had started tail regeneration (i.e., 1 week after autotomy). After tail loss, the wound is rapidly closed by the surrounding tissues and muscle fibres, which will dry within the next hours and form a scab that will cover the injury during the first days (Bellairs & Bryant 1985). The scab detaches within approximately a week in this species (authors' personal observation), leaving the blastema (which is covered only by a very thin epithelium) exposed to environmental conditions. Therefore, one week was deemed sufficient resting period between the second and third trial. After finishing the third trial, all the lizards were released in their capture site.

Preferred temperature

Animals were placed individually in acrylic terraria (100 L x 30 W x 40 H cm) without refuges, with a 0.5 cm layer of a mixture of sand and vermiculite on the bottom and a 150 W infrared reflector bulb placed 25 cm high at one end. The thermal gradient in the terrariums ranged from ± 20—50 °C, according to the methodology by Carretero (2012). The animals were then allowed to freely thermoregulate for a 10-hour period (from 9:00 to 19:00 hours). Lizards were not fed during the trials but had water available in a small dish throughout all the experiment (in order to prevent any possible confounding effects from any potential gradual water stress caused by prolonged exposure to the thermal gradient, Sannolo & Carretero 2019). To record the thermoregulatory behaviour, a thermal (InfraRed) photograph was taken every hour with a thermographic camera FLIR

T335 (sensitivity: < 0.05 °C; accuracy: ± 2%; IR image resolution: 320×240 pixels; Flir Systems Inc., Wilsonville, Oregon, USA), at a distance of around 30 cm from the animal.

Thermal photographs were later analysed using the software FLIR Tools 2.1 (Copyright 2014 FLIR Systems, Inc; http:// www.flir.com). For this post-processing, skin emissivity was established at 0.96 and a correction for the reflective temperature was performed by taking the mean temperature of thermal photographs of a crinkled aluminium surface (taken from inside the gradients when the lizards were photographed). Following the methodology of Barroso *et al.* (2016), the Spotmeter tool was used to measure eye temperature in order to estimate the temperature of the lizard, as this has been shown to be an accurate proxy of internal temperature (calibrated for *Podarcis* sp., Barroso *et al.* 2016). At the end of the test, lizards were returned to their holding terraria where they were fed, provided water *ad libitum* and sprayed in order to re-establish any potential water losses.

Evaporative Water Loss

Evaporative water loss rate trials were performed the day after from the preferred temperature trials. According to Sannolo *et al.* (2018), lizards were placed in individual plastic containers (10 H × 9 W cm), with ventilation holes on the top and the bottom, and kept in an incubator for 8 hours (from 9:00 to 5:00 hours) fitted with 125 g of silica gel to ensure a dry environment (relative humidity < 20 %) inside the incubator throughout the experiment. Since the preferred temperature for *P. bocagei* has been shown to range between 29.4—32.0 °C (Sannolo *et al.* 2018), the incubator was set to 32 °C. During the trial period, lizards were weighed to the nearest 0.0001 g every hour in a precision balance (Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany). Lizards that reached 5 % decrease in mass (i.e., water lost) were removed from the incubator and the trial ceased for that individual, as this has been previously used as safety cut-off to prevent exaggerated hydric stress (Sannolo *et al.* 2018). Faeces found in the plastic containers during the trials were not removed, according to previous studies (Carneiro *et al.* 2015), but its presence was recorded and accounted for.

Statistical analysis

All the variables were checked for normality and homogeneity of variance of the residuals (Kolmogorov-Smirnov and Levene tests). Homogeneity of sizes (SVL) between tailed and tailless groups was tested by a one-way ANOVA with SVL as the response variable and tail group as a factor.

For the preferred temperature data, mean and median temperatures were calculated for each lizard in each of the three trials. To test for possible differences between tailed and autotomized lizards in the mean and median temperature of each individual through the three trials, repeated measures ANOVAs were carried out with tail group as inter-subject factor and SVL as the covariate. Besides, a general linear mixed model was used to check if there were differences between tailed and tailless lizards in the variation of temperature preference of each individual through the 10 hours of the trials, and among the three different trials. In this model, absolute temperatures were the response variable while tail group (tailed/tailless), hour (1-10), trial (1-3) and SVL were fixed factors and the individual was a random factor.

As lizards of the experimental group have less mass and volume due to tail loss, they were expected to have less surface to lose water through, which could mask differences in desiccation rates. To determine whether the possible effects of tail loss on desiccation rates would be due to physiological shifts and not just to differences in morphology (i.e., the lack of the tail), the theoretical water loss expected for the removed tail was estimated and included in the analysis. As such, we applied the following correction to the desiccation data for each tailless individual: 1 + TM / M; were TM is the mass of the shed tail, and M is the lizard's mass (before losing the tail). A general linear mixed model was fitted to analyse differences between tailed and tailless individuals in the desiccation rate through time in each trial and through the different trials. Accumulated absolute mass loss (in each measure) was the response variable, tail group (tailed/tailless), hour (1-8), trial (1-3), SVL and initial mass (at the beginning of each trial) were fixed factors, and the individual was a random factor. A Pearson's Chi-squared test was run to determine if there were differences in the presence of faeces between tailed and tailless lizards.

Statistical analyses were performed in SPSS (version 20) and in R software version 3.6.0 (R Core Team, https://www.r.project.org) where the nlme package (Pinheiro *et al.* 2017) was used to fit the general linear mixed models and the graphs produced with the ggplot2 package (Wickham 2009).

Results

All variables were normally distributed and fit the assumption of residual homoscedasticity (Kolmogorov-Smirnov and Levene tests: $p > 0.05$ in all cases). Tailed and tailless groups were homogeneous regarding snout-vent length (mean ± SD SVL Tailed: 62.3 ± 4.3 mm; Tailless: 60.4 ± 4.0 mm; one-way ANOVA: $F_{1,43} = 0.456$, $p = 0.503$) and mass before autotomy (mean ± SD mass before autotomy Tailed: 4.53 ± 0.84 g; Tailless: 4.26 ± 0.83 g; one-way ANOVA: $F_{1,43} = 1.149$, $p = 0.290$). Tailless individuals lost up to around 15 % of their weight after autotomy (mean ± SD: 15.25 ± 3.11 %).

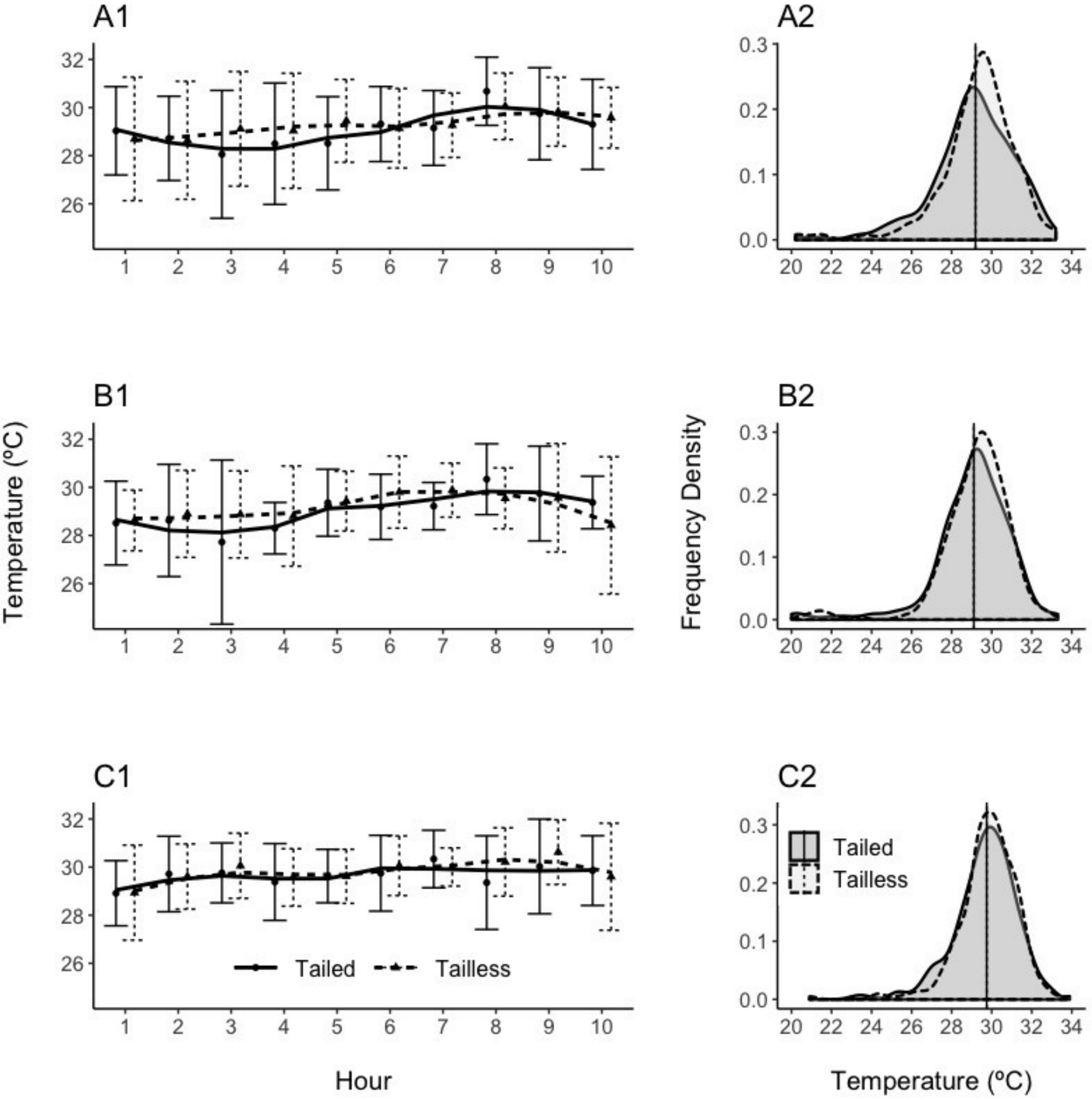


Figure 4.1. A1, B1, C1, Body temperatures of tailed (circles, continuous line) and tailless (triangles, discontinuous line) lizards during the tested 10-hour period. Values are means ± SD with a LOESS curve fitted to demonstrate the general pattern. **A2, B2, C2**, Frequency density plot of body temperatures registered for tailed (grey, continuous line) and tailless (white, discontinuous line) individuals during all the trial. Vertical lines represent the mean values for tailed (continuous line) and tailless (discontinuous line), which overlap as they are very similar for both groups. The A graphs correspond to the first trial before inducing tail autotomy to the experimental (tailless group); the B graphs represent the second trial, a day after autotomy on tailless group; the C graphs correspond to the third trial, a week after inducing autotomy to the experimental group.

Preferred temperature

The pattern of temperature variation across the 10 hours did not vary through the three different trials between tailed and tailless lizards neither among the trials, nor during the 10 hours (general linear mixed model, tail group factor, tail group*trial and tail group*time interactions, $p > 0.05$ in all cases; Figure 4.1, Table 4.1). However, preferred temperature fluctuated significantly through time and through the three trials (general linear mixed model, trial and time factors, $p < 0.001$ in both cases; Figure 4.1, Table 4.1). Moreover, both the mean and the median preferred temperature did not vary between tailed and tailless groups (Figure 4.2), through the three different trials (repeated measures ANOVAs with tail group as inter-subject factor and SVL as covariate, mean temperature: $F_{1,42} = 0.003$, $p = 0.997$; median temperature: $F_{1,42} = 0.015$, $p = 0.985$).

Table 4.1. Statistical results of the general linear mixed model of the preferred temperature variation between tailed and tailless lizards through the 10 hours of the trials, among the three different trials. The absolute temperatures were the response variable of the model, tail group (tailed/tailless), time (1-10 hours), trial (1-3) and SVL were fixed factors, and the individual was a random factor.

	DF	F-value	p-value
Trial	2	19.40	< 0.001
Tail group	1	0.61	0.439
Time	1	57.00	< 0.001
SVL	1	2.59	0.115
Trial*Tail group	2	0.00	0.999
Trial*Time	2	1.41	0.244
Tail group*Time	1	1.59	0.207
Trial*Tail group*Time	2	1.92	0.147

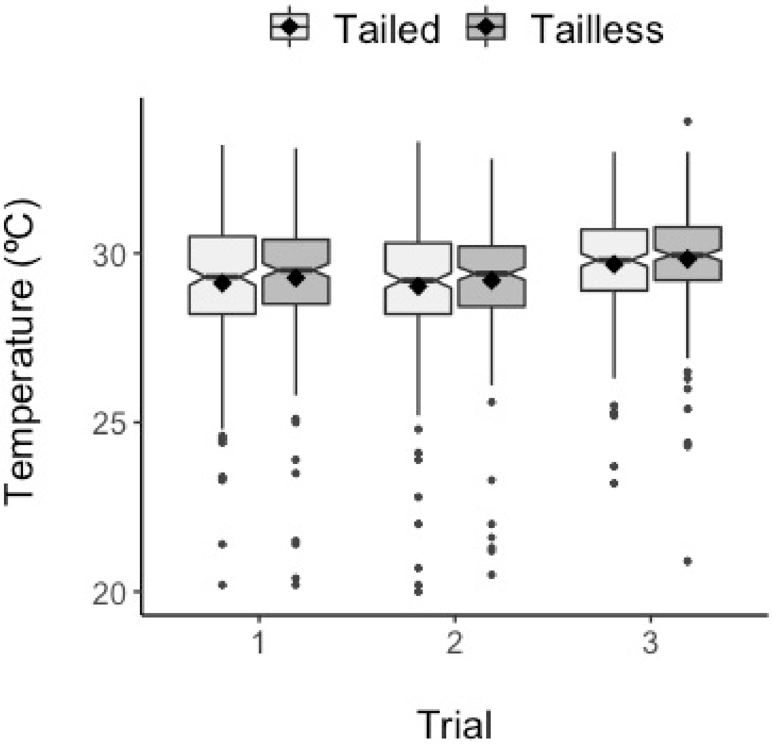


Figure 4.2. Temperature boxplots of tailed (light grey) and tailless (dark grey) for the three trials (1, first trial, before inducing tail autotomy to the tailless group; 2, second trial, a day after autotomy; 3, third trial, a week after autotomy). Rhombuses represent the mean values, horizontal black lines inside the boxes represent the median values, and box notches gives visual indication of whether boxplots are significantly different or not (if notches overlap, they may not be significantly different).

Evaporative Water Loss

No differences were found in the desiccation rates between tailed and autotomized lizards, nor in their desiccation rates through time and through the three trials (general linear mixed model, tail group factor, tail group*trial and tail group*time interactions, $p > 0.05$ in all cases; Figure 4.3, Table 4.2). Nonetheless, desiccation rates of all individuals increased significantly with time within each trial and all lizards lost significantly more water during the second and third trial than during the first one (general linear mixed model, trial and time factors, $p < 0.001$ in both cases, Table 4.2). Defecation during the experiment was not included in the model, as there were no differences between tailed and tailless lizards after tail loss (Pearson's Chi-squared tests, second trial: $X^2 = 0.178$, $p = 0.673$; third trial: $X^2 = 2.002$, $p = 0.157$).

Table 4.2. Statistical results of the general mixed model of the evaporative water loss rates variation between tailed and tailless lizards through time, in each trial and through the different trials. Accumulated absolute mass loss (in each measure) was the response variable, tail group (tailed/tailless), time (1-8 hours), trial (1-3), SVL and the initial mass (at the beginning of each trial) were fixed factors, and the individual was a random factor.

	DF	F-value	p-value
Trial	2	53.626	< 0.001
Tail group	1	0.313	0.579
Time	7	83.647	< 0.001
Initial mass	1	39.462	< 0.001
SVL	1	2.159	0.149
Trial*Tail group	2	7.256	< 0.001
Trial*Time	14	1.421	0.137
Tail group*Time	7	1.314	0.241
Initial mass*SVL	1	1.879	0.171
Trial*Tail group*Time	14	0.784	0.688

Discussion

Regulation of thermal and hydric balances and the interaction between these two processes is a cornerstone in the lizard ecophysiology (Sannolo & Carretero 2019). Its examination is essential for a proper understanding of how they interact with the environment. The thermal and hydric priorities on one side, and the individual's body condition (poorer after caudal autotomy) on the other, may both have contributed towards a shift in thermoregulation and water balance. Nonetheless, our results showed that neither the hydric nor the thermal physiology were intrinsically shifted either immediately after caudal autotomy or a week after tail loss.

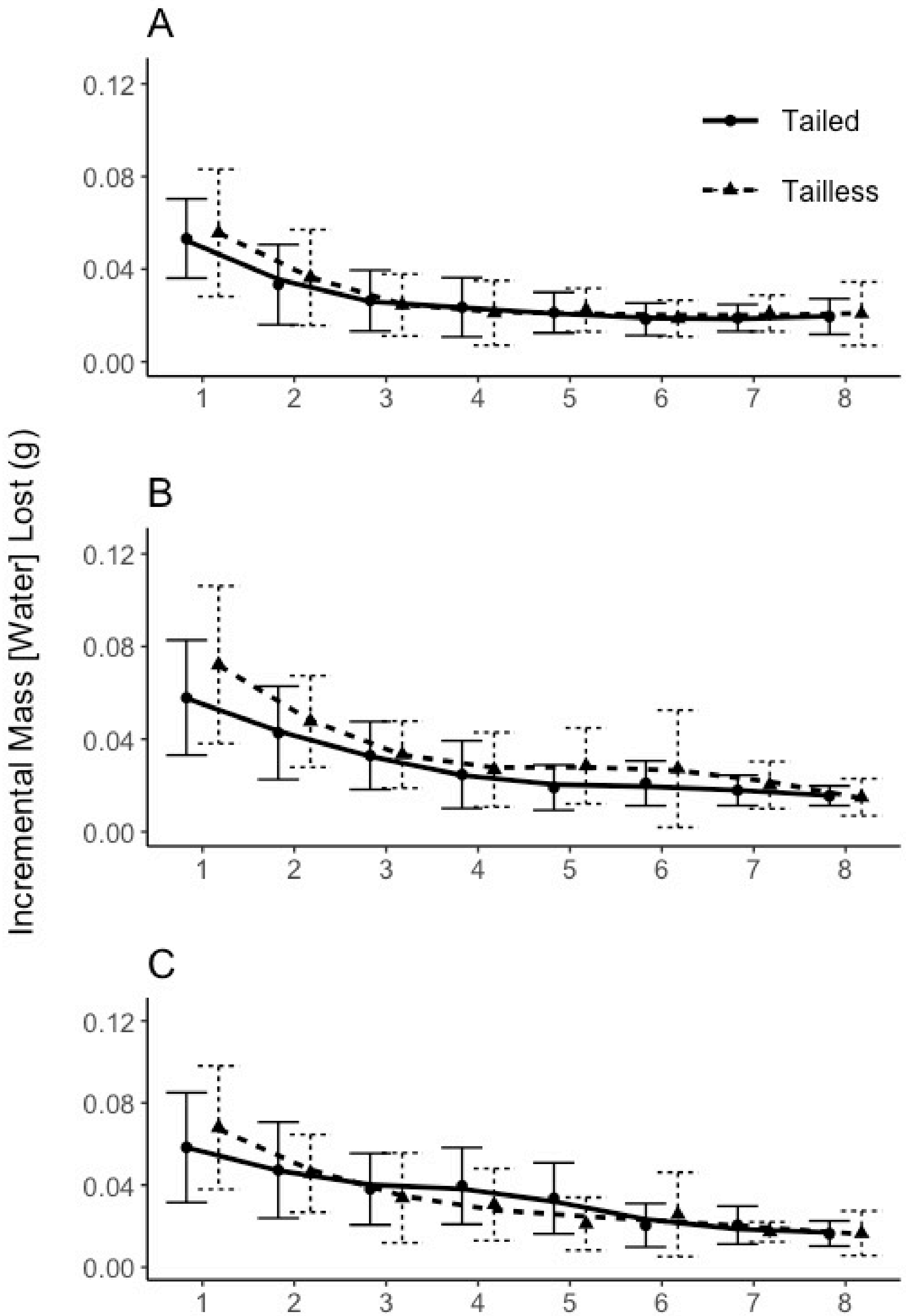


Figure 4.3. Evaporative Water Loss (EWL) rates of tailed (circles, continuous lines) and tailless (triangles, discontinuous lines) individuals at each of the 8 hours tested, in the three trials done (**A**, first trial, before inducing tail autotomy to the tailless group; **B**, second trial, a day after autotomy; **C**, third trial, a week after autotomy). Values are means ± SD, and curves were fitted with LOESS to illustrate the general trend.

Our first experiment was designed to remove the effect of hydration on thermal preferences by eliminating the water constraint. As such, lizards were hydrated before the trial started and they had water available throughout the whole test. Not surprisingly, the time pattern of preferred body temperatures during the trials was horizontal (Figure 4.1), which, according to Sannolo & Carretero (2019), confirmed that lizards were not under hydric stress during the tests (otherwise they would probably have adopted a curvilinear strategy of temperature selection, or a descending pattern as reported by S'khifa *et al.* 2020).

Our thermal results are consistent with previous studies reporting no change on the selected body temperatures after tail loss on other lizard species (Martín & Salvador 1993; Wilson 1994 in Chapple & Swain 2004; Chapple & Swain 2004; Herczeg *et al.* 2004; Cromie & Chapple 2012; Zamora-Camacho *et al.* 2015). The selected body temperatures were also individually consistent during the three different trials, which agrees with previous results that showed that short-term captivity does not influence temperature selection (Díaz-Ricaurte & Serrano 2020). As suggested by Herczeg *et al.* (2004) for *Zootoca vivipara*, the role of the tail in heat exchange seems to be insignificant for small lizards (like *P. bocagei*). Regardless, it could be a relevant heating and cooling organ in large lizards by adjusting the blood flow inside the tail (Dzialowsky & O'Connor 1999). Our results also contradict the hypothesis that lizards would select higher temperatures after tail loss to increase metabolic rates and accelerate tail regeneration (Zamora-Camacho *et al.* 2015). Considering the trade-off between thermoregulation (i.e., maintaining an optimal body temperature) and hydroregulation (i.e., avoiding dehydration), increasing body temperature would probably result in higher EWL rates, which would also increase the risk of overheating and compromise other processes (such as activity rates, growth, or reproduction; Andrews 1982; Lorenzon *et al.* 1999; Wang *et* *al.* 2016; Sannolo & Carretero 2019; Rozen-Rechels *et al.* 2021). This suggests that maintaining the preferred body temperatures (rather than increasing them) would be the most plausible and less costly situation after tail loss, provided that water loss rates are not affected by tail loss.

Contrary to our initial expectations, EWL rates remained unaffected by autotomy at different timeframes. Although some species of lizards can lose a considerable amount of water through the skin (Pirtle *et al.* 2019), a high percentage of water loss through evaporation occurs in the airways and the lungs, during breathing (Thompson & Withers 1997). In this context, our results suggest that autotomy might not provoke a relevant physiological shift in the overall EWL rates (although further physiological studies that measure metabolic rates in specific tissues are needed to disentangle the physiological responses after tail loss). The loss of water through the injury also seemed insignificant. This is likely due to its small surface, and the minimization mediated by the evolution of several adaptations allowed the reduction of the physical trauma of autotomy. Indeed, immediately after tail loss, there is a strong vasoconstriction and constriction of the muscle fibres surrounding the wound to prevent the lizard from losing much blood and internal fluids (Bellairs & Bryant 1985). After a few hours, the external layer of muscles and tissue will dry and constitute a scab that protects the injury. Underneath the scab, the blastema will start growing. After approximately a week, in the case of *P. bocagei* (authors' personal observation), but, for instance, 4–5 weeks in the leopard gecko *E. macularius* (see McLean & Vickaryous 2011), the scab will detach and the blastema will be in contact with the air (Bellairs & Bryant 1985; Bryant *et al.* 2002). Although the blastema is covered by a thin layer of epithelium without scales, water loss through that surface was insignificant, possibly due to its small area.

While there were no apparent consequences of tail loss on thermoregulation and water loss in the lab, the well documented differences observed between tailed and tailless lizards in the field (see below) must therefore have an underlying behavioural (rather than physiological) mechanism. Indeed, in our thermal preference experiment, we did provide water *ad libitum,* yet we did not monitor if autotomized lizards drank more frequently, which could be compensating for a potential increase in water loss. Moreover, although our study assessed the preferred body temperatures, it did not include other thermal parameters, such as the mean basking time, which tailless lizards may increase to maintain body temperatures (Martín & Salvador 1993).

More importantly, the experiments performed here took place with no predation risk. Thermoregulation is costly in terms of time and exposure to predators (Withers & Campbell 1985; Alford & Lutterschmidt 2012) and tailless lizards often face higher predation risks and are more vulnerable to predation. This is because shifts in locomotor dynamics, and because once their tails are lost, they cannot rely on autotomy during a new predator encounter until a long-enough tail has regenerated; Dial & Fitzpatrick 1984; Fox & McCoy 2000; Fernández-Rodríguez & Braña 2020). As a consequence, it is expectable that lizards exhibit behavioural adjustments to minimize predation risk, such as decreased activity, different microhabitat use or adopt a more cryptic and wary behaviour (Formanowicz *et al.* 1990; Martín & Salvador 1993; Cooper 2003; Cooper 2007). In turn, these behavioural shifts may affect thermoregulatory performance and, hence, alter the body temperature of lizards in the wild. A poor thermoregulation would constrain locomotion and foraging performances (Avery & Mynott 1990; Angilletta *et al.* 2002), which are relevant tasks for survival and fitness (Garland & Losos 1994; Robson & Miles 2000).

Our study showed that, under controlled conditions, tail autotomy had no measurable short to mid-term effects on the thermal and hydric ecology of the small lizard *P. bocagei*. This seems to contradict observations in the field (Martín & Salvador 1993), thus suggesting that such compensatory mechanisms must have an underlying behavioural mechanism as opposed to a physiological one. Certainly, more divergence in ecophysiology is expected in the field than in the laboratory: while our animals had continuous water availability during the thermal preference experiments, lizards in the field would probably be more dehydrated throughout the day or after consecutive dry days. Furthermore, lizards in the field are exposed to a range of other pressures, such as risk of predation and unstable availability of food/water, which may push the animals' physiology to an extreme not contemplated by the "ideal" and stable laboratory conditions in which these animals were tested.

Our results suggest that the tested ecophysiological parameters are similar in tailed and tailless lizards. Nonetheless, autotomized lizards may incur additional metabolic costs due to the loss of both weight (e.g., our individuals had already lost up to around 15 % of their weight after autotomy) and fat reserves (which are also a reservoir of metabolic water, Price 2017). These biochemical processes are expected to be important in the mid to long-term and not necessarily immediately after autotomy. Besides, as it has been shown before, the consequences of dehydration for thermoregulation could be more severe in those areas with drought periods, as it may happen in our studied population (Sannolo *et al.* 2018).Ultimately, future studies should aim to determine the medium to long-term effects of caudal autotomy on the thermal and hydric ecology of these animals while also investigating (or at least controlling) for potential effects of different hydration states or for any behavioural mechanisms which may be buffering underlying physiological differences.

References

Aguado, S. & Braña. F. (2014). Thermoregulation in a cold-adapted species (Cyren's Rock Lizard, *Iberolacerta cyreni*): influence of thermal environment and associated costs. *Canadian Journal of Zoology*, 92: 955–964.

Alford, J.G. & Lutterschmidt, W.I. (2012). Modelling energetic and theoretical costs of thermoregulatory strategy. *Journal of Biological Dynamics*, 6: 63–79.

Andrews, R.M. (1982). Patterns of growth in reptiles. In: Gans, C. & Pough, F.H. (Eds), *Biology of the Reptilia*. Vol. 13, Physiology D, Physiological Ecology. Academic Press, New York, pp. 273–320.

Angilletta, M.J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis.* Oxford University Press Inc. York, USA.

Angilletta, M.J. Jr. (2017). Biochemical and Physiological Adaptations. In: Losos, J.B. (Ed.), *The Princeton Guide to Evolution.* Princeton University Press, Princeton, New Jersey, pp. 285–289.

Angilletta, M.J.; Hill, T. & Robson, M.A. (2002). Is physiological performance optimized by thermoregulatory behavior?: a case study of the eastern fence lizard, *Sceloporus undulatus*. *Journal of Thermal Biology*, 27: 199–204.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Avery, R.A. & Mynott, A. (1990). The effects of temperature on prey handling time in the common lizard, *Lacerta vivipara*. *Amphibia-Reptilia*, 11: 111–122.

Barrios, J.V.; Gaymer, C.F.; Vásquez, J.A. & Brokordt, K.B. (2008). Effect of the degree of autotomy on feeding, growth, and reproductive capacity in the multi-armed sea star *Heliaster helianthus*. *Journal of Experimental Marine Biology and Ecology*, 361: 21–27.

Barroso, F.M.; Carretero, A.M.; Silva, F. & Sannolo, M. (2016). Assessing the reliability of thermography to infer internal body temperatures of lizards. *Journal of Thermal Biology*, 62: 90–96.

Bateman, P.W. & Fleming, A. (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. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Braña, F. (1993). Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos*, 66: 216–222.

Brown, R. P. & Griffin, S. (2003). Lower selected body temperatures after food deprivation in the lizard *Anolis carolinensis*. *Journal of Thermal Biology*, 30: 79-83.

Brown, R.P. & Au, T. (2009). The influence of metabolic heat production on body temperature of a small lizard, *Anolis carolinensis*. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 153(2): 181–184.

Bryant, S.V.; Endo, T. & Gardiner, D.M. (2002). Vertebrate limb regeneration and the origin of limb stem cells. *International Journal of Developmental Biology*, 46: 887–896.

Carneiro, D.; García-Muñoz, E.; Kaliontzopoulou, A.; Llorente, G.A. & Carretero, M.A. (2015). Comparing ecophysiological traits in two Podarcis wall lizards with overlapping ranges. *Salamandra*, 51(4): 335–344.

Carrascal, L.M.; López, P.; Martín, J. & Salvador, A. (1992). Basking and antipredator behaviour in a high altitude lizard: implications of heat-exchange rate. *Ethology*, 92(2): 143–154.

Carretero, M. A.; Ribeiro, R.; Barbosa, D.; Sá-Sousa, P. & Harris, D. J. (2006): Spermatogenesis in two Iberian *Podarcis* lizards: Relationships with male traits. *Animal Biology*, 56(1): 1–12.

Carretero, M.A. (2012). Measuring body temperatures in small lacertids: infrared vs. contact thermometers. *Basic and Applied Herpetology*, 26: 99–105.

Chapple, D.G. & Swain, R. (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. & Swain, R. (2004). Caudal autotomy does not influence thermoregulatory characteristics in the metallic skink, *Niveoscincus metallicus*. *Amphibia-Reptilia*, 25: 326–333.

Clusella-Trullas, S.; van Wyk, J.H. & Spotila, J.R. (2009). Thermal benefits of melanism in cordylid lizards: a theoretical and field test. *Ecology*, 90(8): 2297–2312.

Cooper, W.E. (2003). Shifted balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (*Holbrookia propinqua*). *Behavioral Ecology and Sociobiology*, 54: 179–187.

Cooper, W.E. Jr. (2007). Compensatory changes in escape and refuge use following autotomy in the lizard *Sceloporus virgatus*. *Canadian Journal of Zoology*, 85: 99–107.

Cromie, G.L. & Chapple, D.G. (2012). Impact of tail loss on the behaviour and locomotor performance of two sympatric *Lampropholis* skinks species. *PLoS ONE*, 7: e34732.

Crowley, S. (1987). The effect of desiccation upon the preferred body temperature and activity level of the lizard *Sceloporus undulatus*. *Copeia*, 1987: 25–32.

Dial, B.E. & Fitzpatrick, L.C. (1984). Predator escape in tailed versus tailless *Scincella lateralis* (Sauria: Scincidae). *Animal Behaviour*, 32: 301–302.

Díaz-Ricaurte, J.C. & Serrano, F.C. (2020). Short-term captivity does not affect immediate voluntary thermal maximum of a neotropical pitviper: Implications for behavioral thermoregulation. *Journal of Experimental Zoology*: 1–8.

Dzialowsky, E.M. & O'Connor, M.P. (1999). Utility of blood flow to the appendages in physiological control of heat exchange in reptiles. *Journal of Thermal Biology*, 24: 21–32.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15: 511–521.

Formanowicz, D.R.J.; Brodie, E.D.J. & Bradley, P.J. (1990). Behavioural compensation for tail loss in the ground skink, *Scincella lateralis*. *Animal Behaviour*, 40: 782–784.

Fox, S.F. & McCoy, K.J. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia,* 122: 327–334.

Fox, S.F. & Rostker, M.A. (1982). Social costs of tail loss in *Uta stansburiana*. *Science*, 218: 692–693.

Fox, S.F.; Rose, E. & Myers, R. (1981). Dominance and the acquisition of superior home ranges in the lizard *Uta stansburiana*. *Ecology,* 62: 888–893.

Garland, T. & Losos, J.B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P.C. & Reilly, S.M. (Eds.), *Ecological morphology.* University of Chicago Press, Chicago, pp. 240–302.

Gilbert, A.L. & Miles, D.B. (2016). Food, temperature and endurance: effects of food deprivation on the thermal sensitivity of physiological performance. *Functional Ecology*, 30: 1790–1799.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Herczeg, G.; Gonda, A.; Saarikivi, J. & Merilä, J. (2006). Experimental support for the cost-benefit model of lizard thermoregulation. *Behavioral Ecology and Sociobiology*, 60: 405–414.

Herczeg, G.; Kovács, T.; Tóth, T.; Török, J.; Korsós, Z. & Merilä, J. (2004). Tail loss and thermoregulation in the common lizard *Zootoca vivipara*. *Naturwissenschaften*, 91: 485–488.

Hillman, S.S.; Withers, P.C.; Drewes, R. & Hillyard, S.D. (2009). *Ecological and environmental physiology of amphibians*. Oxford University Press, Oxford.

Huey, R.B. & Stevenson, R.D. (1979). Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist*, 19: 357–366.

Huey, R.B. (1982). Temperature, Physiology and the Ecology of Reptiles. In: Gans, C. & Pough, F.H. (Eds), *Biology of the Reptilia*. Vol. 12, Physiology C, Physiological Ecology. Academic Press, London, pp. 25–91.

Kuo, C.; Yao, C. & Lin, T. (2013). Tail loss compromises immunity in the many-lined skink, *Eutropis multifasciata*. *Naturwissenschaften*, 100: 379–384.

Lin, J.-W.; Chen, Y.-R.; Wang, Y.-H.; Hung, K.-C. & Lin, S.-M. (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(1847): 20162538.

Lorenzon, P.; Clobert, J.; Oppliger, A. & John-Adler, H. (1999). Effect of water constraint on growth rate, activity and body temperature of yearling common lizard. *Oecologia*, 118: 423–430.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Martín, J. & Salvador, A. (1993). Thermoregulatory behaviour of rock lizards in response to tail loss. *Behaviour*, 124: 123–126.

McConnachie, S. & Whiting, M.J. (2003). Costs associated with tail autotomy in an ambush foraging lizard, *Cordylus melanotus melanotus. African Zoology*, 38(1): 57–65.

McLean, K.E. & Vickaryous, M.K. (2011). A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius*. *BMC Developmental Biology*, 11: 50.

Naya, D.E.; Veloso, C.; Muñoz, J.L.P. & Bozinovic, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 146: 189–193.

Pinheiro, J.C.; Bates, D.M.; DebRoy, S.; Sarkar, D. & R Core Team (2017). *nlme: Linear and Nonlinear Mixed Effects Models.* R package version 3. 1-129, https://CRAN.R- project.org/package=nlme.

Pirtle, E.I.; Tracy, C.R. & Kearney, M.R. (2019). Hydroregulation: a neglected behavioural response of lizards to climate change? In: Bels, V.L. & Russell, A.P. (Eds.), *Behavior of Lizards: Evolutionary and Mechanistic Perspectives*. CRC Press, Taylor & Francis Group, pp. 343–375.

Price, E.R. (2017). The physiology of lipid storage and use in reptiles. *Biological Reviews*, 92: 1406-1426.

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Ramsay, K.; Kaiser, M.J. & Richardson, C.A. (2001). Invest in arms: behavioural and energetic implications of multiple autotomy in starfish (*Asterias rubens*). *Behavioral Ecology and Sociobiology*, 50: 360–365.

Robson, M.A. & Miles, D.B. (2000). Locomotor performance and dominance in male Tree Lizards, *Urosautrus ornatus*. *Functional Ecology*, 14: 338–344.

Rodríguez-Díaz, T.; González, F.; Ji, X. & Braña, F. (2010). Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible? *Zoology*, 113(1): 33–38.

Rozen-Rechels, D.; Rutschmann, A.; Dupoué, A.; Blaimont, P.; Chauveau, V.; Miles, D.; Guillon, M.; Richard, M.; Badiane, A.; Meylan, S.; Clobert, J. & Le Galliard, J.F. (2021). Interaction of hydric and thermal conditions drive geographic variation in thermoregulation in a widespread lizard. *Ecological Monographs*, 91(2): e01440.

Ryan, M.J.; Latella, I.M.; Giermakowski, J.T.; Snell, H.; Poe, S.; Pangle, R.E.; Gehres, N.; Pockman, W.T. & McDowell, N.G. (2016). Too dry for lizards: short-term rainfall influence on lizard microhabitat use in an experimental rainfall manipulation within a piñon-juniper. *Functional Ecology*, 30: 964–973.

S'khifa, A.; Koziel, G.; Vences, M.; Carretero, M. A. & Slimani, T. (2020). Ecophysiology of a lacertid community in the high Moroccan mountains suggests conservation guidelines. *Journal of Thermal Biology*, 94: 102743.

Sagonas, K., Karambotsi, N., Bletsa, A., Reppa, A., Pafilis, P., and Valakos, E. D. (2017). Tail regeneration affects the digestive performance of a Mediterranean lizard. *The Science of Nature*, 104: 22.

Sannolo, M. & Carretero, M.A. (2019). Dehydration constrains thermoregulation and space use in lizards. *PLoS ONE*, 14(7): e0220384.

Sannolo, M.; Barroso, F.M. & Carretero, A.M. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, 126: 58–64.

Sannolo, M.; Civantos, E.; Martín, J. & Carretero, M.A. (2019). Variation in field body temperature and total evaporative water loss along an environmental gradient in a diurnal ectotherm. *Journal of Zoology*, 310(3): 221–231.

Seebacher, F. & Franklin, C.E. (2005). Physiological mechanisms of thermoregulation in reptiles: a review. *Journal of Comparative Physiology B*, 175(8): 533–541.

Stevenson, R.D. (1985). Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*, 125(1): 102–117.

Thompson, G.G. & Withers, P.C. (1997). Evaporative water loss of Australian goannas (Squamata: Varanidae). *Amphibia-Reptilia*, 18: 177-190.

Verwaijen, D. & Van Damme, R. (2007). Correlated evolution of thermal characteristics and foraging strategy in lacertid lizards. *Journal of Thermal Biology*, 32: 388–395.

Wang, Y.; Zeng, Z.G.; Li, S.R.; Bi, J.H. & Du, W.G. (2016). Low precipitation aggravates the impact of extreme high temperatures on lizard reproduction. *Oecologia*, 182: 961–971.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York.

Wilson, R.S. (1994). *Physiological responses to caudal autotomy in* Eulamprus quoyii. Honours Thesis, University of Queensland, Brisbane, Australia.

Withers, P.C. & Campbell, J.D. (1985). Effects of environmental costs on thermoregulation in the desert iguana. *Physiological Zoology*, 58: 329–339.

Zamora-Camacho, F.J.; Reguera, S. & Moreno-Rueda, G. (2015). Does tail autotomy affect thermoregulation in an accurately thermoregulating lizard? Lessons from a 2200-m elevational gradient. *Journal of Zoology*, 297(3): 204–210.

Zamora-Camacho, F.J.; Rubiño-Hispán, M.V.; Reguera, S. & Moreno-Rueda, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid *Psammodromus algirus*. *Herpetological Journal*, 26: 213–218.

Chapter 3: Supplementary material

Our experimental design included only lizards with intact tails in the control group, and lizards with intact or regenerated tails in the experimental group. We decided not to include regenerated lizards in the control group because one of our hypotheses was that regenerated tails might behave differently regarding dehydration. For that reason, we maintained a completely homogeneous control group in order to compare autotomized lizards (experimental group) to completely intact individuals. Including regenerated lizards could introduce noise in that group, in the case that they presented differences due to their regenerated tails.

To ensure that these different backgrounds do not have consequences and lizards do not face differently their first and their subsequent tail detachments (regarding the thermal and hydric variables studied in this paper), we performed extra statistics. All the analysis carried out (of both the thermal and hydric experiments) were repeated using only the lizards from the experimental group and considering their tail condition (intact or regenerated) at the moment of capture. All these results (listed below) show that there are no differences between intact and previously regenerated lizards (these key comparisons are highlighted in red colour), indicating that lizards in our experiment responded equally (for dehydration rates and preferred temperatures) after tail autotomy, regardless of their previous tail condition.

Temperature:

Mean temperature: Repeated measures ANOVAs with SVL as covariate, trial * tail condition, $F_{1,19} = 0.156$, $p = 0.857$.

Median temperature: Repeated measures ANOVAs with SVL as covariate, trial * tail condition $F_{1,19} = 0.508$, $p = 0.610$.

Table S4.1. Statistical results of the GLMM of the preferred temperature variation between **tailless lizards with previously original or regenerated tails (tail condition)** through the 10 hours of the trials, among the three different trials. Absolute temperatures were the response variable of the model, tail condition (intact/regenerated), time (1-10 hours), trial (1-3) and SVL were fixed factors, and the individual was a random factor.

	DF	F-value	p-value
Trial	2	10.49	< 0.001
Tail condition	**1**	**2.08**	**0.165**
Time	1	21.72	< 0.001
SVL	1	0.13	0.719
Trial*Tail condition	**2**	**0.53**	**0.588**
Trial*Time	2	0.57	0.568
Tail condition*Time	**1**	**2.70**	**0.101**
Trial*Tail condition*Time	**2**	**0.92**	**0.401**

Desiccation:

Table S4.2. Statistical results of the GLMM of the evaporative water loss rates variation between **tailless lizards with previously original or regenerated tails (tail condition)** through time, in each trial and through the different trials. Accumulated absolute mass loss (in each measure) was the response variable, tail condition (intact/regenerated), time (1-8 hours), trial (1-3), SVL and the initial mass (at the beginning of each trial) were fixed factors, and the individual was a random factor.

	DF	F-value	p-value
Trial	1	35.319	<0.001
Tail condition	**2**	**2.833**	**0.108**
Time	7	39.045	<0.001
Initial mass	1	17.433	<0.001
SVL	1	0.784	0.386
Trial*Tail condition	**2**	**0.577**	**0.562**
Trial*Time	14	1.069	0.384
Tail condition*Time	**7**	**1.569**	**0.143**
Initial mass*SVL	1	0.293	0.589
Trial*Tail condition*Time	**14**	**0.575**	**0.884**

“If there were no regeneration, there could be no life. If everything regenerated there would be no death. All organisms exist between these two extremes”. Goss, 1969 (*Principles of Regeneration*).

“Si no hubiese regeneración, no podría existir la vida. Si todo regenerase, no habría muerte. Todos los organismos existen entre estos dos extremos”.

Cover photo: *Podarcis muralis* hatching

V. Chapter 4

Allocation costs of regeneration: tail regeneration constrains body growth under low food availability in juvenile lizards

Under Review in *Oecologia*: Fernández-Rodríguez, Irene & Braña, Florentino. Allocation costs of regeneration: tail regeneration constrains body growth under low food availability in juvenile lizards.

Allocation costs of regeneration: tail regeneration constrains body growth under low food availability in juvenile lizards

Abstract

The balance of energy allocated to development and growth of different body compartments may incur allocation conflicts and can thereby entail physiological and evolutionary consequences. Regeneration after autotomy restores the functionality lost after shedding a body part but requires a strong energy investment that may trade-off with other processes, like reproduction or growth. Caudal autotomy is a widespread antipredator strategy in lizards, but regeneration may provoke decreased growth rates in juveniles that could have subsequent consequences. Here, we assessed the growth of intact and regenerating hatchling wall lizards (*Podarcis muralis*) exposed to different food regimens. Regenerating juveniles presented slightly but significantly lower body growth rates than individuals with intact tails when facing low food availability, but there were no differences when food was supplied *ad libitum*. Regenerating individuals fed ad libitum increased their ingestion rates compared to intact ones during the period of greatest tail growth, which also reveals a cost of tail regeneration. When resources were scarce, hatchlings invested more in tail regeneration in relation to body growth, rather than delay regeneration to give priority to body growth. We propose that, in juvenile lizards, regeneration could be prioritized even at the expense of body growth to restore the functionality of the lost tail, likely increasing survivorship and the probability to reach reproductive maturity. Our study indicates that food availability is a key factor for the occurrence of trade-offs between regeneration and other growth processes, so that environmental conditions would be determinant for the severity of the costs of regeneration.

Keywords: autotomy, resource allocation, trade-offs, *Podarcis muralis*, early growth

Introduction

Animal life histories exhibit an outstanding diversity, modulated by decisions about the timing of certain events and the allocation of the assimilated energy (Roff 1992; Reznick 2017). Organisms capture and metabolically process energy and materials that they will later assign to various processes such as body maintenance, somatic growth, reserve accumulation and reproduction (Elliott 1994; van der Meer 2019). Resources are often limited, and it is frequent that several traits require energy or materials simultaneously from the same storage, leading to allocation conflicts, so that a great investment in one trait implies fewer resources available for other competing traits. For this reason, trade-offs may have physiological but also evolutionary consequences, and individuals must balance the proportion (or the timing) of energy allocated to the different traits in a way that maximizes fitness (Stearns 1992; Reznick 2017).

Some animals have the ability to self-mutilate a body part as a reflex response when they are threatened, which is called “autotomy” (Maginnis 2006). Self-mutilation, often followed by the regeneration of the lost parts to restore the organism’s functionality, evolved independently several times in different animal lineages, both invertebrates and vertebrates (Goss 1969; Arnold 1988; Bely & Nyberg 2010; Clause & Capaldi 2006; Lin *et al.* 2017). Caudal autotomy is a particularly frequent antipredator strategy in lizards, occurring in 13 out of 20 families of saurians (Downes & Shine 2001; McConnachie & Whiting 2003). In addition to its antipredator value, lizards’ tail assumes important functions related to lipid storage (Bellairs & Bryant 1985), communication among conspecifics (Peters *et al.* 2007) or locomotion (Arnold 1988; Gillis *et al.* 2013), so that tail loss may negatively impact the performance of relevant ecological functions, thereby affecting fitness (Fox & McCoy 2000; Chapple *et al.* 2004; Medger *et al.* 2008; Fleming

& Bateman 2012; Hsieh 2016). Caudal regeneration after autotomy seems to restore the functional role of the lost tail in different lizard species (Clause & Capaldi 2006; Zamora-Camacho *et al.* 2016; see Fernández-Rodríguez & Braña 2020 for *Podarcis muralis*), but re-growing the lost parts requires a substantial input of energy and materials, and this investment may constrain the resources available for other critical whole-organism functions, such as growth or reproduction (Bellairs & Bryant 1985; Maginnis 2006; Bateman & Fleming 2009).

The conflict that arises over the cost of regeneration is likely to be subject to ontogenetic variations (Bateman & Fleming 2009), since other potentially competing, energy demanding processes strongly vary with age. For example, the age of the individual in relation to the onset of reproduction and to its lifespan is expected to have great importance in elucidating allocation conflicts: while adult lizards invest much of the available energy in reproduction and less so in growth, juveniles do not invest in reproduction and have very high growth rates (Andrews 1982; Avery 1970; Steiner & Pfeiffer 2007). Therefore, energy allocated to regeneration in juvenile lizards may diminish the available resources and may constrain body growth (Bernardo & Agosta 2005), even when body size is an important determinant of age at maturity, social rank and mating success in lizards (Vitt *et al.* 1977). Then, behavioural and physiological changes after tail autotomy are expected to be more extreme in juveniles than in adults (Bateman & Fleming 2009). Besides, stressful environmental conditions, such as low food quality or availability in early stages, may have physiological consequences for the organism, induce accelerated ageing and can have long term consequences, affecting development, behaviour and physiology later in life (Monaghan 2007; Monaghan *et al.* 2012). For these reasons, studying the energy costs of regeneration on body growth and its possible consequences in juvenile individuals is of special interest.

The study of the functional, physiological and ecological implications of regeneration, as well as the possible mechanisms to minimize its costs, is crucial to understand the evolution of autotomy and regeneration in animals. In this context, the aim of the current study was to assess the cost of tail regeneration in early body growth rates in the wall lizards *P. muralis*, comparing growth performance of hatchlings with intact tails with that of regenerating ones. Our experiments were done with newborn lizards that hatched in the laboratory under the same incubation conditions and that had exactly the same age at the beginning of the experiment (two days, see methods). Therefore, since it is a quite homogeneous sample in which, in addition, there is no interference from any reproductive investment, we consider that it is a very suitable model for the study of the effects of tail regeneration on body growth. As food availability may influence growth rates and the occurrence or intensity of trade-offs (Lawrence 2010; Lynn *et al.* 2013), we exposed hatchlings with intact or regenerating tails to two different food supply levels, one of which represents a situation of high food availability and the other a situation of food scarcity, likely imposing a conflict of resource allocation without compromising hatchling's survival and normal development.

Material and methods

Laboratory experiments and measurements

Fifty-six gravid females of *Podarcis muralis* were captured by noose over the course of May 2018 and May 2019 in several close localities of central Asturias (northern Spain), and oviposition occurred in the laboratory between 2 and 20 days after capture in the field. Eggs were incubated individually in covered plastic containers with moistened vermiculite (at a ratio 1:2 of vermiculite to distilled water by weight) at 29 °C, which is the highest temperature at which incubation is the fastest without having negative effects on hatchling phenotypes (Braña & Ji 2000). Hatchlings emerged from the egg after 30-35 days of incubation (mean ± SD: 32.51 ± 0.87 days), and they were weighed (with a digital balance Mettler Toledo AB54 that gave measures to the nearest 0.0001 g) and measured (with a digital calliper Vogel DIN 862 that provided measures to the nearest 0.001 cm) for snout-vent-length (SVL), tail length (TL) and width at the tail base (TW) a few hours after hatching. Hatchlings were sexed by applying a gentle pressure on both sides of the base of the tail, which causes the eversion of hemipenes in males (Harlow 1996; Braña 2008). Sex was confirmed by observing the dimorphic pattern of flank colouration, which is clearly developed in most individuals towards the end of the experimental period. Hatchlings were housed in terraria with water *ad libitum* containing supplementary vitamins and calcium, and 60 W lamps, to allow behavioural thermoregulation. Hatchlings of each clutch were divided as evenly as possible into two different experimental groups: control (tailed) or experimental (tailless) group; and within each tail group, they were subjected to two different food experiments during one month: food supplied *ad libitum*, or restricted food. Hatchlings born in 2018 were assigned to the *ad libitum* treatment, and those born in 2019 were subjected to a restricted food regime; since the trials with both food regimens were in different years, they were considered as two different experiments and analysed separately. Newly hatched lizards were fasted for 2 days to ensure that they had metabolized the remaining residual yolk and were then weighted again. At this point, caudal autotomy was induced to the lizards of the experimental group by firmly grabbing them by the basis of the tail until they detached it. All tailless lizards were left a tail stub of around 0.5 cm (mean ± SD tail stub: 0.482 ± 0.050 cm), corresponding to approximately 7-10 caudal rings/caudal vertebrae.

Hatchlings assigned to the experiment of food *ad libitum* (N_{total} = 89; N_{tailed} = 45, $N_{tailless}$ = 44) were fed daily mainly with crickets, and they were offered also mealworms once per week to provide a more diverse diet. Food intake was estimated every five days by weighing each lizard before and after eating, and then calculating the weight increase. In order to estimate food intake, lizards were fasted for 24 hours, and they were then fed *ad libitum* for 30 min. The mass of prey ingested was calculated by weighing each lizard before and after eating. Hatchlings subjected to food restriction (N_{total} = 80; N_{tailed} = 41, $N_{tailless}$ = 39) were offered one cricket (mean ± SD cricket weight: 0.037 ± 0.006 g) every two days. Once per week, they were offered one mealworm (mean ± SD mealworm weight: 0.021 ± 0.005 g) instead of crickets, to ensure a varied diet. Three days a month (every 10 days) they were fed *ad libitum*.

Every 10 days, the lizards of all experimental treatments were weighed and measured for SVL, TL, and TW for monitoring their growth during the first month of life. Lizards were always fasted for 24 hours before being weighed. To separate the relevant components of total mass of each lizard (i.e., tail and body without tail), we measured (tail length and width) and weighed a sample of shed tails of different sizes and regeneration stages (N_{intact} = 34; $N_{regenerated}$ = 44) to be able to make estimates of tail mass from tail volume. Linear regressions of tail mass on tail volume had very high coefficients of determination both for intact and for regenerated tails ($R^2_{intact\ tails}$ = 0.946, $R^2_{regenerated\ tails}$ = 0.972; $P < 0.0001$ in both cases), and the intercept did not significantly differ from 0 in either case. This indicates a linear isometric relationship between tail mass and tail volume (Packard & Boardman 1987), which allows using the mean ratio mass/volume of the samples of shed tails used in each regression (one for intact and another for regenerated tails), to estimate tail mass from tail volume. Body mass was then calculated by subtracting the calculated values of tail mass from the total mass.

Statistical analysis

The assumptions of normality and homoscedasticity were tested by Kolmogorov-Smirnov and Levene tests, respectively. To test for differences in total mass of hatchlings, general linear mixed models were done with tail group and sex as fixed factors and the mother identity as a random factor, for total mass of hatchlings at day 0 (just after inducing tail autotomy to the experimental group) and 30 days after.

To study the investment in body growth vs. tail regeneration, the whole animal was divided into two main compartments: body (without tail) and tail. To test possible differences in longitudinal (SVL) growth, general linear mixed models were done with tail group and sex as fixed factors and the mother as random factor, for SVL at day 0 and for the increase in SVL in 30 days (i.e., SVL at day 30 – SVL at day 0). Besides, a general linear mixed model was done for SVL at days 0, 10, 20 and 30, with tail group, sex and time as fixed factors and mother as random factor. Differences in body mass at day 0 and in the increase in body mass in 30 days were tested by means of general linear mixed models with tail group and sex as fixed factors and mother as random factor.

To study tail growth, we used the increase of tail length and estimated tail mass in 30 days, which adjusted to normality and homogeneity of variances. General linear mixed models with tail group and sex as fixed factors and mother as random factor were done to test for possible differences in growth between intact and regenerated tails. Linear regressions were done separately for tailless and tailed hatchlings of both food experiments to test if tail growth (i.e., the increase of estimated tail mass in 30 days) was related to body growth (i.e., increase of body mass in 30 days). Estimated tail mass increase from day 20 to 30 was tested by a general linear mixed model with tail group as fixed factor, mother as random factor and body mass increase as covariate.

Food intake of animals fed *ad libitum* was analysed by grouping the six feeding measures taken for each individual in two fortnightly periods of three measures each, considering that these periods correspond to two significant stages of the regeneration process, namely the initial latency phase in which tail regeneration has just started, and the effective regeneration that involves a substantial elongation of the tail. A general linear mixed model with food intake in these two periods as the response variable was carried out to test for possible differences between tailed and tailless animals, and between males and females (tail group, sex and fortnight as fixed factors, mother as random factor).

Results

Total growth

As expected, total growth (body and tail) was much more intense in hatchlings from the *ad libitum* feeding experiment (mean ± SD, Tailed: 0.306 ± 0.099 g; Tailless: 0.334 ± 0.097 g; GLMM with tail group and sex as fixed factors, and mother as a random factor: $F_{1,57} = 1.596$, $p = 0.217$) than in those on the food restriction experiment (mean ± SD Tailed: 0.052 ± 0.033 g; Tailless: 0.059 ± 0.029 g; $F_{1,51} = 1.527$, $p = 0.222$). Obviously, total mass of tailed hatchlings (at day 0) was higher than that of tailless ones that had just lost their tail, and that difference was maintained until the end of the experiment (day 30) both in lizards from the food restriction experiment (Figure 5.1A; GLMM: $F_{1,57} = 36.450$, $p < 0.001$) and from the fed *ad libitum* experiment (GLMM: $F_{1,57} = 5.882$, $p = 0.018$). Sex was not a significant factor explaining differences of total mass at day 30 between males and females fed *ad libitum* or with food restriction (GLMMs: $p > 0.05$ in all cases). The interactions between tail group and sex were not significant in any of the former tests.

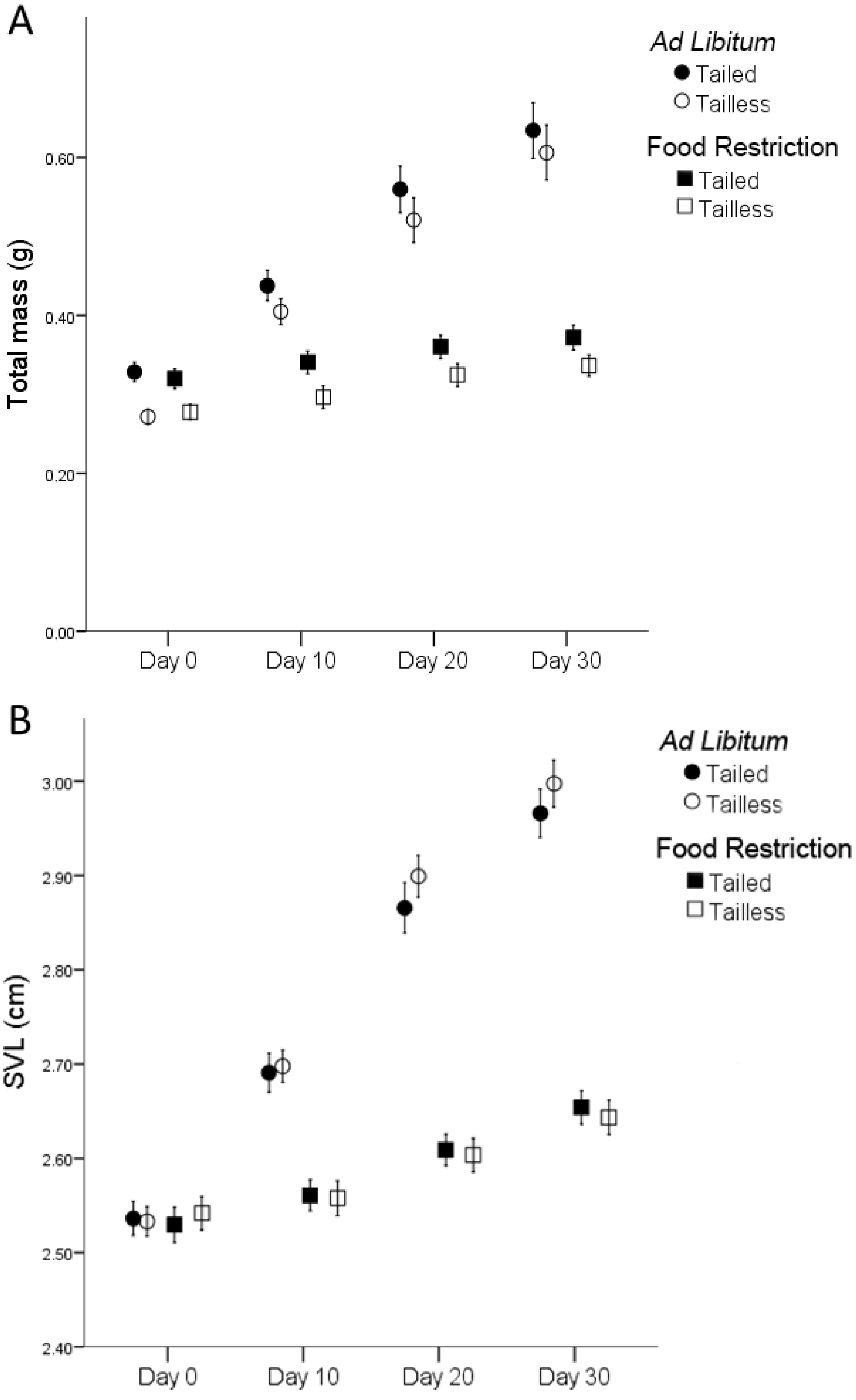


Figure 5.1. Total mass (body and tail, **A**) and SVL (**B**) in successive time intervals during the first month of life (from day 0 to day 30) of tailed and tailless hatchlings either fed *ad libitum* or subjected to food restriction. Values are means ± 2SE in A and 1SE in B.

Body growth (without tail)

Tailed and tailless lizards of both *ad libitum* and restricted food experiments did not differ in SVL at hatching, but tailless lizards' body mass at birth was slightly lower in both experiments (Table 5.1). Growth in length (SVL) was not significantly different for tailless and tailed lizards fed *ad libitum*, neither after 30 days (Table 5.1), nor in the successive measurements of body length during one month (GLMM with tail group, sex and time as fixed factors, and mother as a random factor: $F_{1,57} = 0.054$, $p = 0.817$). Regarding body mass, no differences were found in the increase on body mass in 30 days between tailed and tailless hatchlings fed *ad libitum* (Table 5.1).

Regarding the food restriction experiment, the effect of tail group on the successive measurements of body length during one month was marginally significant (GLMM of SVL at days 0, 10, 20 and 30 with tail group, sex and time as fixed factors and mother as a random factor: $F_{1,51} = 3.552$, $p = 0.065$). Besides, there was a significant interaction between tail and time ($F_{1,236} = 5.574$, $p = 0.019$), as tailed lizards were slightly smaller in SVL at the beginning of the experiment but significantly larger at the end, and the differences in SVL between tailed and tailless lizards increased with time (Figure 5.1B). In addition, the total increase of SVL in these 30 days was significantly higher in tailed lizards (Table 5.1). There were no differences in body mass growth after 30 days between tailed and tailless hatchlings (Table 5.1).

Females of both *ad libitum* and restricted food experiments were significantly longer at birth than males (Table 5.1). These differences tended to disappear after 30 days in lizards fed *ad libitum* (GLMM: $F_{1,57} = 2.945$, $p = 0.092$), although growth was statistically not significantly greater in males than in females (Table 5.1). Regarding the restricted food experiment, although males grew significantly more than females in SVL

(Table 5.1), females remained bigger than males in SVL after 30 days (GLMM: $F_{1,51}$ = 5.279, p = 0.026).

No differences were found between males and females in body mass in any of the two experiments, neither at the beginning of the experiment, nor in the growth in body mass for 30 days (Table 5.1). The interactions between tail group and sex were not significant in any of the former tests.

Tail growth

Regeneration rate in tailless hatchlings fed *ad libitum* was much faster than in those subjected to food restriction (mean ± SD tail regenerated in 30 days, *Ad Libitum*: 0.055 ± 0.022 g; Food restriction: 0.015 ± 0.007 g). There were no significant differences in the increase of estimated tail mass in 30 days between intact (tailed individuals) and regenerated tails (tailless ones) of hatchlings from the *ad libitum* and food restriction experiments (Table 5.1). Tail length increase was not different for tailless and tailed lizards in the food restriction experiment but was significantly higher for tailless lizards from the ad libitum experiment (Table 5.1). No between-sex differences were found in the increase of tail length or estimated tail mass for hatchlings fed *ad libitum* or with food restriction (Table 5.1). The interactions between tail group and sex were not significant in any of the former tests.

There was a positive relationship between estimated body and tail mass growth for tailed hatchlings, both for *ad libitum* and restricted food experiments (Figure 5.2A; *ad libitum*: R^2 = 0.441, slope = 0.197 ± 0.034 (standard error), p < 0.001; restricted food: R^2 = 0.309, slope = 0.173 ± 0.041, p < 0.001), with similar slopes.

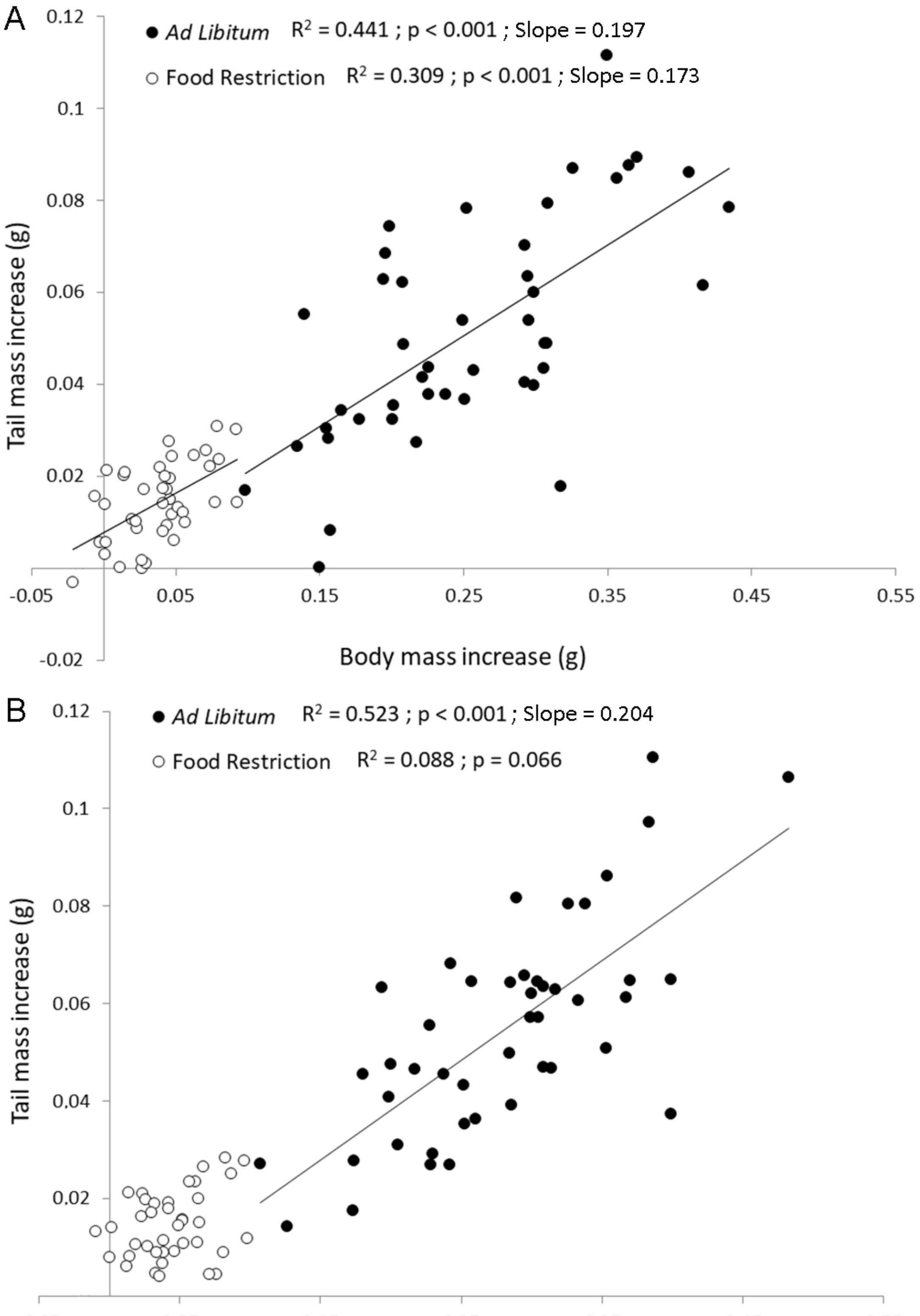


Figure 5.2. Body growth against tail growth (i.e., estimated increase of mass) in 30 days of hatchlings that were fed *ad libitum* or underwent a food restriction regime. The data for the two feeding regimes come from two different experiments. **A**, Tailed hatchlings; **B**, Tailless hatchlings.

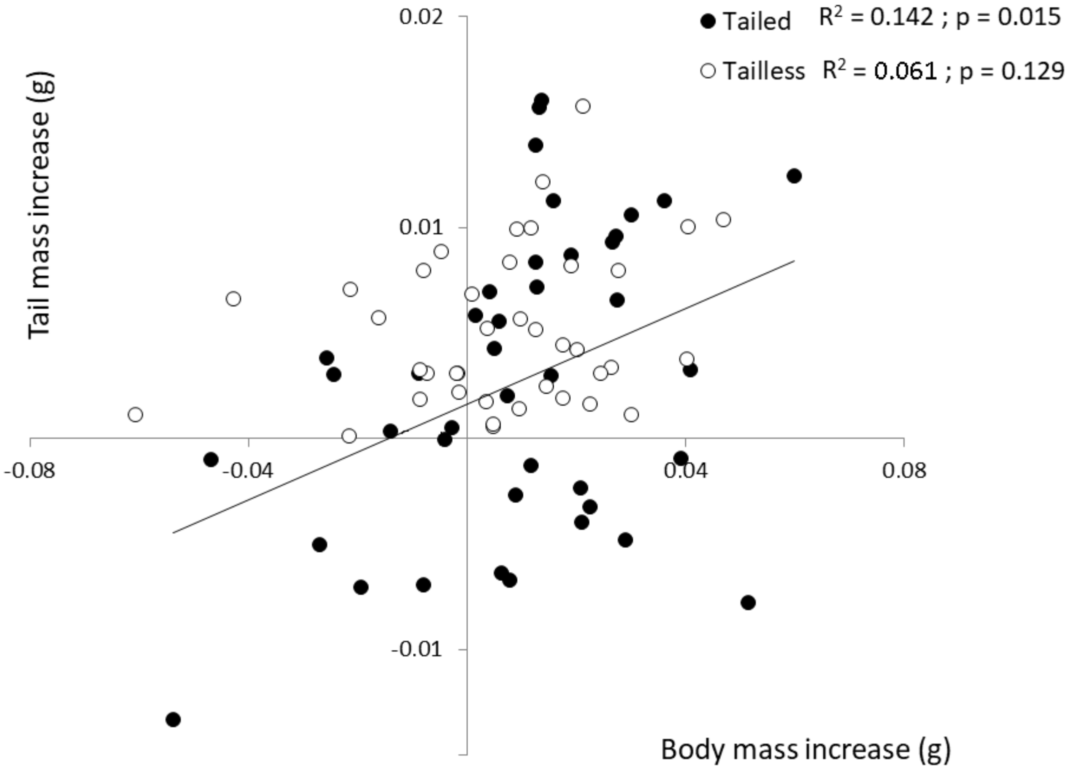


Figure 5.3. Estimated body growth (tail excluded) against tail growth during the third experimental period (from day 20 to day 30 after hatching) of tailed and tailless hatchlings subjected to food restriction. The regression line corresponds to the relationship for tailed lizards.

Tailless individuals fed *ad libitum* also showed a positive relationship between these variables, but no significant relationship was found for lizards subjected to food restriction (Figure 5.2B; *ad libitum*: $R^2 = 0.523$, slope = 0.204 ± 0.029, $p < 0.001$; restricted food: $R^2 = 0.088$, $p = 0.066$). Besides, estimated tail growth investment of lizards with food restriction was low during the first 10 days (although slightly higher in tailed individuals than in regenerating ones), both in absolute values and in relation to body growth, but estimated tail growth (both for intact and regenerated tails) reached its maximum during the days 10 to 20, being higher in tailed individuals in relation to estimated body growth. Finally, in the third period measured (from day 20 to 30), regenerating individuals invested more in tail growth in relation to body growth than did

Table 5.1. Descriptive statistics for size and growth of lizards subjected to *Ad Libitum* (above) and Food Restriction (below) regimens, for female and male tailed and tailless hatchlings. The increases refer to the total increase from day 0 to day 30. General linear mixed models were done with tail group and sex as fixed factors and mother as random factor in all cases. Abbreviations: Init. = initial; leng. = length; incr. = increase. Values are means ± SD.

Ad Libitum

	Tailed			Tailless			Tail group		Sex	
	Females	Males	Total	Females	Males	Total	F	p	F	p
Initial SVL (cm)	2.580 ± 0.118	2.494 ± 0.109	2.536 ± 0.121	2.543 ± 0.087	2.522 ± 0.123	2.532 ± 0.105	0.408	0.526	**6.738**	**0.012**
SVL increase (cm)	0.418 ± 0.113	0.442 ± 0.194	0.429 ± 0.103	0.469 ± 0.123	0.455 ± 0.105	0.462 ± 0.114	2.194	0.144	0.073	0.788
Init. body mass (g)	0.295 ± 0.028	0.266 ± 0.039	0.280 ± 0.037	0.271 ± 0.025	0.273 ± 0.039	0.272 ± 0.032	**6.337**	**0.015**	1.823	0.182
Body mass incr. (g)	0.258 ± 0.094	0.249 ± 0.069	0.254 ± 0.081	0.267 ± 0.072	0.291 ± 0.086	0.279 ± 0.079	1.862	0.178	0.722	0.399
Tail leng. incr. (cm)	1.698 ± 0.332	1.731 ± 0.273	1.731 ± 0.301	2.123 ± 0.455	2.269 ± 0.491	2.194 ± 0.472	**56.47**	**< 0.001**	0.999	0.322
Tail mass incr. (g)	0.059 ± 0.024	0.045 ± 0.023	0.052 ± 0.024	0.052 ± 0.018	0.057 ± 0.026	0.055 ± 0.022	0.275	0.612	0.359	0.551

Food Restriction

	Tailed			Tailless			Tail group		Sex	
	Females	Males	Total	Females	Males	Total	F	p	F	p
Initial SVL (cm)	2.590 ± 0.097	2.459 ± 0.104	2.529 ± 0.119	2.557 ± 0.129	2.524 ± 0.087	2.542 ± 0.112	0.719	0.400	**20.395**	**< 0.001**
SVL increase (cm)	0.108 ± 0.053	0.144 ± 0.053	0.125 ± 0.055	0.092 ± 0.095	0.114 ± 0.056	0.102 ± 0.051	**4.833**	**0.033**	**6.935**	**0.011**
Init. body mass (g)	0.292 ± 0.031	0.267 ± 0.031	0.280 ± 0.033	0.271 ± 0.027	0.285 ± 0.033	0.278 ± 0.030	**4.510**	**0.039**	0.032	0.858
Body mass incr. (g)	0.038 ± 0.027	0.038 ± 0.029	0.038 ± 0.028	0.042 ± 0.017	0.048 ± 0.035	0.044 ± 0.026	2.015	0.163	0.623	0.434
Tail leng. incr. (cm)	0.958 ± 0.324	1.043 ± 0.309	0.997 ± 0.316	1.003 ± 0.327	0.961 ± 0.369	0.983 ± 0.343	1.048	0.311	0.853	0.360
Tail mass incr. (g)	0.013 ± 0.009	0.016 ± 0.008	0.014 ± 0.009	0.014 ± 0.007	0.015 ± 0.008	0.015 ± 0.007	0.011	0.915	1.247	0.269

intact ones (Figure 5.3; GLMM with $\log_{10}$-transformed estimated tail mass increase from day 20 to 30, with tail group as fixed factor, mother as random factor and $\log_{10}$-transformed body mass increase as covariate: $F_{1,23} = 6.669$, $p = 0.017$).

Food intake

There were not significant differences in food intake between tailed and tailless lizards fed *ad libitum* in the two different fortnights (GLMM with tail group, sex and fortnight as fixed factors, and mother as a random factor: $p > 0.05$). However, there was a significant interaction between periods of the ingestion rate of tailed and tailless individuals (GLMM, interaction between fortnights and tail group: $F_{1,84} = 4.113$, $p = 0.046$): tailless hatchlings increased their ingestion rate relative to tailed ones in the second fortnight (Figure 5.4).

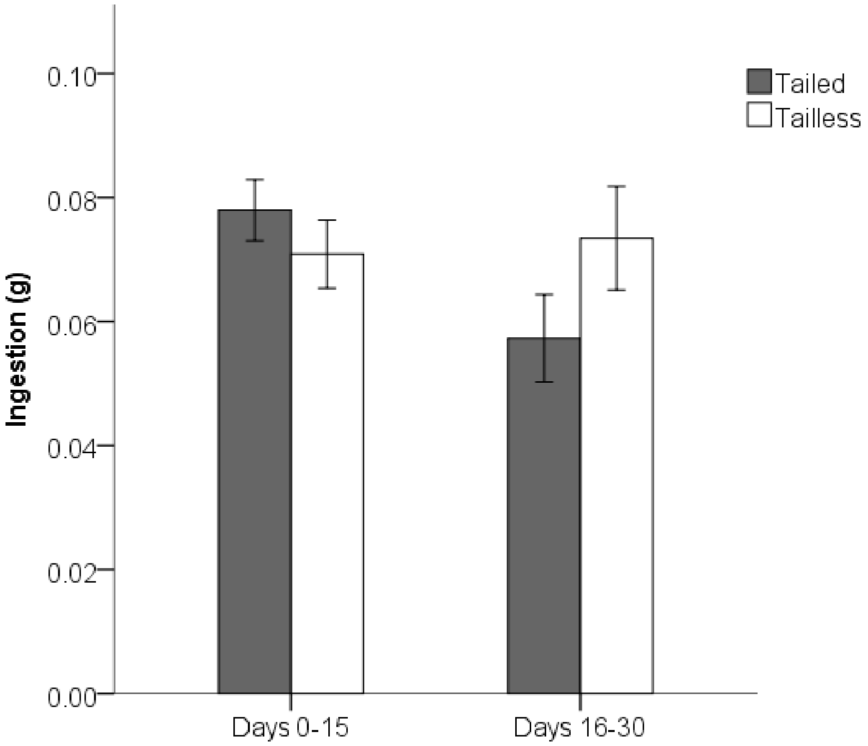


Figure 5.4. Mass of prey ingested by tailed and tailless hatchlings fed *ad libitum*, grouped in two fortnightly periods. Values are means ± SE of three measures made to all lizards in each period.

Ingestion of hatchlings subjected to food restriction was fixed and they ingested a mean of 0.368 ± 0.048 g (mean ± SD) of prey in the whole month. Food intake was homogeneous for tailed and tailless hatchlings and for males and females of the restricted food experiment (GLMM with tail group and sex as fixed factors, and mother as a random factor: $p > 0.05$ in both cases).

Discussion

Caudal autotomy has been shown to entail significant functional costs in many species of lizards, affecting locomotion, foraging habits, mating success, habitat use and social status (Fox & Rostker 1982; Fox *et al.* 1981; Bateman & Fleming 2009). Some long-term studies evidenced that these costs can decrease survivorship and thus the overall lifetime fitness of the individual (Fox & McCoy 2000; Lin *et al.* 2017). However, experimental studies have given less attention to the investment of energy and materials associated to regeneration following autotomy and the potential subsequent costs for growth or reproduction. Theoretical predictions state that regeneration may trade off with other processes, such as reproduction or growth, that occur simultaneously and that have also a high demand on energy and materials (Maginnis 2006), but the consequences of re-growing the tail on body growth remains rather unknown. Our study revealed that tailless (i.e., regenerating) juvenile wall lizards had slightly but significantly lower growth rates in body length than individuals with intact tails when facing situations of low food availability, but there were not such differences when food was supplied *ad libitum*. Similarly, Lynn *et al.* (2013) found that juvenile leopard geckos (*Eublepharis macularius*) had reduced body growth rates after autotomy when they had limited food resources. Although the differences in growth (SVL) between tailed and tailless lizards observed in this study might seem minimal and therefore of low biological relevance, these differences could thus probably increase with time if a regime of low food availability is maintained, likely attaining higher biological relevance. Regarding sex-related differences, females were larger in SVL than males at birth in both experiments, and males grew more than females during the experiment with food restriction but did not reach females' size after 30 days. It is worth noting that no interaction between sex and tail group was significant in any of the measured variables. This model of sexual dimorphism in size at birth (being females longer than males) and initial body growth (higher in males) has been previously reported for this species (Braña & Ji 2000).

In contrast to our results, most of the few studies that have addressed the costs of tail regeneration on body growth in lizards have not found evidence of such costs. Some of these studies were laboratory experiments performed under controlled conditions, but food was supplied *ad libitum*, which could have masked the possible trade-off between tail regeneration and body growth (Ballinger & Tinkle 1979; Chapple *et al.* 2004; Goodman 2006; Iraeta *et al.* 2012; Starostová *et al.* 2017), as suggested by our own data, since the slopes of body growth against tail growth were almost equivalent for tailed and tailless lizards fed *ad libitum*. Althoff & Thompson (1994) made similar experiments to ours, subjecting individuals of the side-blotched lizard (*Uta stansburiana*) to different food treatments (low, medium and *ad libitum* food supply) in order to avoid overlooking a possible trade-off; however, they did not find differences in growth rates, neither among tailed and tailless lizards, nor between food treatments. This contrasts sharply with the results obtained in our experiment, in which growth rates of lizards fed *ad libitum* were 3.9 times higher than those of lizards subjected to food restriction. The most plausible explanation for that discrepancy is that the amount of food that Althoff & Thompson (1994) provided to their group with the most severe food restriction was almost twice the amount provided to hatchlings in our restricted food experiment (for lizard species of similar size), so it is likely that that restriction was not enough to impose a major constraint on growth.

With regard to field studies, some of them have reported diminished body growth rates in regenerating lizards, which were generally attributed to possible limitations of food intake during regeneration (Ballinger & Tinkle 1979; Smith 1996; Niewiarowski *et al.* 1997; Salvador & Veiga 2005). However, other field studies have found no effect of tail regeneration on body growth rates of lizards, and some authors speculated about possible higher ingestion rates of regenerating individuals (Van Sluys 1998; Fox & McCoy 2000; Webb 2006). Environmental conditions, including prey abundance, will likely determine the severity of the costs of regeneration in juveniles under natural conditions.

Hatchlings and juvenile lizards generally exhibit high growth rates (Andrews 1982; Avery 1970) and have therefore a high energy demand, which may even increase in situations of additional requirements, such as tail regeneration. The regeneration process first begins with the cover and repair of the injury, cell differentiation and blastemal formation (Bellairs & Bryant 1985; Bryant *et al.* 2002), so that cell proliferation and tail elongation start a few weeks after autotomy (e.g., 4–5 weeks in the leopard gecko *E. macularius,* McLean & Vickaryous 2011, and 1–2 weeks in our hatchlings). There is conflicting evidences of how tail regeneration may affect metabolic rates in lizards. For instance, Starostová *et al.* (2017) did not find significant differences between control and regenerating lizards in the gecko *Paroedura picta*, whereas Naya *et al.* (2007) reported a substantial increase (36%) in the standard metabolic rate of *Liolaemus belli*. Our results support the idea that regeneration requires a significant demand of energy, since regenerating individuals grew less (under food restriction) or increased ingestion rates in relation to intact ones (when fed *ad libitum*), precisely at the time when the effective growth phase of regeneration begins. In return for the advantages of acquiring more resources, juveniles that face increased energy requirements may spend more time foraging, increasing exposure and predation risk (Dial & Fitzpatrick 1981; Fox 1978). Besides, juvenile lizards sometimes face agonistic interactions with adults, being restricted to more limited and often suboptimal territories (Brandl & Völkl 1988). This, together with an impaired locomotion due to tail loss (Medger *et al.* 2008; Gillis *et al.* 2009; see Fernández-Rodríguez & Braña 2020 for *Podarcis muralis*), could affect their access to food resources and foraging efficiency.

Our results indicate that body growth and tail regeneration are not positively correlated when resources are scarce (i.e., low food availability), but in these conditions hatchlings seems to invest more energy in tail regeneration in relation to body growth, rather than delay regeneration to give priority to body growth. Contrary to this finding, Vitt *et al.* (1977) suggested that regeneration should be selected to be slow in long-lived species with high probability of surviving to the next reproductive season, and juveniles should prioritize allocation on body growth over tail regeneration more than adults (but see Tinkle 1967; Lynn *et al.* 2013). As predation is usually size-related, juvenile lizards are likely to have more potential predators, and thus face a higher predation risk than adults (Blomberg & Shine 2000). As a consequence, tail autotomy is very frequent in juveniles (Chapple *et al.* 2004), and it is a very important antipredator mechanism, because locomotor performance and other abilities are not yet well developed (Iraeta *et al.* 2012). As an example of the relevance of this mechanism, juveniles of many lizard species exhibit striking colourations in the tail, which may attract predators' attention and deflect the attacks from the head or body, hence increasing the chance of survivorship (Cooper & Vitt 1985; Castilla *et al.* 1999; Pianka & Vitt 2003; Kuriyama *et al.* 2016).

However, lizards are more vulnerable after autotomy, as they have lost one effective defence against predators (Congdon *et al.* 1974; Wilson 1992; Fox & McCoy 2000; but see Daniels 1983; Ding *et al.* 2012) and tail loss has been proved to impair locomotor performance (Chapple *et al.* 2004; Sun *et al.* 2009; Fernández-Rodríguez & Braña 2020). Regeneration has long-term antipredator value (Tsasi *et al.* 2009; Lin *et al.* 2017) and, under this framework, rapid regeneration rates (giving even priority to tail re-growth at the expenses of body growth) would be important for the individuals' fitness and could have been selected in juveniles of some species (like *P. muralis*) to increase the probabilities of survival until the first reproductive season. In such case, investing in tail regeneration would have immediate benefits (e.g., restoring locomotor capacities, which may improve feeding or diminish predation risk) implying lifetime fitness consequences.

Decreased body growth due to energy allocated to regeneration can delay approaching to the asymptotic size and can even lead to a smaller final size, with important potential consequences for lifetime fitness, as body size can affect metabolic rates, age at sexual maturity, social rank, territory use, fecundity, mating success and survival in lizards (Brownikowsi & Arnold 1999; King *et al.* 2016; see Peters 1983 for a general account). Besides, fat reserves and body size reached at the beginning of hibernation are important for winter survival of juvenile lizards (Bauwens 1981; Civantos *et al.* 1999; Iraeta *et al.* 2012), and some authors have suggested that there might be selective advantages to reach early the minimum body size at maturity (Iraeta *et al.* 2008). Reduction of growth rate during tail regeneration could even trigger compensatory growth responses in juveniles once regeneration finishes, in order to reach a minimum body size (Vogel *et al.* 1986; Dmitriew 2011), although compensatory growth is known to affect physiology later in life (e.g., maintaining high metabolic rates in adulthood; Criscuolo *et al.* 2008). Finally, although the high metabolic demands during tail regeneration and its impact on juvenile’s body growth could be finally fulfilled or compensated to diminish or avoid the costs of decreased body size (as suggested by our results), those stressful conditions during early life stages may have long-term consequences in adulthood, affecting physiology later in life, or reducing reproductive investment or lifespan (Monaghan 2007; Inness & Metcalfe 2008). Further research on the consequences of regeneration during early life is needed, considering not only immediate and short-term effects (during juvenile stages), but also long-term effects during adulthood, that could affect reproductive output and life-time individual fitness.

To conclude, in general terms, and according to the results of our study and the available literature, regeneration does not impose extremely high additional energy demands, but it may compromise body growth when environmental conditions (food availability) are unfavourable. Our data provided evidence that food shortage has negative consequences for regeneration and body growth, which could be especially critical for hatchlings, as they have narrow range of potential preys and do not have fully developed predatory skills. Besides, tail loss affects locomotor performance of lizards and therefore reduces their efficiency as predators. For these two reasons, it is likely that this scenario of food scarcity may occur in the wild. It seems, therefore, that the availability of food is a determinant for the occurrence of a trade-off between regeneration and other growth processes, which agrees with some experimental studies conducted in other animals with high regenerative capacities, mainly echinoderms (Díaz-Guisado *et al.* 2006; Barrios *et al.* 2008; Lawrence 2010; Ramsay *et al.* 2001). Caudal autotomy and tail regeneration are very common and key antipredator strategies for juvenile lizards, and from our results we propose that tail regeneration in juveniles may be prioritized even at the expenses of body growth, allowing to restore the lost functionality as soon as possible, and thus diminish vulnerability to predators, increase survivorship and the probability to reach reproductive maturity.

References

Althoff, D.M. & Thompson, J.N. (1994). The effects of tail autotomy on survivorship and body growth of *Uta stansburiana* under conditions of high mortality. *Oecologia*, 100: 250–255.

Andrews, R.M. (1982). Patterns of growth in reptiles. In: Gans, C. & Pough, F.H. (Eds), *Biology of the Reptilia*. Vol. 13, Physiology D, Physiological Ecology. Academic Press, New York, pp. 273–320.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Avery, R.A. (1970). Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. *Comparative Biochemistry and Physiology*, 37: 119–121.

Ballinger, R.E. & Tinkle, D.W. (1979). On the cost of tail regeneration to body growth in lizards. *Journal of Herpetology*, 13: 375–375.

Barrios, J.V.; Gaymer, C.F.; Vásquez, J.A. & Brokordt, K.B. (2008). Effect of the degree of autotomy on feeding, growth, and reproductive capacity in the multi-armed sea star *Heliaster helianthus*. *Journal of Experimental Marine Biology and Ecology,* 361: 21–27.

Bateman, P.W. & Fleming, A. (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.

Bauwens, D. (1981). Survival during hibernation in the European common lizard, *Lacerta vivipara*. *Copeia*, 1981: 741–744.

Bellairs, A. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bely, A.E. & Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170.

Bernardo, J. & Agosta, S.J. (2005). Evolutionary implications of hierarchical impacts of nonlethal injury on reproduction, including maternal effects. *Biological Journal of the Linnean Society*, 86: 309–331.

Blomberg, S.P. & Shine, R. (2000). Size-based predation by kookaburras (*Dacelo novaeguineae*) on lizards (*Eulamprus tympanum*: Scincidae): what determines prey vulnerability? *Behavioral Ecology and Sociobiology*, 48: 484–489.

Braña, F. (2008). Sex of incubation neighbours influences hatchling sexual phenotypes in an oviparous lizard. *Oecologia*, 156: 275–280.

Braña, F. & Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *Journal of Experimental Zoology,* 286: 422–433.

Brandl, R. & Völkl, W. (1988). Tail break rate in the Maderian lizard (*Podarcis dugesii*). *Amphibia-Reptilia*, 9: 213–218.

Brownikowski, A.M. & Arnold, S.J. (1999). The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. *Ecology,* 80: 2314–2325.

Bryant, S.V.; Endo, T. & Gardiner, D.M. (2002). Vertebrate limb regeneration and the origin of limb stem cells. *International Journal of Developmental Biology*, 46: 887–896.

Castilla, A.; Gosá, A.; Galán, P. & Pérez-Mellado, V. (1999). Green tails in lizards of the genus *Podarcis*: do they influence the intensity of predation? *Herpetologica*, 55: 530–537.

Chapple, D.G.; McCoull, C.J. & Swain, R. (2004). Effect of tail loss on sprint speed and growth in newborn skinks, *Niveoscincus metallicus. Journal of Herpetology,* 38: 137–140.

Civantos, E.; Salvador, A. & Veiga, J.P. (1999). Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia*, 1999: 1112–1117.

Clause, A.R. & Capaldi, E.A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology*, 305A: 965–973.

Congdon, J.D.; Vitt, L.J. & King, W.W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science*, 184: 1379–1380.

Cooper, W.E. Jr. & Vitt, L.J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift für Tierpsychologie*, 70: 265–276.

Criscuolo, F.; Monaghan, P.; Nasir, L. & Metcalfe, N.B. (2008). Early nutrition and phenotypic development: ‘catch-up’ growth leads to elevated metabolic rate in adulthood. *Proceedings of the Royal Society of London B: Biological Sciences*, 275: 1565–1570.

Daniels, C.B. (1983). Running: an escape strategy enhanced by autotomy. *Herpetologica*, 39: 162–165.

Dial, B.E. & Fitzpatrick, L.C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia*, 51: 310–317.

Díaz-Guisado, D.; Gaymer, C.F.; Brokordt, K.B. & Lawrence, J.M. (2006). Autotomy reduces feeding, energy storage and growth of the sea star *Stichaster striatus*. *Journal of Experimental Marine Biology and Ecology*, 338: 73–80.

Ding, G.; Fu, T.; Zhou, Z. & Ji, X. (2012). Tail autotomy does not increase locomotor costs in the oriental leaf-toed gecko *Hemidactylus bowringii*. *Asian Herpetological Research*, 3: 141–146.

Dmitriew, C.M. (2011). The evolution of growth trajectories: what limits growth rate? *Biological Reviews*, 86: 97–116.

Downes, S. & Shine, R. (2001). Why does tail loss increase a lizard’s later vulnerability to snake predators? *Ecology,* 82: 1293–1303.

Elliott, J.M. (1994). *Quantitative Ecology and the Brown Trout*. Oxford Series in Ecology and Evolution, Oxford University Press.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15(6): 511–521.

Fleming, P.A. & Bateman, P.W. (2012). Autotomy, tail regeneration and jumping ability in Cape dwarf geckos (*Lygodactylus capensis*) (Gekkonidae). *African Zoology,* 47(1): 55–59.

Fox, S.F. & McCoy, K.J. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia,* 122: 327–334.

Fox, S.F. & Rostker, M.A. (1982). Social costs of tail loss in *Uta stansburiana*. *Science*, 218: 692–693.

Fox, S.F. (1978). Natural selection on behavioral phenotypes of the lizard *Uta stansburiana*. *Ecology*, 59: 834–847.

Fox, S.F.; Rose, E. & Myers, R. (1981). Dominance and the acquisition of superior home ranges in the lizard *Uta stansburiana*. *Ecology*, 62: 888–893.

Gillis, G.B.; Bonvini, L.A. & Irschick, D.J. (2009). Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis. Journal of Experimental Biology*, 212: 604–609.

Gillis, G.B.; Kuo, C.-Y. & Irschick, D.J. (2013). The impact of tail loss on stability during jumping in green anoles (*Anolis carolinensis*). *Physiological and Biochemical Zoology*, 86: 680–689.

Goodman, R.M. (2006). Effects of tail loss on growth and sprint speed of juvenile *Eumeces fasciatus* (Scincidae). *Journal of Herpetology*, 40: 99–102.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Harlow, P.S. (1996). A harmless technique for sexing hatchling lizards. *Herpetological Reviews*, 27: 71–72.

Hsieh, S.-T.T. (2016). Tail loss and narrow surfaces decrease locomotor stability in the arboreal green anole lizard (*Anolis carolinensis*). *Journal of Experimental Biology*, 219: 364–373.

Inness, C.L. & Metcalfe, N.B. (2008). The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish. *Proceedings of the Royal Society of London B: Biological Sciences*, 275: 1703–1708.

Iraeta, P.; Salvador, A. & Díaz, J.A. (2008). A reciprocal transplant study of activity, body size and winter survivorship in juvenile lizards from two sites at different altitude. *Ecoscience*, 15: 298–304.

Iraeta, P.; Salvador, A. & Díaz, J.A. (2012). Effects of caudal autotomy on postnatal growth rates of hatchling *Psammodromus algirus*. *Journal of Herpetology*, 46(3): 342–345.

King, R.B.; Stanford, K.M.; Jones, P.C. & Bekker, K. (2016). Size matters: Individual variation in ectotherm growth and asymptotic size. *PLoS ONE*, 11(1): e0146299.

Kuriyama, T.; Morimoto, G.; Miyaji, K. & Hasegawa, M. (2016). Cellular basis of anti-predator adaptation in a lizard with autotomizable blue tail against specific predators with different color vision. *Journal of Zoology*, 300: 89–98.

Lawrence, J.M. (2010). Energetic costs of loss and regeneration of arms in stellate Echinoderms. *Integrative and Comparative Biology*, 50 (4): 506–514.

Lin, J.W.; Chen, Y.R.; Wang, Y.H.; Hung, K.C. & Lin, S.M. (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(1847): 20162538.

Lynn, S.E.; Borkovic, B.P. & Russell, A.P. (2013). Relative apportioning of resources to the body and regenerating tail in juvenile leopard geckos (*Eublepharis macularius*) maintained on different dietary rations. *Physiological and Biochemical Zoology*, 86(6): 659–668.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

McConnachie, S. & Whiting, M.J. (2003). Costs associated with tail autotomy in an ambush foraging lizard, *Cordylus melanotus melanotus*. *African Zoology*, 38(1): 57–65.

McLean, K.E. & Vickaryous, M.K. (2011). A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius. BMC Developmental Biology*, 11: 50.

Medger, K.; Verburgt, L. & Bateman, P.W. (2008). The influence of tail autotomy on the escape response of the Cape Dwarf Gecko, *Lygodactylus capensis. Ethology*, 114: 42–52.

Monaghan, P. (2007). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363: 1635–1645.

Monaghan, P.; Heidinger, B.J.; D'Alba, L.; Evans, N.P. & Spencer, K.A. (2012). For better or worse: reduced adult lifespan following early-life stress is transmitted to breeding partners. *Proceedings of the Royal Society B: Biological Sciences*, 279: 709–714.

Naya, D.E.; Veloso, C.; Muñoz, J.L.P. & Bozinovic, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 146: 189–193.

Niewiarowski, P.H.; Congdon, J.D.; Dunham, A.E.; Vitt, L.J. & Tinkle, D.W. (1997). Tales of lizard tails: effects of tail autotomy on subsequent survival and growth of free-ranging hatchling *Uta stansburiana. Canadian Journal of Zoology*, 75: 542–548.

Packard, G.C. & Boardman, T.J. (1987). The misuse of ratios, indices, and percentages in ecophysiological research. *Physiological and Biochemical Zoology*, 61(1): 1–9.

Peters, R.A.; Hemmi, J.M. & Zeil, J. (2007). Signaling against the wind: modifying motion-signal structure in response to increased noise. *Current Biology*, 17: 1231–1234.

Peters, R.H. (1983). *The ecological implications of body size*. Cambridge University Press, Cambridge.

Pianka, E.R. & Vitt, L.J. (2003). *Lizards: windows to the Evolution of Diversity*. University of California Press, California.

Ramsay, K.; Kaiser, M.J. & Richardson, C.A. (2001). Invest in arms: Behavioural and energetic implications of multiple autotomy in starfish (*Asterias rubens*). *Behavioral Ecology and Sociobiology,* 50: 360–365.

Reznick, D. (2017). Evolution of life histories. In: Losos, J.B.; Baum, D.A.; Futuyma, D.J.; Hoekstra, H.E.; Lenski, R.E.; Moore, A.J.; Peichel, C.L.; Schluter, D. & Whitlock, M.C. (Eds.), *The Princeton Guide to Evolution*. Princeton University Press, Princeton, New Jersey, 270–277.

Roff, D.A. (1992). *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York.

Salvador, A. & Veiga, J.P. (2005). Activity, tail loss, growth and survivorship of male *Psammodromus algirus. Amphibia-Reptilia,* 26: 583–585.

Smith, G.R. (1996). Tail loss in the striped plateau lizard, *Sceloporus virgatus. Journal of Herpetology*, 30: 552–555.

Starostová, Z.; Gvoždíkb, L. & Kratochvíl, L. (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.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford University Press, London.

Steiner, U.K. & Pfeiffer, T. (2007). Optimizing time and resource allocation trade-offs for investment into morphological and behavioral defense. *The American Naturalist*, 169(1): 118–129.

Sun, Y.-Y.; Yang, J. & Ji, X. (2009). Many-lined sun skinks (*Mabuya multifasciata*) do not compensate for the costs of tail loss by increasing feeding rate or digestive efficiency. *Journal of Experimental Zoology*, 311A: 125–133.

Tinkle, D.W. (1967). The life and demography of the side-blotched lizard, *Uta stansburiana*. *Miscellaneous publications. Museum of Zoology, University of Michigan*, 132: 1–182.

TsasiI, G.; Pafilis, P.; Simou, C. & Valakos, E.D. (2009). Predation pressure, density-induced stress and tail regeneration: a casual-nexus situation or a bunch of independent factors? *Amphibia-Reptilia*, 30: 471–482.

van der Meer, J. (2019). Metabolic theories in ecology: the dynamic energy budget theory and the metabolic theory of ecology. *Encyclopedia of Ecology (Second Edition)*, 3: 463–471.

Van Sluys, M. (1998). Growth and body condition of the saxicolous lizard *Tropidurus itambere* in southeastern Brazil. *Journal of Herpetoly*, 32: 359–365.

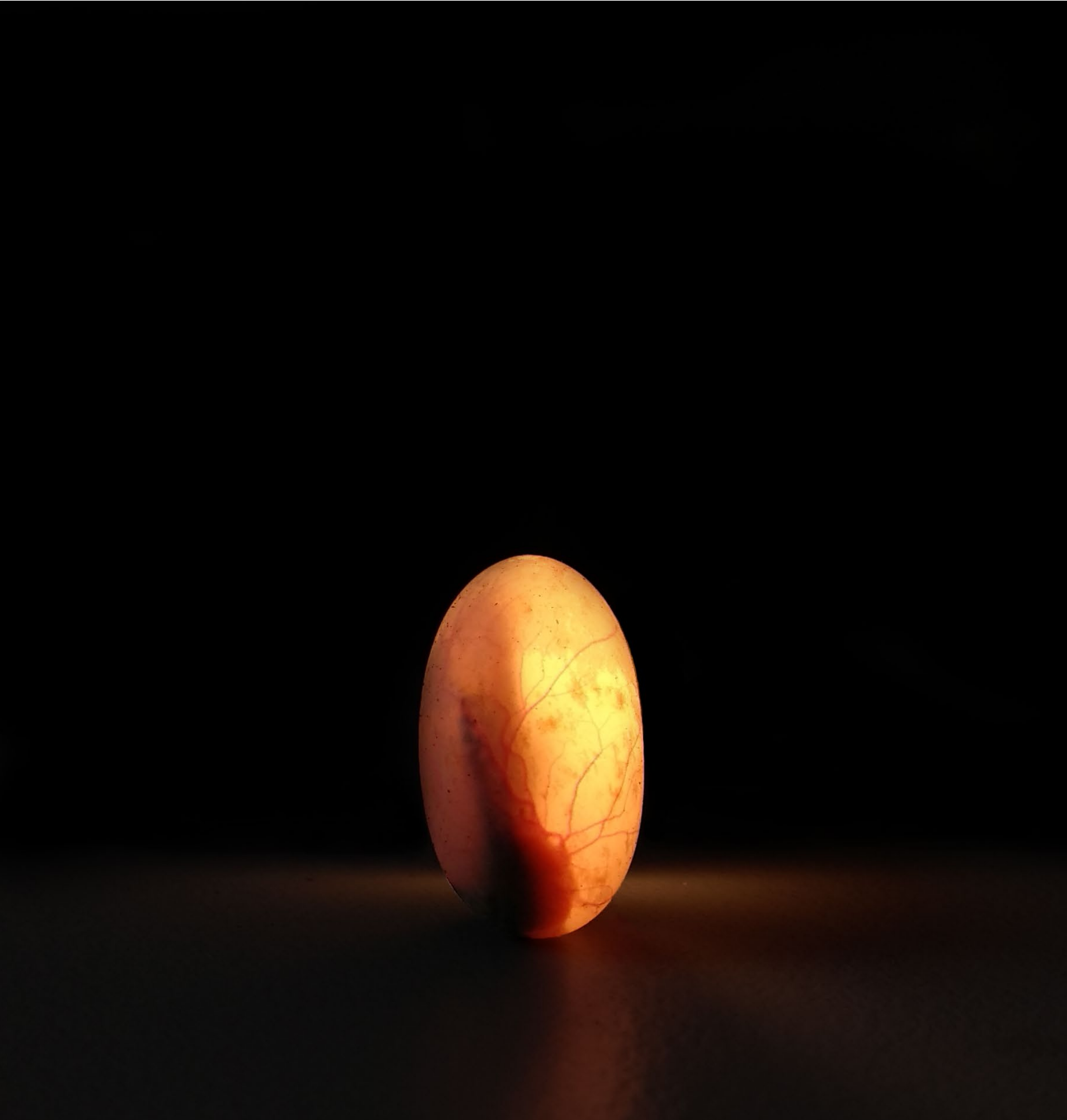
Vitt, L.J.; Congdon, J.D. & Dickson, N.A. (1977). Adaptive strategies and energetics of tail autotomy in lizards. *Ecology,* 58: 326–337.

Vogel, P.; Hettrich, W. & Ricono, K. (1986). Weight Growth of Juvenile Lizards, *Anolis lineatopus*, Maintained on Different Diets. *Journal of Herpetology*, 20(1): 50–58.

Webb, J.K. (2006). Effects of tail autotomy on survival, growth and territory occupation in free-ranging juvenile geckos (*Oedura lesueurii*). *Austral Ecology*, 31: 432–440.

Wilson, B.S. (1992). Tail injuries increase the risk of mortality in free-living lizards (*Uta stansburiana*). *Oecologia*, 92: 145–52.

Zamora-Camacho, F.J.; Rubiño-Hispán, M.V.; Reguera, S. & Moreno-Rueda, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid *Psammodromus algirus*. *Herpetological Journal*, 26: 213–218.



“It is by reproducing that organisms regenerate themselves and their species”. Goss, 1969 (*Principles of Regeneration*).

“Es mediante la reproducción como los organismos se regeneran a sí mismos y a sus especies”.

Cover photo: Egg of *Podarcis muralis* at fourth week of incubation

Photography awarded with 1st prize in the VII Photography Contest “Biología en Movimiento” 2019 of the Faculty of Biology, University of Oviedo

VI. Chapter 5

Short-term and long-term consequences of regeneration on the reproductive investment of a multivoltine lizard

Provisionally accepted in *Journal of Zoology*: Fernández-Rodríguez, I. & Braña, F. Short-term and long-term consequences of tail regeneration on the reproductive investment in females of a multivoltine lizard.

Short-term and long-term consequences of tail regeneration on the reproductive investment in females of a multivoltine lizard

Abstract

Autotomy is the ability to shed a body part when an animal receives the attack of a predator, and is often followed by regeneration of the lost parts to counteract disfunctions related to limb amputation. Caudal autotomy in lizards is frequently used to avoid predation and is followed by tail regeneration, a costly process that can limit the available resources for somatic growth, accumulation of reserves and reproduction. We raised two experiments to assess the short-term and long-term effects of regeneration on the reproductive investment of female wall lizards (*Podarcis muralis*), an iteroparous multivoltine species that produces 1-3 clutches per breeding season. In the short-term experiment we compared the clutches of intact and regenerating females to explore the effects of regeneration during the second clutch development (built under an income breeding strategy). In the long-term experiment we studied the investment to the first spring clutch of intact and regenerating females to assess the effects of regeneration that started prior to wintering, likely affecting the lipid storages necessary for that clutch (built under a capital breeding strategy). Regenerating females of both experiments presented a reduction in clutch mass relative to SVL, but greater in the short-term than in the long-term experiment. Besides, in the short-term experiment the amount of tail regenerated was negatively correlated with the investment in reproduction and regenerating females presented higher egg failure rates. These effects were stronger when clutches were built mostly under an income breeding strategy, which suggests that capital breeding may buffer the costs of regeneration on reproduction.

Keywords: allocation trade-off, income breeding, capital breeding, autotomy, reproductive investment, *Podarcis muralis*

Introduction

Life-histories are shaped by decisions about the timing of major life cycle events and the allocation of the limited energy and materials acquired and metabolically processed by organisms (Roff 1992; van der Meer 2019). When resources are limited, allocation conflicts may arise when several traits require resources from the same source simultaneously, in which a great investment on one trait may imply that there are fewer resources available to allocate to the others (Stearns 1992). A generalized trade-off in organisms' life histories arises from the competition for materials and energy between somatic growth, reproduction and accumulation of reserves (Elliott 1994; Roff 2002; see Tracer 2002, for humans), and the way in which these trade-offs are resolved is an essential component of life histories and may vary through time (age) in the individuals' life (Boggs 2009).

Autotomy, defined as the self-detachment of a body part, is a mechanism that evolved in a wide variety of animal clades, allowing survival in certain unfavourable events such as physical damages, difficulties in shedding the molt, accumulation of toxins, or predatory attacks (Maginnis 2006; Bely & Nyberg 2010). Despite its obvious benefits for immediate survival, autotomy may entail some negative effects, such as the decrease of locomotor performance, mating success, feeding capacity or immune response (Salvador *et al.* 1995; Díaz-Guisado *et al.* 2006; Medger *et al.* 2008; Kuo *et al.* 2013). Besides, after autotomy animals may face an increased vulnerability to predation (Fox & McCoy 2000) and they may exhibit changes in the behaviour, activity patterns or habitat use (Fox & Rostker 1982; Barrios *et al.*, 2008).

Caudal autotomy is commonly used as an antipredator strategy in lizards and occurs in 13 out of 20 families of Sauria (Arnold 1988; McConnachie & Whiting 2003), being particularly common in lacertid lizards, in which the frequencies of tail breakage of adult individuals in natural populations are often around 50% (Bellairs & Bryant 1985; Bateman & Fleming 2009; Pafilis *et al.* 2009). The tail of lizards is important for locomotion, energy storage and social interaction (Bellairs & Bryant 1985; Peters *et al.* 2007; Gillis *et al.* 2013), so its loss may decrease the performance of ecologically relevant tasks (for a review, see Bateman and Fleming 2009). The evolution of regeneration after autotomy allowed the restoration of the lost functionality in many animals, including lizards (e.g., Clause & Capaldi 2006; Zamora-Camacho *et al.* 2016; Fernández-Rodríguez & Braña 2020), thus increasing their long-term survival and fitness (Lin *et al.* 2017; Goss 1969). However, regeneration is not exempt of costs, as it requires a great investment of energy and materials that can compromise other high-demanding physiological processes such as growth or reproduction (Maginnis 2006; Bateman & Fleming 2009; Lawrence 2010; Hoso 2012; Chapter 4; but see Ballinger & Tinkle 1979; Iraeta *et al.* 2012).

Organisms' reproductive investment is partially affected by ecological variations that ultimately translate into gradients of risks and resources (Reznick 1985) and, in this scenario, the great requirements of regeneration in terms of energy and materials can lead to a reduction of the investment in a concurrent reproductive event. Reproductive investment is a key issue in the organisms' life history, as it determines the quantity and quality of an individual's offspring, and thereby its fitness (Stearns 1992), so the potential trade-off between reproduction and caudal regeneration is particularly important for the evolution of life histories. For instance, females with a diminished energy budget due to regeneration could produce smaller or fewer clutches per season, with either smaller, fewer, or lower quality eggs (Maiorana 1977; Pomory & Lawrence 1999; Morgan & Jangoux 2004; Wang *et al.* 2017). With respect to the time and the source from which resources are recruited to support reproductive output, a distinction is usually made between two main strategies (Jönsson 1997; Stephens *et al.* 2009; for ectothermic animals, mainly Squamata, see Braña *et al.* 1992, Bonnet *et al.* 1998, 1999): 1) animals that rely upon resources acquired and stored some time before reproduction (designated as "capital breeders"), and 2) animals that use resources acquired through adjustments in food intake simultaneously with reproduction (named "income breeders"). If lizards that rely on an income breeding strategy suffer from energy shortage during reproduction due to regeneration, they can compensate by increasing their feeding rate and foraging time to face these higher energetic demands. However, this implies a greater exposure which usually means a higher risk of predation (Fox 1978; Dial & Fitzpatrick 1981) and could negatively affect the probability of survival for future reproductive bouts. Thus, considering the trade-off between current and future reproduction that is characteristic of life cycles of iteroparous animals (Roff 1992; Stearns 1992; Messina & Fox 2001), we hypothesize that the current clutch development could be delayed or even skipped due to the resources limitation when facing tail regeneration, in order to ensure a proper development of future reproductions.

Under this framework, the functional, physiological and ecological implications of regeneration are essential to understand how these traits are integrated in the animals' life histories, and to what extent the conflicts that may arise from regeneration may compromise not only current, but also future reproductive investment, thereby influencing lifetime fitness. This study aims to shed light on the former questions by studying the effects of tail regeneration on the reproductive investment of female wall lizards (*Podarcis muralis*). We have chosen to carry out this study with females because their reproductive investment is strongly directed towards vitellogenesis and egg production, so that the final output is easily measurable, while males make a stronger investment in developing behaviours aimed at maximizing mating success and less so in gametogenesis (e.g., Schwarzkopf 1994; see, for lacertid lizards, Braña *et al.* 1992). Our hypothesis is that the reproductive investment of females must be negatively affected by the energy demands of tail regeneration occurring concurrently with vitellogenesis. Additionally, we hypothesize that the way in which resources for reproduction are recruited, either through a "capital breeding" or an "income breeding" strategy, can influence the swiftness of the regeneration and the strength of an eventual trade-off between these processes. To test these hypotheses, we raised two parallel experiments aiming to assess the possible short-term and long-term effects of caudal regeneration on the reproductive investment of female wall lizards. For each experiment we compared intact and experimentally autotomized females for clutch size and mass, individual egg mass, time between clutches, conversion efficiency of eggs (measured as the hatchlings size in relation to egg size) and egg failure rates. Besides, we compared regeneration rates between males and females, as an indirect measure of possible sexual differences in the prioritization between investing resources to regeneration and reproduction.

Material and methods

Study animals

For this study we used adult common wall lizards (*Podarcis muralis*) captured by noose in several nearby locations in central Asturias (northern Spain), which have very similar physical and environmental conditions and have the same community of potential predators. In our study area, wall lizards are active mainly from April to October, and reproduction occurs from the end of April to the beginning of July, period in which this multivoltine lizard can carry out up to three clutches with an approximate interval between clutches of one month, being the first clutch larger than the other two (Saint

Girons & Duguy 1970; Ji & Braña 2000). According to previous studies on the same population of wall lizard studied in this paper (Braña *et al.* 1992), vitellogenesis for the first clutch takes place in spring mainly at the expense of fat body storages acquired in the previous summer and autumn (under a capital breeding strategy), while the second and third clutches mainly depend on the immediate food intake (under an income breeding strategy). After finishing the reproductive period, lizards decrease their activity and forage to accumulate fat reserves to be used for winter brumation and, especially, to build the first clutch in the next breeding season.

Short-term effects of regeneration

For the study of the immediate (short-term) effects of tail regeneration on reproductive investment, 62 gravid females and 34 males (to ensure egg fertilization in the laboratory) were captured by noose in April 2018 and 2019 and were transported to the laboratory of the Zoology Unit (University of Oviedo). All lizards were weighted to the nearest 0.01 g and measured for snout-vent-length (SVL) and tail length (TL) to the nearest 0.01 cm. Lizards were housed in groups of three females and one male in terraria (50 L x 37 W x 25 H, cm) with lamps of 35W, to allow behavioural thermoregulation within the limits of the thermal preference of the species, and water and food (crickets, mealworms, and cockroaches) were provided ad libitum. Lizards were exposed to a natural photoperiod and the environmental temperature inside the room was 22–24 °C.

For this experiment we used female wall lizards that had just completed the vitellogenesis for the first clutch (i.e., either bearing large vitellogenic follicles or oviductal eggs), or had just laid the first clutch in spring. Reproductive stage was evaluated by abdominal palpation, a reliable method to determine the reproductive condition in female lizards (Weiss 2002; Ji & Braña 2000, for a previous application of the method in *P. muralis*), and by checking for the presence of marked post-oviposition abdominal skin folds. Females were equitably divided into a control group (hereafter "tailed", N = 31) and an experimental group (hereafter "tailless", N = 31); autotomy was induced to the experimental group either a few days before laying their first clutch (when the females collected in the field had advanced oviductal eggs), or immediately after laying their first clutch (when females were captured just after laying eggs, with clearly marked post-oviposition skin folds). Tail autotomy was induced by firmly grasping the animals from the base of the tail until they provoked a vigorous shaking and the detachment of their tail, according to the procedure described by Fernández-Rodríguez & Braña (2020). The grasping point was approximately the same for all animals, leaving an average tail stub of 0.802 ± 0.169 cm (mean ± SD).

Feeding rate was measured every six days from the oviposition of the first clutch on a sample of both tailed and tailless females that had laid the first clutch in the laboratory ($N_{tailed} = 12$; $N_{tailless} = 20$), by measuring the body mass increase (to the nearest 0.0001 g) of lizards that were allowed to eat food provided ad libitum for two hours, after a full day of fasting, in order to standardize hunger and to prevent faecal output. Both the first (for females that laid that clutch in the laboratory, $N_{tailed} = 12$; $N_{tailless} = 20$) and second clutches were counted for number of eggs and weighted to the nearest 0.0001 g, and the time elapsed from the oviposition of the first clutch to that of the second clutch was also recorded. The regenerated portion of the tail of tailless females was measured for length and width to the nearest 0.01 cm, immediately after egg laying, to be able to estimate the volume of the regenerated tail, which has been calculated assuming the tail is conical in shape.

Eggs were incubated individually in covered plastic containers with moistened vermiculite (at a ratio 1:2 of vermiculite to distilled water by weight) at 29 °C, which are very suitable conditions for embryo development in this species, according to Braña & Ji

(2000). Hatchlings were weighed to the nearest 0.0001 g a few hours after hatching and before being fed, and the duration of the incubation period was also registered.

Long-term effects of regeneration

For the study of long-term effects of tail regeneration on reproduction, 40 females and 17 males were captured by noose during August and September 2019, after the end of the annual reproductive period and one or two months prior to winter brumation. According to previous studies, wall lizards have highly developed abdominal fat bodies in this phase of the cycle (Saint Girons & Duguy 1970; Braña 1984). The lizards were initially transported to the Zoology laboratory of the University of Oviedo, where their housing and feeding conditions were the same as those described for the short-term experiment.

Females were divided into a control (hereafter "tailed", N= 21) and an experimental group (hereafter "tailless", N = 19), and tail autotomy was induced in September 2019 to the females belonging to the experimental group and also to all the males (for posterior comparisons between sexes regarding the regeneration rate), following the same procedure as for the short-term experiments, leaving a tail stub of 0.816 ± 0.124 cm (mean ± SD). All the lizards were weighted and measured for SVL, TL and TW, and maintained at the laboratory for 30 days since tail loss was induced to the experimental group. After that (in October 2019) they were housed in outdoor terraria, where they stayed for most of the autumn and winter (around seven months) under natural weather conditions, allowing them to hibernate in shelters inside rock clusters as they do in their surrounding natural habitats. During this period the lizards had water permanently available and, in addition to the free prey that entered the terraria from the natural soils, lizards were provided with supplementary food whenever the presence of active animals was detected, especially on days with milder temperatures.

At the end of April 2020, males and gravid females were brought back to the laboratory and weighted and measured for SVL, TL and TW, and the first clutches of all the females were weighted to the nearest 0.0001 g and counted for the number of laid eggs. Females were also weighed, and the tailless ones were measured for regenerated TL and TW after oviposition. To test if tailless females took more time than tailed ones for preparing their first clutch, the days until the first clutch was laid were registered for each female, considering day 1 as the day when the first female oviposited. The volume of the regenerated portion of the tail was estimated from TL and TW at the time of oviposition. Eggs were incubated following the same protocol described for the short-term experiments (Braña & Ji 2000), hatchlings were weighed to the nearest 0.0001 g a few hours after hatching, and the duration of incubation of each egg was recorded.

Statistical analysis

The data from the two experiments described above were analysed separatedly following essentially the same methodology. The assumptions of normality and homogeneity of variances were tested by Kolmogorov-Smirnov and Levene tests. To test for differences in reproductive investment of tailed and tailless lizards, one-way ANCOVAs were done with tail group as factor and SVL as covariate for both experiments; these tests were done for the following response variables: number of eggs, total clutch mass, mean egg mass and either the time elapsed between the oviposition of the first and the second clutch (for the short-term experiment) or the days until the first clutch was laid (for the long-term experiment). For tailless females, linear regressions were done of the total clutch mass (first clutch in the long-term experiment and second clutch in the short-term experiment) on the volume of regenerated tail, as a potential indicator of the cost that regeneration could impose on reproductive investment. Moreover, linear regressions were done with either the time elapsed between the oviposition of the first and the second clutch (for the short-term experiment) or the days until the first clutch was laid (for the long-term experiment) as dependent variable, and the volume of the regenerated tail as predictor variable. As the amount of regenerated tail may depend on the time spent between the first and second clutches, we also tested the relationship of the time interval between clutches with the residuals of the linear regression of the volume of regenerated tail on the regeneration time. For the short-term experiment, comparisons between the first and second clutches of tailed and tailless lizards for the total clutch mass, the number of eggs laid and the mean mass of the eggs of each female were done by means of repeated measures ANCOVAs with tail group as intersubject factor and SVL as the covariate.

To test the possible differences in the quality of eggs of tailed and tailless females in both the short-term and long-term experiments, we used one-way ANCOVAs, with tail group as factor and egg mass as covariate, to test for differences in hatchlings mass and incubation time. To avoid pseudo-replication, we used the mean values of all eggs or juveniles from each female (i.e., from each clutch) for the analysis of all traits (days of incubation, egg mass and hatchling mass). A Pearson's Chi-Squared test was done to test for possible differences in egg incubation success between tailed and tailless females. For the short-term experiment, the food ingestion rates of tailed and tailless lizards were analysed by means of a repeated measures ANOVA of all ingestion measures taken per female, with tail group as the intersubject factor.

For the long-term experiment, regeneration rates (i.e., per day increases in length and volume of regenerated tail) of males and females in the period from autotomy to the end of the brumation period (hereafter "total regeneration rates") were compared by means of one-way ANCOVAs, with sex as factor and SVL as covariate. A log10 transformation was applied to the total regeneration rates in length to accomplish the assumption of homogeneity of variances. Partial regeneration rates during the two time-intervals, i.e., the autumn period (from autotomy to the transfer to the outdoor terraria for winter brumation) and the brumation period (from the transfer to the outdoor terrariums to the return to the laboratory, before laying the first clutch), were also analysed with one-way ANCOVAs with sex as factor and SVL as covariate.

Results

Short-term effects

All the variables accomplished the assumptions of normality and homogeneity of variances (Kolmogorov-Smirnov and Levene tests, $p > 0.05$ in all cases). Tailless females laid clutches significantly lighter (in terms of total mass) than tailed ones (Table 6.1), and this difference tended to increase with females' SVL (Figure 6.1A; one-way ANCOVA with SVL as covariate: $F_{1,59} = 4.078$, $p = 0.048$ for the main effect; $F_{1,59} = 9.432$, $p = 0.003$ for the effect of the covariate). Overall, tailless females showed a reduction of 12.94% in their clutch mass compared to tailed ones. No significant differences were found between tail groups in the clutch size, time interval between the oviposition of the first and the second clutch or mean mass of the eggs (one-way ANCOVAs with SVL as covariate, $p > 0.05$ in all cases), although in all these aspects tailed females tended to perform slightly better than tailless ones, and the differences were marginally not significant for the number of eggs ($F_{1,57} = 3.177$, $p = 0.080$). For tailless females, the amount of regenerated tail in the time between clutches (i.e., volume of tail regenerated concurrently with the development of the second clutch) was negatively correlated with total mass of the second clutch (Figure 6.2A; $R^2 = 0.167$, $p = 0.023$) and positively correlated with the time elapsed between the oviposition of the first and second clutches ($R^2 = 0.267$, $p = 0.017$), as expected, because regeneration time was very close to the time between clutches. The residuals from the linear regression of the volume of regenerated tail on regeneration time ($R^2 = 0.335$, $p = 0.006$) were no longer significantly correlated with the time elapsed between the first and the second clutches ($R^2 < 0.001$, $p = 0.985$).

The mean mass of eggs was quite similar in the first and second clutches (repeated measures ANCOVA with tail group as intersubject factor: $F_{1,29} = 0.045$, $p = 0.833$), but the number of laid eggs and the total clutch mass of the second clutch were lower than those of the first one, both for females with tail and without tail (repeated measures ANCOVAs with tail group as intersubject factor, $p < 0.005$ in both cases; Figure 6.3). The decrease in clutch mass from the first to the second clutch was significantly greater in tailless females than in tailed ones (Figure 6.3; interaction between clutch order and tail group: $F_{1,29} = 6.650$, $p = 0.015$), but no differences were found between tail groups in the mean egg mass or the number of laid eggs ($p > 0.19$ in both cases). No differences were found in the ingestion rate between tailed and tailless females (repeated measures ANOVA with tail group as intersubject factor: $F_{1,22} = 0.603$, $p = 0.665$).

There were no differences between the mass of hatchlings born from tailed and tailless females neither in absolute values (Table 6.1; one-way ANOVA: $F_{1,22} = 0.636$, $p = 0.434$) or in relation to the egg mass (one-way ANCOVA with egg mass as covariate: $F_{1,21} = 0.119$, $p = 0.733$), nor between the incubation time of eggs from tailed and tailless females (Table 6.1; one-way ANOVA: $F_{1,22} = 3.515$, $p = 0.074$). The rate of incubation failure, either due to failure in fertilization or early embryonic mortality, was significantly higher for eggs laid by tailless females (74 failed eggs out of 125 eggs laid by tailed females, against 79 out of 108 for those laid by tailless females: $X^2_1 = 4.990$, $p = 0.025$).

Long-term effects

All the variables accomplished the assumptions of normality and homogeneity of variances (Kolmogorov-Smirnov and Levene tests, $p > 0.05$ in all cases). Total mass of the first clutch was significantly higher for tailed females than for tailless ones (Table 6.1), and this difference increased with females' SVL (Figure 6.1B; one-way ANCOVA with SVL as covariate: $F_{1,37} = 10.104$, $p = 0.003$ for the main effect; $F_{1,37} = 66.196$, $p < 0.001$ for the effect of the covariate). On average, tailless females laid clutches 6.57 % lighter than tailed ones. However, there were no differences between tailed and tailless females neither in the number and mean mass of the eggs (although tailless females tended to perform worse than tailed ones), nor in the time in which they laid the first clutch since the first female did (Table 6.1; one-way ANCOVAs with SVL as covariate, $p > 0.05$ in all cases). The amount of tail regeneration of tailless females was not correlated neither with total mass of the first clutch (Figure 6.2B; $R^2 = 0.173$, $p = 0.086$), nor with the days until the first clutch was laid (i.e., considering day 1 as the day on which the first female laid the first clutch; $R^2 = 0.002$, $p = 0.871$).

No differences were found either between the mass of hatchlings born from tailed and tailless females in absolute values (Table 6.1; one-way ANCOVA with egg mass as covariate: $F_{1,24} < 0.001$, $p = 0.999$), in relation to their egg mass (one-way ANCOVA with egg mass as covariate: $F_{1,23} = 0.005$, $p = 0.945$) or between the incubation time of eggs from tailed and tailless females (Table 6.1; one-way ANOVA: $F_{1,24} = 1.083$, $p = 0.308$).

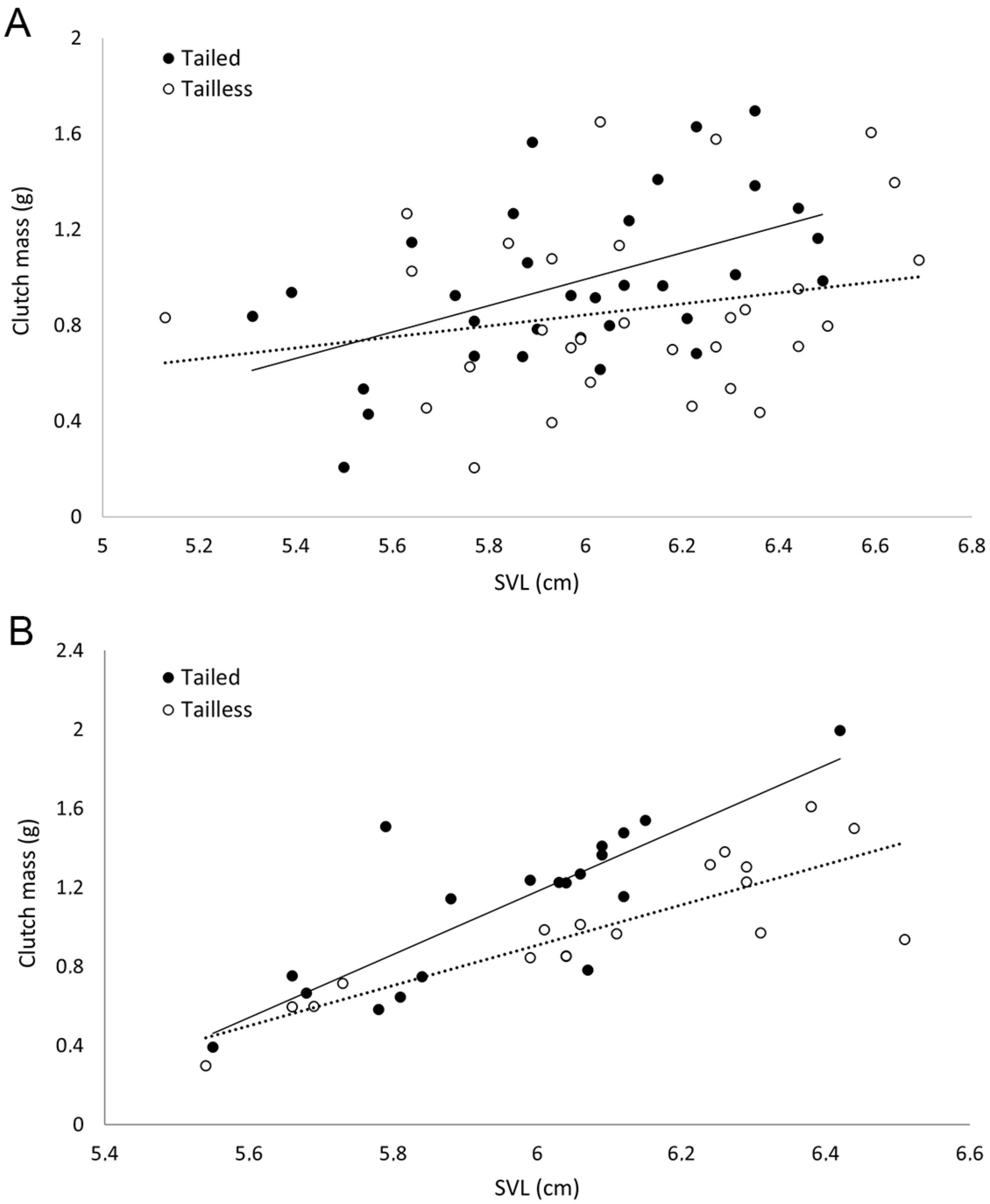


Figure 6.1. Clutch mass of tailed (black) and tailless (white) females in relation to their size (SVL: snout-vent length). **A**, Short-term experiment: caudal autotomy was induced a few days before/after laying the first clutch in the spring, and regeneration occurred simultaneously with the development of the vitellogenesis for the second clutch, about a month later. **B**, Long-term experiment: tail autotomy was induced to females at the end of the breeding season, and regeneration occurred from September until the time when the females laid their first clutch after winter brumation, about eight months later.

Incubation failure (i.e., eggs incubated that did not hatch) was not different for eggs laid by tailless or tailed females (34 failed eggs out of 76 eggs from tailed females, against 36 failed eggs out of 66 from tailless ones: X^2_1 = 1,359, p = 0.244).

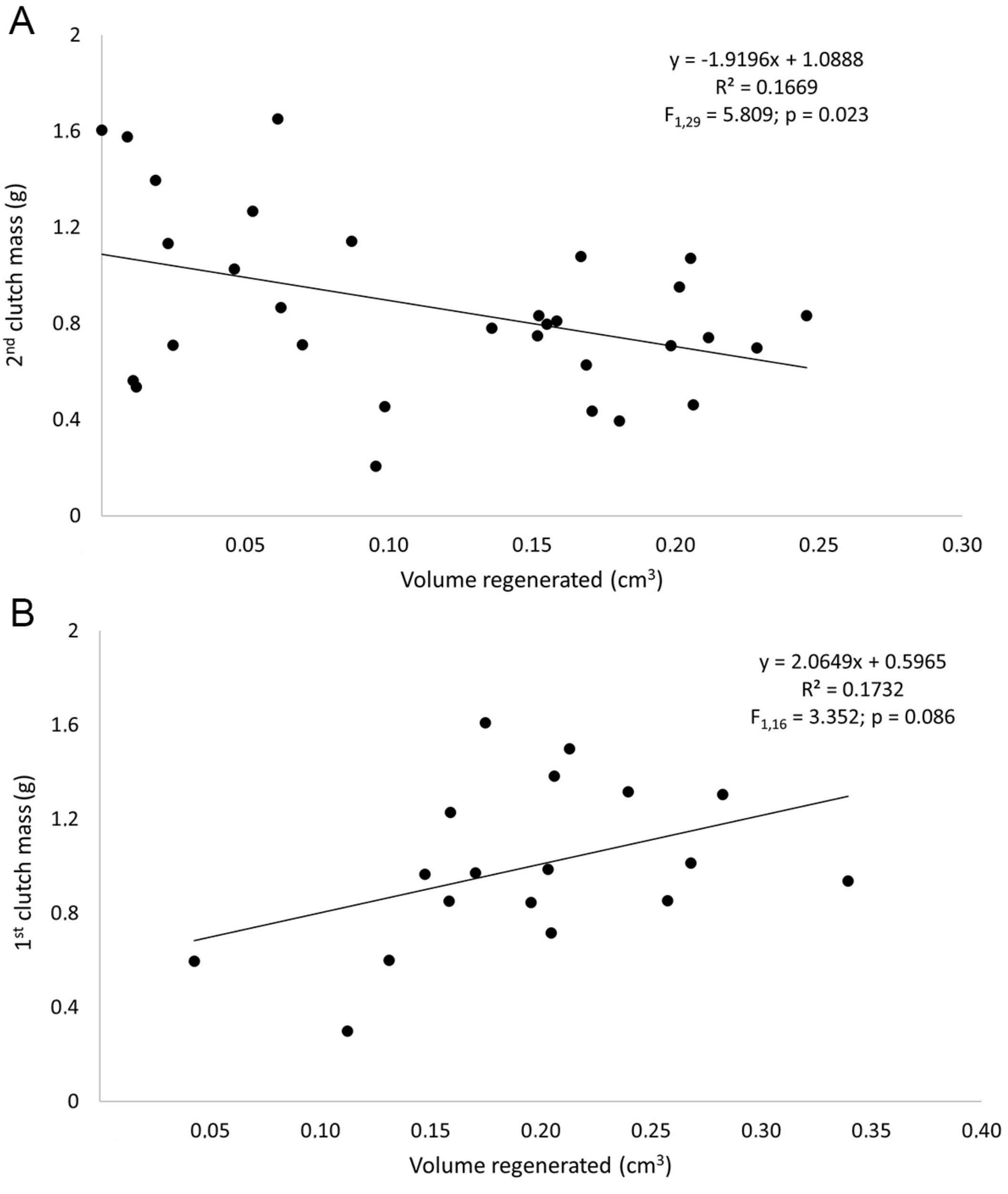


Figure 6.2. Volume of regenerated tail against total clutch mass of tailless females. **A**, Short-term experiment, in which caudal autotomy was induced a few days before/after laying the first clutch, and regeneration occurred simultaneously to the development of the second clutch. **B**, Long-term experiment, in which tail autotomy was induced to females at the end of the breeding season, and regeneration occurred from September to the end of the experiment, when females laid their first clutch in the spring, after a period of winter brumation.

Table 6.1. Descriptive statistics (means ± SD, the sample size is indicated in brackets) from the main reproductive variables (direct values, not corrected values with SVL) measured on the short- and long-term experiments for both tailed and tailless females. Variables of the short-term experiment correspond to the second clutch, and those of the long-term belong to the first clutch. The variable "time clutches" represents the time spent between the first and second clutch in the short-term experiment, and the time until the first clutch was laid (considering day 1 as the day when the first female laid eggs) in the long-term experiment.

	Short-term		**Long-term**	
	Tailed	**Tailless**	**Tailed**	**Tailless**
Clutch mass (g)	0.979 ± 0.346 (31)	0.854 ± 0.359 (31)	1.076 ± 0.411 (21)	1.005 ± 0.333 (19)
Clutch size (nº eggs)	4.033 ± 1.245 (30)	3.583 ± 1.175 (30)	3.619 ± 1.359 (21)	3.474 ± 0.964 (19)
Mean egg mass (g)	0.254 ± 0.051 (30)	0.248 ± 0.113 (30)	0.306 ± 0.070 (21)	0.289 ± 0.048 (19)
Time clutches (days)	32.629 ± 10.570 (12)	35.714 ± 5.875 (20)	10.429 ± 7.691 (21)	10.842 ± 7.741 (19)
Hatchlings' mass (g)	0.315 ± 0.049 (15)	0.299 ± 0.034 (9)	0.343 ± 0.039 (15)	0.343 ± 0.021 (11)
Egg incubation time (days)	33.067 ± 1.033 (15)	32.333 ± 0.707 (9)	33.533 ± 0.915 (15)	33.181 ± 0.750 (11)
Snout-vent-length (cm)	5.975 ± 0.321 (31)	6.093 ± 0.344 (31)	5.941 ± 0.223 (21)	6.081 ± 0.277 (19)

Regarding tail regeneration rates (both in length and volume) during the autumn (i.e., from autotomy to the transfer to the outdoor terraria for winter brumation) and brumation (i.e., from the transfer to the outdoor terrariums to the return to the laboratory, before laying the first clutch) periods, no differences were found between males and females (one-way ANCOVAs with sex as factor and SVL as covariate, $p > 0.05$ in all cases). However, total regeneration rates (i.e., from autotomy to the end of the brumation period) were higher for males than for females, both for tail length (one-way ANCOVA with sex as factor and SVL as covariate; $F_{1,34} = 9.212$, $p = 0.002$) and volume ($F_{1,34} = 7.747$, $p = 0.009$).

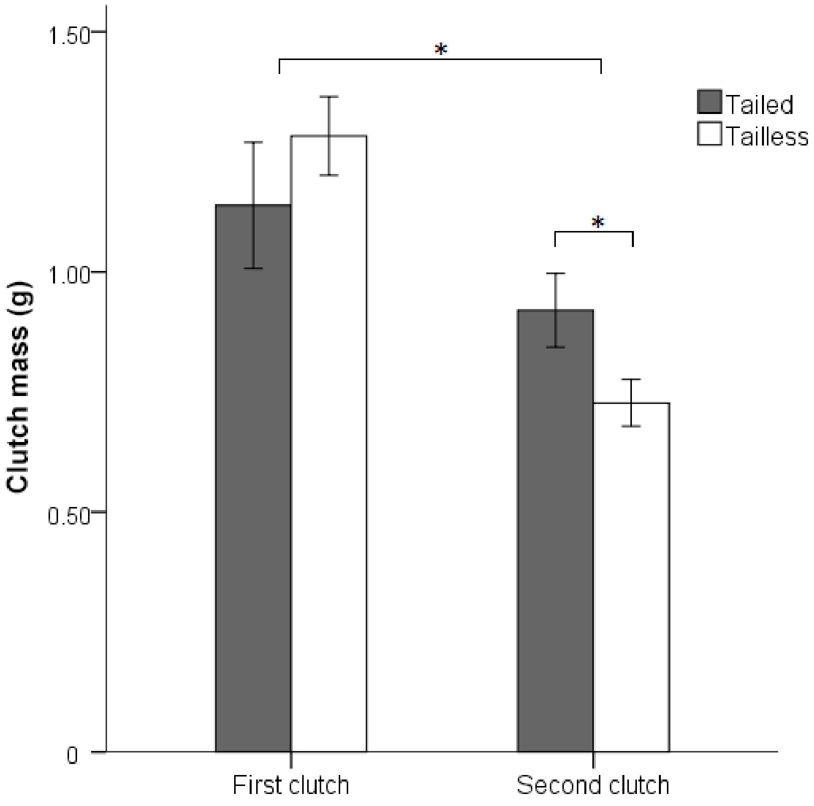


Figure 6.3. Total mass of the first and second clutches of tailed (grey) and tailless (white) females. Tailless (i.e., regenerating) females were induced caudal autotomy a few days before/after laying the first clutch, and regeneration occurred simultaneously to the development of the second clutch (short-term experiment). Values of clutch mass are means ± SE; only data from females that laid both the first and second clutch in the laboratory are included; * $p < 0.05$.

Discussion

Caudal autotomy is a useful antipredator strategy that has obvious benefits for immediate survival but also some negative effects, and it is known that tail regeneration largely restores the functions lost after autotomy (Lin *et al.* 2017; Zamora-Camacho *et al.* 2016; Fernández-Rodríguez & Braña 2020). However, regeneration implies significant costs (Naya *et al.* 2007), so it can interfere with other processes posing high energy demands, such as reproduction or growth (Chapple *et al.* 2002; Chapter 4; but see Ballinger & Tinkle 1979; Goodman 2006, for no effects on somatic growth). Our results evidenced a negative effect of regeneration on females' reproductive investment in both the short and long-term scales: regenerating females laid clutches that were 12.94% and 6.57% lighter than those laid by tailed females in the short- and long-term experiments, respectively. These findings are consistent with most previous studies done with lizards and salamanders, which frequently reported reductions of clutch mass ranging from 6 to 75 % (Smyth 1974; Dial & Fitzpatrick 1981; Taylor 1984; Wilson & Booth 1998; Chapple *et al.* 2002; Bernardo & Agosta 2005), or even a complete inhibition of the current reproduction in some salamander and lizard species (Maiorana 1977; Taylor 1984). On the contrary, only a few studies have found no effects of tail loss on reproductive investment of female lizards (Taylor 1984); or even partially positive results in some components of the reproductive investment (Fox & McCoy 2000; Beatty *et al.* 2021). However, the valuable field experiment by Fox and McCoy (2000) reported that tailless females of *Uta stansburiana* produced heavier offspring than tailed ones, but also that tailless females had significantly reduced winter survival, thus we can assume that the higher quality individuals survived and reproduced, which could partly explain the higher quality (size) of their offspring. Other recent study found an increase in females' reproductive investment during tail regeneration (Beatty *et al.* 2021), which the authors explained by a possible increase in the energetic efficiency during regeneration.

Among the forementioned studies, Bernardo & Agosta´s (2005) evaluated the effect of tail regeneration on reproductive investment and showed a reduction in the clutch size of regenerating females. However, in contrast to our results, they failed to find any significant relationship between the amount of tail regenerated and the degree of clutch reduction. However, their analysis was based on an estimation of the amount of regenerated tail relative to the expected tail length, using animals with intact tails for the prediction of tail length for regenerating individuals of a given size, which could have biased their results and led to misleading conclusions. In many lizard species the completely regenerated tail may result shorter than the intact one (Zamora-Camacho *et al.* 2016; authors' personal observations), so using that estimated proportion as a measure of the investment in regeneration may not be completely accurate. Most of the other studies cited above do not refer to the costs of caudal regeneration itself, but to the consequences of the loss of fat reserves stored in the tail (Smyth 1974; Maiorana 1977; Dial & Fitzpatrick 1981; Doughty *et al.* 2003). The magnitude of the impact of autotomy on reproductive investment seems to be greater in those species that have reduced abdominal fat bodies or lack them, as these species frequently depend on caudal lipid stores for reproduction (Bernardo & Agosta 2005). Our results conform to what would be expected under this hypothesis, as *Podarcis muralis* have well developed abdominal fat bodies (Saint Girons & Duguy 1970; Braña 1983; Braña *et al.* 1991) and, even though we found some reduction in the females' reproductive investment associated with regeneration, it was less severe than that found in species lacking prominent abdominal lipid stores (Bernardo & Agosta 2005).

There was a significant reduction in the total mass of clutches laid by tailless females, and tailed females tended to lay slightly larger clutches and larger eggs than tailless (i.e., regenerating) ones, although these differences did not reach the conventional level of statistical significance. The absence of clear differences in clutch size and egg size might be explained by individual variability in the stage in the vitellogenesis cycle at the beginning of regeneration: those females that started regeneration after clutch size was set might not be able to readjust the number of eggs, but only the average mass of the eggs. On the contrary, those females that started regeneration in an early stage of vitellogenesis would have been able to adjust clutch size, as suggested by Sinervo & Licht (1991) and Bernardo & Agosta (2005). Dial & Fitzpatrick (1981) found that the eggs from tailless females of *Coleonyx brevis* were lower in energy content, both in absolute values and relative to egg mass. In contrast, we did not find differences in the conversion efficiency (i.e., the hatchling´s mass relative to initial egg mass) between hatchlings from tailed and tailless females, neither in the short-term nor in the long-term experiment. Moreover, our results indicate that, although the energetic demands of regeneration seem to constrain reproductive investment in *P. muralis*, they do not affect the incubation time. This probably indicates that there were no differences between intact and regenerating females in the retention time and the level of development reached by the embryos at the time of egg laying, although this trait may have a certain plasticity in lacertid lizards (Braña *et al.* 1991; Rodríguez-Díaz & Braña 2011). Previous studies showed that in female`s wall lizards oviposition occurs when embryos reached stages 25–29, according to the Dufaure & Hubert´s (1961) development table (Braña *et al.* 1991), and that the incubation time at 29ºC ranges from 33 to 35 days and is not affected by egg mass (Ji & Braña 1999), which is in accordance with our results. Regarding the success of incubations, failure rates were significantly higher in eggs from tailless females than in those from tailed ones in the short-term experiment (although we did not find this pattern in the long-term experiment). For the eggs that did not hatch, we were not able to discern whether the cause was fertilization failure or early embryonic mortality. However, previous studies proved that tailless females were less courted and had lower copulation rates than tailed ones, thus facing a diminished mating success (for *Iberolacerta monticola*, see Martín & Salvador 1993). This would support the possibility that tailless females may have had lower fertilization rates in our short-term experiment, focused on the second clutch that takes place in a very short time after the first one (Ji & Braña 2000; present study) and could have therefore fewer opportunities for copulation.

Many reptiles use fat reserves (caudal lipid stores, abdominal fat bodies, or both) to support metabolic expenditure and water acquisition during winter brumation (Avery 1970; Derickson 1976), but in most lizard species the predominant function of these stores seems to be related to reproduction (Derickson 1976; Vitt & Cooper 1985; see Braña *et al.* 1992 for lacertid lizards). For instance, fat reserves allow males to emerge and maintain activity in early spring, when food resources may be still limited, and it is not possible to perform an optimal thermoregulation to complete the spermatogenesis before females become active (Saint Girons & Duguy 1970; Braña 1983). We did observe this pattern in our outdoor terraria during sunny days of March and April, when almost only males showed any activity, and this longer period of activity could be the main explanation for the higher rates of tail regeneration in males as compared to females; the same pattern has been repeatedly reported for other lizards (e.g., Congdon *et al.* 1974; Dial & Fitzpatrick 1981; Fox & McCoy 2000).

Females of many lizard species base the development of their first clutch of the season on abdominal and/or caudal fat reserves (Hahn & Tinkle 1965; Derickson 1976). Previous studies on the same population of wall lizards studied here (Braña *et al.* 1992) showed that fat bodies are blooming in the fall and are hardly reduced during wintering, but they suffer a rapid decline to exhaustion during the vitellogenesis for the first clutch, so the energy allocated to the second and third clutches is obtained from concomitant food intake. Thus, female wall lizards switch from a capital (first clutch) to an income breeding strategy (second and third clutches), as was also reported for other reptiles and invertebrate living in markedly seasonal environments (Bonnet *et al.* 1998; Varpe *et al.* 2009; Sainmont *et al.* 2014). Our short-term experiment showed that, regardless of the tail condition of females (i.e., tailed/tailless), second clutches were lighter than first ones, according to the results of previous studies on the same population (Ji & Braña 2000) and other lizards (e.g., *Sceloropus formosus*, Guillette & Sullivan 1985). However, it is worth noting that the decrease in mass from the first to the second clutch was significantly greater for tailless females, which were regenerating their tails simultaneously with the development of vitellogenesis, revealing a constraint of the energy available to be allocated to reproduction. Contrary to the results reported by Dial & Fitzpatrick (1981) for *Coleonyx brevis*, we did not find evidence that the investment in regeneration has led to an increase in food intake by tailless lizards to take over the simultaneous requirements of reproduction.

Our experiments posed two scenarios that represent two alternative tactics of provision of resources for reproduction: firstly, the long-term experiment focused on reproductive investment based on a capital breeding strategy, as the first clutch is done with fat reserves accumulated prior to winter brumation (Braña *et al.* 1992); secondly, the short-term experiment examined the investment in the second clutch, developed under an income breeding strategy (Braña *et al.* 1992). It is worth noting that the reduction in reproductive investment due to tail regeneration was much stronger in the short-term experiment (reduction of 12.94 %, against 6.57 % in the long-term one). In such regard, it seems that the negative effect of the energy demands of regeneration on reproductive investment may be buffered under a capital breeding strategy. It is important to remember that, in this situation, the energy demands of regeneration and reproduction are partially unsynchronized, as tail regrowth started and progressed several months before the onset of the reproductive season. On the contrary, in the scenario of an income breeding strategy posed in the short-term experiment, regeneration occurred concurrently with clutch development, so both processes probably suffer from a greater competition for the incoming resources, which would be conditioned by the current resource availability. Contrary to this argument, Dial & Fitzpatrick (1981) suggested that, because females develop their second and third clutches as income breeders and would not suffer from the loss of caudal fat reserves after autotomy, the reproductive investment on these clutches might not differ significantly between tailed and tailless females. Still, Dial & Fitzpatrick did not consider the competing demands of regeneration on reproduction, but only the role of the reserves accumulated in the tail.

Further evidence on the trade-off between regeneration and reproduction comes from the negative relationship we found between the regenerative tail growth and the reproductive investment in the group of tailless (regenerating) females that underwent autotomy before developing their second clutch, for which regeneration occurred concurrently with vitellogenesis. On the contrary, females that lost their tails and initiated regeneration in autumn, approximately eight months before laying their first clutch, did not shown such negative relationship, although they experienced a reduction in clutch mass compared to females with intact tail. Negative correlations between two traits may evidence a trade-off due to the dominance of resources allocation over acquisition, but an absence of a negative correlation does not necessarily imply that there is not a competition for the resources (van Noordwicjk & de Jong 1986; Reznick *et al.* 2000). Our results also support the idea that the negative effects on reproduction were stronger when regeneration occurred concurrently with clutch development under an income breeding strategy than when regeneration occurred months before reproduction, during the phase of accumulation of reserves.

Criteria for energy allocation between somatic (here including regeneration) and reproductive recipients are expected to vary depending on the age-specific reproductive value of an organism (Harshman & Zera 2007; Tracer 2002), which implies the consideration of the effects of reproduction on survival and the trade-off between current and future reproductive investment (Williams 1966; Pianka 1976). Thus, animals may adjust the amount of energy allocated to each trait in a way that maximizes its life-time fitness, i.e., the total number of its offspring that survive to sexual maturity (Dial & Fitzpatrick 1981). In this context, the hierarchy in the allocation of resources in the conflict between regeneration and reproduction, is important for the understanding of the evolution of autotomy and regeneration. Previous studies raised the idea that reproduction would have priority on energy allocation over tail regeneration in short-lived species with low probability of future reproduction, while regeneration would have priority in long-lived species with high probability of future reproductive seasons (Maiorana 1977; Vitt *et al.* 1977; Dial & Fitzpatrick 1981). Female wall lizards do not interrupt or postpone regeneration during the phases of high reproductive investment and, from the negative relationship we found between the investment in regeneration and reproduction, we deduce that investment in regeneration is prioritized over reproduction.

To conclude, *P. muralis* is an iteroparous multivoltine species that usually lays 2–3 clutches per season, usually reaches sexual maturity at the age of 2-3 and lives 6-13 years (depending on the population, see Castanet & Roche 1981; Barbault & Mou 1988; Eroglu *et al.* 2018), so this species may have 4-10 reproductive seasons. In such regard and considering the benefits of regeneration for future survival, a reduction of the current clutch quality in favour of regenerating the tail may not imply a great decrease in the relative fitness of tailless females. Besides, the overall negative effects of the energy costs of regeneration were stronger under an income breeding strategy, which suggest that capital breeding may be more resilient to variations in the availability of resources for clutch development.

References

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Avery, R.A. (1970). Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. *Comparative Biochemistry and Physiology*, 37: 119–121.

Ballinger, R.E. & Tinkle, D.W. (1979). On the cost of tail regeneration to body growth in lizards. *Journal of Herpetology*, 13: 375–375.

Barbault, R. & Mou, Y.-P. (1988). Population dynamics of the common wall lizard, *Podarcis muralis*, in southwestern France. *Herpetologica*, 44: 38–47.

Barrios, J.V.; Gaymer, C.F.; Vásquez, J.A. & Brokordt, K.B. (2008). Effect of the degree of autotomy on feeding, growth and reproductive capacity in the multi-armed sea star *Heliaster helianthus. Journal of Experimental Marine Biology and Ecology,* 361: 21–27.

Bateman, P.W. & Fleming, A. (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.

Beatty, A.E.; Mote, D.M. & Schwartz, T.S. (2021). Tail of reproduction: Regeneration leads to increased reproductive investment. *Journal of Experimental Zoology Part A Comparative Experimental Biology*, 335(5): 522–528.

Bellairs, A. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bely, A.E. & Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170.

Bernardo, J. & Agosta, S.J. (2005). Evolutionary implications of hierarchical impacts of nonlethal injury on reproduction, including maternal effects. *Biological Journal of the Linnean Society*, 86: 309–331.

Boggs, C.L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, 23(1): 27–37.

Bonnet, X.; Bradshaw, D. & Shine, R. (1998). Capital versus income breeding: an ectothermic perspective. *Oikos*, 83(2): 333–342.

Bonnet, X.; Naulleau, G.; Shine, R. & Lourdais, O. (1999). What is the appropriate timescale for measuring costs of reproduction in a ‘capital breeder’ such as the aspic viper? *Evolutionary Ecology*, 13: 485–497.

Braña, F. & Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *Journal of Experimental Zoology,* 286: 422–433.

Braña, F. (1983). La reproducción en los Saurios de Asturias (Reptilia: Squamata): ciclos gonadales, fecundidad y modalidades reproductoras. *Revista de Biología de la Universidad de Oviedo*, 1: 29–50.

Braña, F. (1984). *Biogeografía, biología y estructura de nichos de la taxocenosis de saurios de Asturias.* PhD Thesis, University of Oviedo, Spain.

Braña, F.; Bea, A. & Arrayago, M.J. (1991). Egg retention in lacertid lizards: relationships with reproductive ecology and the evolution of viviparity. *Herpetologica*, 47(2): 218–226.

Braña, F.; González, F. & Barahona, A. (1992). Relationship between ovarian and fat body weights during vitellogenesis for three species of lacertid lizards. *Journal of Herpetology*, 26: 515–518.

Castanet, J. & Roche, E. (1981). Détermination de l'âge chez le lézard des murailles, *Lacerta muralis* (Laurenti, 1768) au moyen de la squelettochronologie. *Revue Suisse de Zoologie*, 88: 215–226.

Chapple, D.G.; McCoull, C.J. & Swain, R. (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. & Capaldi, E.A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology*, 305A: 965–973.

Congdon, J.D.; Vitt, L.J. & King, W.W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science*, 184: 1379–1380.

Derickson, W.K. (1976). Lipid storage and utilization in Reptiles. *American Zoologist*, 16(4): 711–723.

Dial, B.E. & Fitzpatrick, L.C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia*, 51: 310–317.

Díaz-Guisado, D.; Gaymer, C.F.; Brokordt, K.B. & Lawrence, J.M. (2006). Autotomy reduces feeding, energy storage and growth of the sea star *Stichaster striatus*. *Journal of Experimental Marine Biology and Ecology*, 338: 73–80.

Doughty, P.; Shine, R. & Lee, M.S.Y. (2003). Energetic costs of tail loss in a montane scincid lizard. *Comparative Biochemistry and Physiology, Part A*, 135: 215–219.

Dufaure, J.P. & Hubert, J. (1961). Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* jacquin. *Archives d'anatomie microscopique et de morphologie expérimentale*, 50: 309–328.

Elliott, J.M. (1994). *Quantitative Ecology and the Brown Trout*. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Eroglu, A.I.; Bülbül, U.; Kurnaz, M. & Odaba, Y. (2018). Age and growth of the common wall lizard, *Podarcis muralis* (Laurenti, 1768). *Animal Biology*, 68: 147–159.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15(6): 511–521.

Fox, S.F. & McCoy, K.J. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia,* 122: 327–334.

Fox, S.F. & Rostker, M.A. (1982). Social costs of tail loss in *Uta stansburiana*. *Science*, 218: 692–693.

Fox, S.F. (1978). Natural selection on behavioral phenotypes of the lizard *Uta stansburiana*. *Ecology*, 59: 834–847.

Gillis, G.B.; Kuo, C.-Y. & Irschick, D.J. (2013). The impact of tail loss on stability during jumping in green anoles (*Anolis carolinensis*). *Physiological and Biochemical Zoology*, 86: 680–689.

Goodman, R.M. (2006). Effects of tail loss on growth and sprint speed of juvenile *Eumeces fasciatus* (Scincidae). *Journal of Herpetology*, 40: 99–102.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Guillette, L.J. Jr. & Sullivan, W.P. (1985). The reproductive and fat body cycles of the lizard, *Sceloporus formosus. Journal of Herpetology*, 19(4): 474–480.

Hahn, W.E. & Tinkle, D.W. (1965). Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard *Uta stansburiana*. *Journal of Experimental Zoology*, 158: 79–86.

Harshman, L.G. & Zera, A.J. (2007). The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution*, 22: 80–86.

Hoso, M. (2012). Cost of autotomy drives ontogenetic switching of anti-predator mechanisms under developmental constraints in a land snail. *Proceedings of the Royal Society B*, 279(1748): 4811–4816.

Iraeta, P.; Salvador, A. & Díaz, J.A. (2012). Effects of caudal autotomy on postnatal growth rates of hatchling *Psammodromus algirus. Journal of Herpetology*, 46(3): 342–345.

Ji, X. & Braña, F. (1999). The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). *Comparative Biochemistry and Physiology, Part A*, 124: 205–213.

Ji, X. & Braña, F. (2000). Among clutch variation in reproductive output and egg size in the wall lizard (*Podarcis muralis*) from a lowland population of Northern Spain. *Journal of Herpetology*, 34(1): 54–60.

Jönsson, K.I. (1997). Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, 78: 57–66.

Kuo, C.; Yao, C. & Lin, T. (2013). Tail loss compromises immunity in the many-lined skink, *Eutropis multifasciata*. *Naturwissenschaften*, 100: 379–384.

Lawrence, J.M. (2010). Energetic costs of loss and regeneration of arms in stellate Echinoderms. *Integrative and Comparative Biology*, 50(4): 506–514.

Lin, J.W.; Chen, Y.R.; Wang, Y.H.; Hung, K.C. & Lin, S.M. (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(1847): 20162538.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Maiorana, V.C. (1977). Tail autotomy, functional conflicts and their resolution by a salamander. *Nature*, 265: 533–535.

Martín, J. & Salvador, A. (1993). Tail loss reduces mating success in the Iberian rock-lizard, *Lacerta monticola*. *Behavioral Ecology and Sociobiology*, 32: 185–189.

McConnachie, S. & Whiting, M.J. (2003). Costs associated with tail autotomy in an ambush foraging lizard, *Cordylus melanotus melanotus*. *African Zoology*, 38(1): 57–65.

Medger, K.; Verburgt, L. & Bateman, P.W. (2008). The influence of tail autotomy on the escape response of the Cape Dwarf Gecko, *Lygodactylus capensis*. *Ethology*, 114: 42–52.

Messina, F.J. & Fox, C.W. (2001). Offspring size and number. In: Fox, C.W.; Roff, D.A. & Fairbairn, D.J. (Eds.), *Evolutionary Ecology: Concepts and Case Studies*. Oxford University Press, Oxford, pp. 113–127.

Morgan, R. & Jangoux, M. (2004). Assessing arm regeneration and its effect during the reproductive cycle in the gregarious brittle-star *Ophiothrix fragilis*. *Cahiers de Biologie Marine*, 45: 277–280.

Naya, D.E.; Veloso, C.; Muñoz, J.L.P. & Bozinovic, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 146: 189–193.

Pafilis, P.; Foufopoluos, J.; Poulakakis, N.; Lymberakis, P. & Valakos, E.D. (2009). Tail shedding in island lizards (Lacertilia, Reptilia): Decline of antipredator defenses in relaxed predation environments. *Evolution*, 63(5): 1262–1278.

Peters, R.A.; Hemmi, J.M. & Zeil, J. (2007). Signaling against the wind: modifying motion- signal structure in response to increased noise. *Current Biology*, 17: 1231–1234.

Pianka, E.R. (1976). Natural selection of optimal reproductive tactics. *American Zoologist*, 16: 775–784.

Pomory, C.M. & Lawrence, J.M. (1999). Effect of arm regeneration on energy storage and gonad production in *Ophiocoma echinata* (Echinodermata: Ophiuroidea). *Marine Biology*, 135: 57–63.

Reznick, D. (1985). Costs of reproduction: an evaluation of the empirical evidence. *Oikos*, 44: 257–267.

Reznick, D.N.; Nunney, L. & Tessier, A. (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution*, 15: 421–425.

Rodríguez-Díaz, T. & Braña, F. (2011). Plasticity and limitations of extended egg retention in oviparous *Zootoca vivipara* (Reptilia: Lacertidae). *Biological Journal of the Linnean Society*, 102(1): 75–82.

Roff, D.A. (1992). *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York.

Roff, D.A. (2002). *Life History Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Sainmont, J.; Andersen, K.H.; Varpe, Ø. & Visser, A.W. (2014). Capital versus income breeding in a seasonal environment. *The American Naturalist*, 184(4): 466–476.

Saint Girons, H. & Duguy, R. (1970). Le cycle sexuel de *Lacerta muralis* L. en plaine et en montagne. *Bulletin du Muséum national d'histoire naturelle*, 42: 609–625.

Salvador, A.; Martín, J. & López, P. (1995). Tail loss reduces home range size and access to females in male lizards, *Psammodromus algirus*. *Behavioural Ecology*, 6: 382–387.

Schwarzkopf, L. (1994). Measuring trade-offs: a review of studies of costs of reproduction in lizards. In: Vitt, L.J. & Pianka, E.R. (Eds.), *Lizard ecology: historical and experimental perspectives*. Princeton University Press, Princeton, N.J., pp. 7–29.

Sinervo, B. & Licht, P. (1991). Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *Journal of Experimental Zoology*, 257: 252–264.

Smyth, M. (1974). Changes in the fat stores of the skinks *Morethia boulengeri* and *Hemiergis peronii* (Lacertilia). *Australian Journal of Zoology*, 22: 135–145.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford University Press, London.

Stephens, P.A.; Boyd, I.L.; McNamara, J.M. & Houston, A.I. (2009). Capital breeding and income breeding: their meaning, measurement, and worth. *Ecology*, 90: 2057–2067.

Taylor, J.A. (1984). Ecology of the lizard, *Ctenotus taeniolatus*; interaction of life history, energy storage and tail autotomy. Ph.D. Thesis, University of New England, Armidale, Australia.

Tracer D.P. (2002). Somatic versus reproductive energy allocation in Papua New Guinea: life history theory and public health policy. *American Journal of Human Biology*, 14: 621–626.

van der Meer, J. (2019). Metabolic theories in ecology: the dynamic energy budget theory and the metabolic theory of ecology. *Encyclopedia of Ecology (Second Edition)*, 3: 463–471.

van Noordwicjk, A.J. & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, 128(1): 137–142.

Varpe, Ø.; Jørgensen, C.; Tarling, G. & Fiksen, Ø. (2009). The adaptive value of energy storage and capital breeding in seasonal environments. *Oikos*, 118: 363–370.

Vitt, L.J. & Cooper, W.E. (1985). The relationship between reproduction and lipid cycling in the skink *Eumeces laticeps* with comments on brooding ecology. *Herpetologica*, 41: 419–432.

Vitt, L.J.; Congdon, J.D. & Dickson, N.A. (1977). Adaptive strategies and energetics of tail autotomy in lizards. *Ecology,* 58: 326–337.

Wang, Y.; Zeng, Z.-G.; Ma, L.; Li, S.-R. & Du, W.-G. (2017). Food restriction affects maternal investment but not neonate phenotypes in a viviparous lizard. *Zoological Research*, 38(2): 81–87.

Weiss, S.L. (2002). Reproductive signals of female lizards: pattern of trait expression and male response. *Ethology*, 108: 793–813.

Williams, G.C. (1966) Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist*, 100: 687–692.

Wilson, R.S. & Booth, D.T. (1998). Effect of tail loss on reproductive output and its ecological significance in the skink *Eulamprus quoyii*. *Journal of Herpetology*, 32: 128–131.

Zamora-Camacho, F.J.; Rubiño-Hispán, M.V.; Reguera, S. & Moreno-Rueda, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid *Psammodromus algirus*. *The Herpetological Journal*, 26: 213–218.

“In the course of evolution, the pros and cons have been weighed by each group of animals. Some have found regeneration to be indispensable (…), others have opted to get along without it and have derived the benefits of investing their energies in other directions.” Goss, 1969 (*Principles of Regeneration*).

“En el curso de la evolución, cada grupo de animales ha sopesado los pros y los contras. Algunos consideraron la regeneración indispensable (...), otros optaron por prescindir de ella y obtuvieron beneficios de invertir sus energías en otras direcciones”.

Cover photo: Eye of *Podarcis muralis*

VII. GENERAL DISCUSSION

General Discussion

Autotomy, which consists of the loss of a non-vital part of the body, has evolved several times in different animal lineages (Bely & Nyberg 2010), as it provides some advantages, being the most prevalent the avoidance of predators (Maginnis 2006; for lizards, see Clause & Capaldi 2006). The shed body part is not vital but can have an important role in the performance of relevant ecological tasks, so in many linages capable of autotomizing body parts, the evolution of regeneration enabled the restoration of the lost capacities (Goss 1969). Organisms always manage a limited amount of resources that must be allocated to different traits and functions in a way that optimizes their fitness. In this resource-limited scenario, life-history theory predicts the existence of trade-offs between competing bodily functions. The allocation to each trait will depend both on the available resources and on the allocation strategies between the different traits that ultimately influence survival and reproduction (Zajitschek & Connallon 2017). Some particularly noteworthy trade-offs are those that are established between different compartments of the organism's growth that involve production of tissues, whether they are consumables (reserves), consolidated structures (skeleton, etc), removable (sexual products) or regenerable tissues. The formation of these structures necessarily comes into conflict, as all of them demand nutrients and energy from the same sources. The balance between the costs and benefits of autotomy and regeneration will determine its evolution and maintenance (Goss 1969), so that the study of their physiological and ecological implications can shed light on their evolution across different animal lineages.

Under this framework, this PhD Thesis focused on the phenomena of autotomy and regeneration after autotomy, examining the effects of caudal autotomy on functional aspects like locomotor performance, antipredator behaviour, thermoregulation or hydroregulation, as well as the conflicts with other compartments of growth, such as general body growth and reproduction. These aspects were chosen as the focus of our studies because they represent some of the main components of animals' life-histories and whole-organism functions with high ecological relevance, that have a repercussion on all the organism and are therefore target for natural selection. Moreover, these relationships have been assessed under the assumption that growth and reproduction require a great amount of energy and materials, so that they may compete with the demands of regeneration and incur a conflict of resource allocation, which will possibly be resolved through some hierarchical distribution system, either simultaneously or deferred over time.

The balance between the costs and benefits of tail loss in wall lizards

The previous chapters of this memory assessed how tail autotomy and/or caudal regeneration affected several functions and life-history traits in *Podarcis muralis*. Chapters 1 and 2 focused on the effects of caudal autotomy on locomotor performance and on the behavioural adjustments after tail loss, while the costs of regeneration for growth and reproduction were studied in Chapters 4 and 5. Additionally, Chapter 3 assessed the effects of tail loss on the thermal preferences and dehydration rates of Bocage's wall lizards (*P. bocagei*), even though the other chapters were focused on *P. muralis.* This part of the study was focused on *P. bocagei* because it was carried out during an international stay in the research center CIBIO-InBIO, in northern Portugal, were there is no presence of *P. muralis*. *Podarcis bocagei* is closely related to *P.* muralis, occupies similar spaces and has similar ecological requirements (Harris y Sá-Sousa 2002; García-Porta *et al.* 2019; see General Introduction). Although there are many other processes and functions that may be affected by tail autotomy and/or regeneration (such as the immune function, Argaez *et al.* 2018), the studies included in this memory dealt with some of the main aspects of animals' life-histories and whole-organism functions of high ecological relevance (locomotion, antipredatory behaviour, thermoregulation, hydroregulation, reproduction and body growth), so that the engagement of all those results will allow to have a fairly broad vision on evolution of autotomy and regeneration in wall lizards through this discussion.

The effects of caudal autotomy

Tail autotomy is very common and taxonomically widespread in lizards, and it is frequent that more than the 50% of the individuals of natural populations have autotomized their tail at least once in life (Downes & Shine 2001; McConnachie & Whiting 2003; Fernández-Rodríguez & Braña 2020, for the herein studied population of *P. muralis*). Caudal autotomy provides an immediate benefit that increases survival, facilitating the escape of the lizard by distracting the predator (Arnold 1988; Higham & Russell 2010). After surviving the predatory event by shedding the tail, the first immediate consequence of autotomy is related to the locomotor capacities of the animal (Figure 7.1). The tail holds important roles for locomotion in lizards, acting as a counterbalance, providing stability during running and climbing, and influencing jumping dynamics (Arnold 1988; Jusufi *et al.* 2008), so its loss may alter the centre of body mass and the biomechanics of locomotion. In particular, tail autotomy impairs some aspects of the locomotor performance of adult wall lizards, mainly by decreasing the effectiveness of jumping dynamics (Chapter 1; Fernández-Rodríguez & Braña 2020). This locomotor impairment will likely affect routine activities of lizards that depend on mobility (such as patrolling, foraging, mating, etc; Garland & Losos 1994; Braña 2003; Husak 2006) and, of course, will difficult the escape from predator attacks. In such circumstances, lizards probably face a higher predation risk after autotomy, increased by the impossibility of resorting again to autotomy in future encounters with predators.

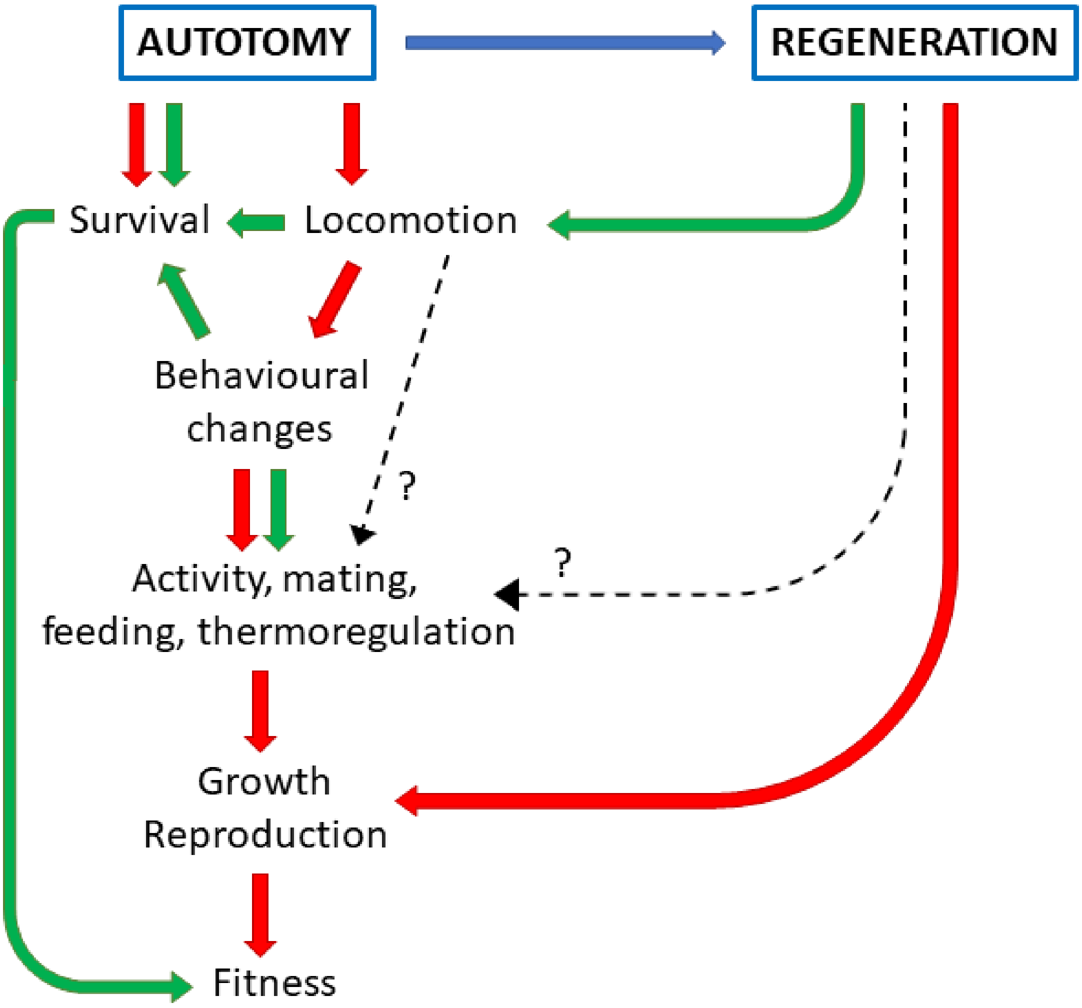


Figure 7.1. Summary of the proven effects (green: positive, red: negative) of tail autotomy and regeneration on several aspects of lizards' biology. Autotomy enhances survival immediately after tail detachment by enabling the escape of the lizard from a predator, but lacking the tail constrains locomotor performance, which also reduces the probability of survival against predator attacks and likely affects some key activities like mating, foraging or patrolling. As a consequence, lizards resort to behavioural adjustments to compensate for the higher risk that they face when lacking the tail, and these changes may affect activity levels, habitat use, mating success, feeding rates or thermoregulation. These alterations can also have negative effects on early growth or reproductive investment, and finally on the individual's fitness. Caudal regeneration initiates shortly after autotomy, and the restoration of the tail recovers the locomotor capacities that were impaired after autotomy, which also increases the probability of future survival. On the other hand, the investment in tail regeneration incurs in allocation trade-offs that can lead to decreased juvenile growth rates or reduced females' reproductive investment, with a potential negative effect on the lizard's lifetime fitness.

However, tailless lizards frequently resort to compensatory behaviours that reduce the risk of predation. According to the data and analyses presented in Chapter 2, some of these behavioural alterations include the intensification of their early-stage predator-avoidance mechanisms (see Putman *et al.* 2015), in order to diminish the probability of detection by a predator and reduce the frequency of attacks. These behavioural changes, such as reducing mobility, increasing shelter use, or decreasing exploration (Fox *et al.* 1981; Martín & Salvador 1993c; Michelangeli *et al.* 2020), would likely increase the probability of immediate survival of tailless lizards, but at the cost of impairing the performance of other relevant activities, such as foraging or mating (Martín & Salvador 1993b; Martín & Avery 1997). Decreasing performance of these relevant activities could secondarily affect growth rates of juveniles and reproductive success of adults, with a potential overall effect on the individuals' lifetime fitness (Figure 7.1).

Other behavioural and/or physiological aspects, like the thermal preferences and body temperature selection, were apparently not affected by tail loss in Bocage's wall lizards (*P. bocagei*; studied in Chapter 3, Fernández-Rodríguez *et al.* 2021). Hydration state is a key factor for thermoregulation, as dehydration increases the risk of overheating and compromises activity rates, growth, or reproduction (Lorenzon *et al.* 1999; Wang *et al.* 2016; Sannolo & Carretero 2019). Selecting higher body temperatures after autotomy would result in faster desiccation rates, which can be even more severe in some regions with either short or relatively long periods of summer droughts (Sannolo *et al.* 2018). On the other hand, decreasing body temperatures would decelerate metabolism and impact the performance of the individuals. Thus, it seems plausible that maintaining the preferred body temperatures after autotomy is the most beneficial option, in terms of maintaining the homeostasis. Another important factor to consider is that this study did only focus on body temperatures, while other behavioural and physiological factors of lizards' thermoregulation could be affected by tail loss. Some of these aspects could be the time and frequency of basking events (that determine the accuracy of thermoregulation), the selected microhabitats for thermoregulation or the heating and cooling rates (that could be affected as tail loss implies a drastic change of morphology, mass and relation volume/surface). Dehydration rates of *P. bocagei* were also not affected by tail loss (Chapter 3), although again our results provide only a preliminary (and novel) insight into the potential effects of tail autotomy on lizards' hydric balance. More factors must be taken in account, such as different hydration states and its consequences for thermoregulation after autotomy, to shed light on the short-term consequences of tail autotomy for thermal and hydric balances.

The effects of regeneration

The short- to medium-term functional and behavioural costs of tail autotomy are likely just temporary and are expected to progressively disappear as caudal regeneration progresses. In *P. muralis* the regenerated tail has almost finished the elongation period after 2-3 months, and the external differentiation of the scales is completed after 3-4 months (authors' personal observations). Regeneration after autotomy allows the restoration of the functionalities lost with the shed part (Goss 1969; Clause & Capaldi 2006), as shown in our study system with regard to the recovery of locomotor capacities impaired after tail loss (Chapter 1; Fernández-Rodríguez & Braña 2020). Therefore, regeneration would increase the probability of survival through the restoration of some functional capacities of the animal (Lin *et al.* 2017). However, the regenerated tail is unlikely to fully maintain its protective value in future encounters with predators, in which a new autotomy could be required. Firstly, a new fracture is only possible in the intact proximal tail portion, anterior to the previous fracture zone, since the central skeleton of the regenerated fragment of the tail is a continuous cartilage axis in which there are no fracture planes (see General Introduction). Secondly, some key characteristics that increases the efficacy of autotomy (i.e., increase its benefit, Emberts *et al.* 2019) are the violent and twitching movements of the shed part, that are especially strong during the first seconds after the detachment (Dial & Fitzpatrick 1983; Bellairs & Bryant 1985; Fernández-Rodríguez & Braña 2020). These initial forceful movements, together with striking tail colours (which are more frequent during juvenile stages), enhance substantially the distraction of the predator, increasing the prey's chances of surviving the attack and escaping (Bellairs & Bryant 1985; Cooper & Vitt 1985; Castilla *et al.* 1999; Higham & Russell 2010). As shown in Chapter 1, regenerated tails moved significantly less and during less time that intact ones, so the antipredator value of regenerated tails remains surely inferior. This fact is probably linked to the structural differences between intact and regenerated tails, that restrict the bending movements of regenerated ones (Hughes & New 1959). In conclusion, caudal regeneration increases the efficiency of autotomy by minimizing the (medium/long-term) costs associated to tail loss, but it does not fully restore the efficacy of future autotomy events.

Tail regeneration implies a great demand of energy and resources that can cause an allocation trade-off between investing either in regeneration or in other processes which also have a high demand for energy and materials, like reproduction or growth (Figure 7.1). In Chapter 4 we have seen that the investment in tail regrowth provokes a decrease in body growth rates of juvenile wall lizards when they face a situation of low food availability. Such limitation in food availability is likely to occur quite frequently in natural conditions for hatchling lizards, considering that: i) hatchlings do not know the territory and have not acquired the skills to successfully identify, tackle and capture prey (Iraeta *et al.* 2012); ii) young lizards have a smaller range of food available, which is restricted both in size and in the taxonomic spectrum of the prey (see, for *Podarcis* *muralis*, Braña, 1981); iii) juveniles do not have their locomotor capacities fully developed (Iraeta *et al.* 2012), and this limitation must be greater for tailless individuals, that must have therefore a lower foraging efficiency (Fernández-Rodríguez & Braña 2020).

Indeed, the value of autotomy and regeneration relies in the balance between its costs and benefits, and this balance will determine its selection and maintenance (Arnold 1988). Reaching a large body size during the first months of life is important in several ways: it increases the spectrum of potential prey, reduces the risk of predation and allows reaching the first winter dormancy in good condition, in terms of size and energy reserves. This increases the probability of survival in juveniles and reduces the time needed for reaching sexual maturity (Bauwens 1981; Peters 1983; Brownikowsi & Arnold 1999). Then, investing on body growth seems to be priority, but even at the price of a certain reduction in body growth rates, it seems plausible that the fully restoration of the lost capacities provides greater benefits that will be relevant in the long-term, as these functional capacities will be essential during adulthood as well.

Regarding the costs of regeneration during adulthood, we have shown that caudal regeneration has both short and long-term negative effects on the reproductive investment of adult females, causing a reduction in their clutch weight (Chapter 5). Besides, this reduction was more severe when regeneration occurs concurrently with clutch development under an income breeding strategy. Although the cost in *P. muralis* is not as extreme as in other species such as *Ctenotus taeniolatus,* in which caudal regeneration leads to a complete skip of reproduction (Taylor 1984), these negative effects of caudal regeneration are not negligible at all. Thus, in order to understand the balance between the costs and benefits of regeneration in adulthood it is important to consider its impact on reproductive investment, both concurrent and future. This means that, although tail regeneration may cause a reduction in the concurrent reproductive investment, it will increase the probability of survival (as explained in Chapter 1) and the future reproductive output, which will ultimately translate into a greater overall lifetime fitness.

Growth is the main demanding process during juvenile stages, while adult females invest the majority of their resources primarily on clutch development during the breeding season, and much less (even negligible during those months) on reserves accumulation and body growth, so we can consider reproduction as their main demanding process during the breeding season. In fact, maybe there is a hierarchy through which the periods of body growth and reproduction alternate during adulthood, and in that sense it would be interesting to investigate if there is any compensatory growth mechanism, by which post-reproductive females have particularly high growth rates. In any case, it is worth noting the different impact that tail regeneration implies for juveniles and adult females on their main demanding processes, as the consequences seemed to be remarkably more severe for the reproductive investment of females than for juveniles' growth rates. In fact, juveniles only decreased their growth rates when they were facing low food availability, while females showed a reduction in the reproductive investment during tail regeneration, despite being fed *ad libitum*. Female wall lizards develop up to three clutches at monthly intervals, each representing 30-40 % of their body mass (Saint Girons & Duguy 1970; Ji & Braña 2000), which implies an intense investment of resources in a short period of time. On the other hand, juvenile growth, although being fast, is a more continuous process in which the investment is more extended in time, so it likely poses a less compulsive demand than clutch development. The high demand for resources that reproduction implies, and especially its high concentration in a short period, may partially explain the fact that females, unlike juveniles, were unable to satisfy the energy needs of reproduction and regeneration simultaneously, even with a high availability of food.

Another possible explanation is that growth is much more unavoidable than reproduction: while females may skip the development of one clutch under unfavourable conditions to divert resources to another process like regeneration (Smyth 1974), juveniles cannot stop growth and development. Besides, as reaching a minimum body size has future important consequences for lifetime fitness (Bauwens 1981; Brownikowsi & Arnold 1999; King *et al.* 2016), so it may be expectable that a great impact of regeneration on juvenile growth would have been under a strong negative selection pressure.

The velocity of tail regeneration in juveniles vs. adults may also give information about how the importance of this phenomenon may change through the life history of the animal. Regeneration rates were measured during the growth and reproduction experiments (Chapters 4 and 5) for 44 juveniles and 41 adults (both males and females). If we consider the absolute value of the increase in length of the regenerated tail of both age groups (considering only the juveniles fed ad libitum, to allow comparison with the adult females that had the same feeding regime), rates of tail regeneration in length were similar for adults and juveniles (mean for juveniles: 0.074 cm/day; adults: 0.078 cm/day). But if we compare the length regenerated per day in relation to the size (SVL) of the animal, relative growth was much greater in juveniles, being almost twice than that of adults (juveniles: 0.024 cm/day in relation to SVL; adults: 0.013 cm/day in relation to SVL). Besides, if we consider the volume or biomass regenerated, the same amount of tail length regrowth implies a faster regeneration rate in adults than in juveniles, as shown by our data (mean for juveniles: 0.0013 cm^3/day; adults: 0.0044 cm^3/day). This is expectable, as the width of the remaining tail stub is much bigger in adults than in juveniles. Interestingly, when comparing the volume of tail regenerated per day in relation to their size, adults regenerated almost twice than juveniles the volume of tail per day in relation to their SVL (mean for juveniles: 0.0004 cm^3/day in relation to SVL; adults: 0.0007 cm^3/day in relation to SVL). These results have two important implications: first, regarding that locomotor capacities are closely related to tail length (Zamora-Camacho *et al.* 2016; Fernández-Rodríguez & Braña 2020 for *P. muralis*), this implies that juveniles restore their locomotor capacities in a shorter period of time than adults. Second, as noted by Bellairs & Bryant (1985), given two lizards of different sizes but same proportions that autotomized the same linear proportion of the tail, the absolute loss of tail length and volume would be greater in the larger one. Although tail elongation in adults is slower than in juveniles, they regenerate more volume of tail per day than juveniles in relation to their body size and need to regenerate a greater volume of tail than juveniles to reach a given length. These two implications mean that tail regeneration should be more costly in adult than in young lizards, both in terms of time (to reach a minimum length that enables functionality) and of demands of energy and resources. This can also contribute to explain the greater impact of the costs of tail regeneration on the reproduction of adult females than on body growth rates of juveniles.

General trends on the costs and benefits of autotomy and regeneration in lizards

The evolution of autotomy and regeneration have captured the attention of scientists for decades, and lizards are one of the most recognised models for this kind of studies. Consequently, many studies have tried to assess the effects that tail loss and its regeneration entail in several aspects of the biology of a number of lizard species that differ in morphology, ecological specialization and life history characteristics. A short review of these studies is summarized in Table 7.1. The effects of tail loss on locomotor performance are by far the best known, as most of the studies have focused on them (Table 7.1), followed by the behavioural adjustments that lizards may adopt after caudal autotomy. On the other hand, other aspects such as the dehydration rates, activity level, habitat use, or mating success have deserved much less attention. Moreover, the effects of regeneration have been less studied than the effects of tail loss, probably because they require longer experiments.

The most studied species have been the lacertid lizards *Psammodromus algirus*, *Iberolacerta monticola* and *Podarcis muralis* (mainly in the present document), and the skink *Lampropholis guichenoti*. These four species constitute rather complete models to compare the different costs and benefits of autotomy and regeneration among species with different life histories. The studies carried out with the Algerian sand racer (*P. algirus*) cover many functional and behavioural consequences of tail loss, showing a decrease in sprint speed, mating success and foraging efficiency, changes in the antipredator behaviour (increased flight distances) and habitat use (reduced home range) and no effects on thermoregulation (body temperature) or survival rate (Table 7.1). Besides, caudal regeneration restores the locomotor capacities in *P. algirus*, and there is evidence that the allocation costs of regeneration may provoke a reduction in their growth rates. The Iberian rock lizard (*I. monticola*) provides the most detailed insights into the behavioural changes after tail loss and its implications in a single species: after tail autotomy lizards presented reduced home range and patrolling, used safer microhabitats and more optimal areas for thermoregulation, decreased their activity levels, social status and mating success, increased basking time but did not change body temperature or foraging efficiency. However, there is no information about the effects of caudal regeneration in this species. Regarding the common garden skink (*L. guichenoti*), its information is mainly related to the functional and behavioural effects of caudal autotomy: tail loss decreases sprint speed and activity levels, changes antipredator behaviour (increased flight distances), but does not affect habitat use or thermoregulation (basking areas). Regarding caudal regeneration, it seems to have no effects on the reproductive investment of female *L. guichenoti*. The effects of autotomy and regeneration in the common wall lizard (*P. muralis*) have been largely explained above, and it is the species in which the effects of caudal regeneration have been most completely studied. Finally, it is worth noting that any of the studies carried out with the gecko *Amalosia lesueurii* reported significant effects of autotomy or regeneration in the locomotor performance, antipredator behaviour, habitat use, survival or growth rates.

The studies summarized in Table 7.1 refer to 57 lizard species from four different clades (according to the classifications of Pyron *et al.* 2013; Hedges 2014; Zheng & Wiens 2016) in which caudal autotomy and regeneration are widespread and frequent: Gekkota (geckos), Iguania (anoles and iguanids), Lacertoidea (teiids and lacertids) and Scincoidea (skinks). These species have distinct lifestyles and adaptations, as well as differences in their morphology and role of the tail (locomotion, accumulation of reserves, etc), which may shed light on how and why the consequences of tail loss and regeneration may vary phylogenetically among different lizards. Here we focused on some of the most studied effects of autotomy (locomotion, antipredator behaviour) and regeneration (reproduction, growth) to make comparisons among the four clades (Table 7.1).

Table 7.1. Effects of autotomy (i.e., interpreted as the effects of lacking the tail shortly after autotomy) and regeneration (i.e., costs during regeneration and effects of the regenerated tail on functional aspects) on several aspects of lizards' biology. **Loc** = locomotion (sprint speed, climbing, jumping or stability); **Ant B** = antipredator behaviour (considered as negative shorter distances to refuge, larger escape distances and increase of wariness); **Ther** = thermoregulation (body temperature, precision or basking time, considering as negative a longer basking time); **Deh** = dehydration rates; **Hab** = habitat and microhabitat use (considered as negative any shift on habitat use); **For** = foraging (decrease on rates, ability or success, or increase on rates); **Mat** = mating success; **Sur** = survival; **Soc** = social status; **Rep** = reproduction (females' reproductive investment); **Gro** = growth rates; • = no effects; x = negative effects; + = positive effects.

Family	Species	AUTOTOMY										REGENERATION				Ref.
		Loc	Ant B	Ther	Deh	Act	Hab	For	Mat	Sur	Soc	Rep	Gro	Loc	Sur	
Diplodactylidae	*Amalosia lesueurii*	•	•				•			•			•			1,2
	Diplodactylus tessellatus									•			•			94
Eublepharidae	*Coleonyx brevis*							+				x				3
	Coleonyx variegatus									x						4
	Eublepharis macularius	•											x	•		5,6
Gekkonidae	*Christinus marmoratus*	+														7
	Hemidactylus bowringii	+														8
	Lygodactylus capensis	•/x														9-12
	Paroedura picta												•			13
	Christinus marmoratus									x						14
	Gehyra variegata											x				95
Sphaerodactylidae	*Gonatodes albogularis*		x													15
	Teratoscincus scincus	•	•			•										16
Dactyloidae	*Anolis carolinensis*	•/x														17-21
	Anolis sagrei										•	+				22,96
Iguanidae	*Dipsosaurus dorsalis*	x														23
Liolaemidae	*Liolaemus nigromaculatus*		•/x													24
Phrynosomatidae	*Cophosaurus texanus scitulus*	x														25
	Holbrookia maculata	•														20

Family	Species	AUTOTOMY										REGENERATION				Ref.
		Loc	Ant B	Ther	Deh	Act	Hab	For	Mat	Sur	Soc	Rep	Gro	Loc	Sur	
Phrynosomatidae	*Holbrookia propinqua*		•/x			•		x								26
	Sceloporus jarrovi												•			27
	Sceloporus merriami	•														28
	Sceloporus scalaris												x			27
	Sceloporus undulatus												x			27
	Sceloporus virgatus	x	x			x							x			29-31
	Uma notata notata	x														25
	Uta stansburiana									•/x	x	+	•/x			32-37
Tropiduridae	*Tropidurus itambere*												•			38
Lacertidae	*Iberolacerta horvathi*		•													39
	Iberolacerta monticola		x	•/x		•/x	x	•	x		x					40-45
	Lacerta agilis	+														46
	Podarcis bocagei			•	•											47
	Podarcis lilfordi	x														48,49
	Podarcis muralis	x	x					•/+				x	x	+		50-54
	Psammodromus algirus	x	x	•			x	•/x	x	•			•/x	+		48,55-62
	Takydromus septentrionalis	x														48,63
	Takydromus sexlineatus	•														20
	Takydromus viridipunctatus														+	64
	Zootoca vivipara	x	x	•												39,65,93
Teiidae	*Aspidoscelis sonorae*	•														20
	Aspidoscelis sexlineatus	x														66
Cordylidae	*Pseudocordylus melanotus*	•	•			•										67
Scincidae	*Carlia jarnoldae*		•						x							68
	Ctenotus taeniolatus											x				69
	Eulamprus quoyii	x		•								x				70-72
	Eulamprus tympanum											x				73,74

Family	Species	AUTOTOMY										REGENERATION				Ref.
		Loc	Ant B	Ther	Deh	Act	Hab	For	Mat	Sur	Soc	Rep	Gro	Loc	Sur	
Scincidae	*Plestiodon chinensis*	x														75
	Plestiodon elegans	x														76
	Plestiodon fasciatus									x			•			77,78
	Plestiodon laticeps									x			•			77
	Hemiergis peronii											x				79
	Lampropholis delicata	x	x	•												80,81
	Lampropholis guichenoti	x	x	•		x	•					•				69,80-83
	Morethia boulengeri											x				79
	Carinascincus metallicus	x		•								x	•	+		84-87
	Scincella lateralis	x	x			x				x						88,89
	Sphenomorphus indicus	x														90
	Trachylepis maculilabris	x														91
	Eutropis multifasciata	x														92

1: Kelehear & Webb (2006), **2**: Webb (2006), **3**: Dial & Fitzpatrick (1981), **4**: Congdon *et al.* (1974), **5**: Jangnadan *et al.* (2014), **6**: Lynn *et al.* (2013), **7**: Daniels (1983), **8**: Ding *et al.* (2012), **9**: Medger *et al.* (2008), **10**: Jusufi *et al.* (2008), **11**: Fleming *et al.* (2009), **12**: Fleming & Bateman (2012), **13**: Starostová *et al.* (2017), **14**: Daniels *et al.* (1983), **15**: Domínguez-López *et al.* (2015), **16**: Lu *et al.* (2010), **17**: Bonvini (2007), **18**: Gillis *et al.* (2009), **19**: Gillis *et al.* (2013), **20**: McElroy & Bergman (2013), **21**: Hsieh (2016), **22**: Kaiser & Mushinksky (1994), **23**: Pond (1978), **24**: Kelt *et al.* (2002), **25**: Punzo (1982), **26**: Cooper (2003), **27**: Ballinger & Tinkle (1979), **28**: Huey *et al.* (1990), **29**: Smith (1996), **30**: Cooper (2007), **31**: Cooper *et al.* (2009), **32**: Fox & Rotsker (1982), **33**: Fox *et al.* (1990), **34**: Wilson (1992), **35**: Althoff & Thompson (1994), **36**: Niewiarowsky *et al.* (1997), **37**: Fox & McCoy (2000), **38**: Van Sluys (1998), **39**: Capizzi *et al.* (2007), **40**: Martín & Salvador (1992), **41**: Martín & Salvador (1993a), **42**: Martín & Salvador (1993b), **43**: Martín & Salvador (1993c), **44**: Martín & Salvador (1995), **45**: Martín & Salvador (1997), **46**: Ekner-Grzyb *et al.* (2013), **47**: Fernández-Rodríguez *et al.* (2021), **48**: Arnold (1998), **49**: Cooper *et al.* (2004), **50**: Brown *et al.* (1995), **51**: Fernández-Rodríguez & Braña (2020), **52**: Chapter 2 of this memory, **53**: Chapter 4 of this memory, **54**: Chapter 5 of this memory, **55**: Salvador *et al.* (1995), **56**: Martín & Avery (1997), **57**: Martín & Avery (1998), **58**: Civantos *et al.* (1999), **59**: Salvador & Veiga (2005), **60**: Iraeta *et al.* (2012), **61**: Zamora-Camacho *et al.* (2015), **62**: Zamora-Camacho *et al.* (2016), **63**: Lin & Ji (2005), **64**: Lin *et al.* (2017), **65**: Herczeg *et al.* (2004), **66**: Ballinger *et al.* (1979), **67**: McConachie & Whiting (2003), **68**: Langkilde *et al.* (2005), **69**: Taylor (1984), **70**: Daniels (1985), **71**: Wilson (1994), **72**: Wilson & Booth (1998), **73**: Doughty & Shine (1997), **74**: Doughty & Shine (1998), **75**: Lin *et al.* (2006), **76**: Lin *et al.* (2010), **77**: Vitt & Cooper (1986), **78**: Goodman (2006), **79**: Smyth (1974), **80**: Cromie & Chapple (2012), **81**: Michelangeli *et al.* (2020), **82**: Downes & Shine (2001), **83**: Shine (2003), **84**: Chapple *et al.* (2002), **85**: Chapple & Swain (2002), **86**: Chapple *et al.* (2004), **87**: Chapple & Swain (2004), **88**: Dial & Fitzpatrick (1983), **89**: Formanowicz *et al.* (1990), **90**: Lu *et al.* (2013), **91**: Cooper & Smith (2009), **92**: Sun *et al.* (2009), **93**: Liu *et al.* (2011), **94**: Henle (1990b), **95**: Henle (1990a), **96**: Beatty *et al.* (2021).

Quantitative analyses would require considering the phylogenetic context, but the available data do not allow this, as there are important gaps and a taxonomic bias with a lack of representation of some groups against an over-representation of other groups. Besides, the methodologies used to measure the effects of autotomy and regeneration, together with experimental conditions (temperature, feeding, etc), age and sex of the individuals differed among the different studies, so that the comparisons among species will be done qualitatively, considering just the effects (negative, neutral or positive) reported for each species.

The effects of autotomy on locomotion

The short-term consequences of tail loss for locomotor performance seem to be considerably less severe in geckos than in skinks, iguanians and lacertoids (Figure 7.2A), as only 14.28 % of the studied species of geckos presented impaired locomotor capacities after tail autotomy, in contrast to the 62.5 % of the studied species of iguanians, the 66.66% of studied lacertoids and the 90 % of studied skink species that suffered such locomotor impairment. The differences between geckos and the other clades are based on data from a rather small number of species, not large enough to reach a strong statistical significance if we consider the positive, negative or neutral effects of tail loss as three different categories (Pearson's Chi-Squared test, $X^2 = 12.799$, $p = 0.046$); however, these differences turn out to be highly significant if we consider the joint frequency of positive and neutral effects within a single category, against the negative effects ($X^2 = 10.837$, $p = 0.013$).

These differences in the severity of the effects of tail loss for locomotor performance among clades are related both to the morphological differences of the body and tail and its different role for locomotion, as proposed by Bergman & Irschick (2012) and McElroy & Bergmann (2013). The meta-analysis carried out by McElroy &

Bergmann (2013) revealed two main patterns of changes in sprint speed after tail loss, related to body and tail morphology: i) lizards that drag their tails during running may experience an increased sprint speed after autotomy (Willey *et al.* 2004), and ii) lizards with long tails may experience a reduction in sprint speed after tail loss due to changes in the center of body mass (Ballinger *et al.* 1979) or in the undulatory movements of their body (Cromie & Chapple 2012).

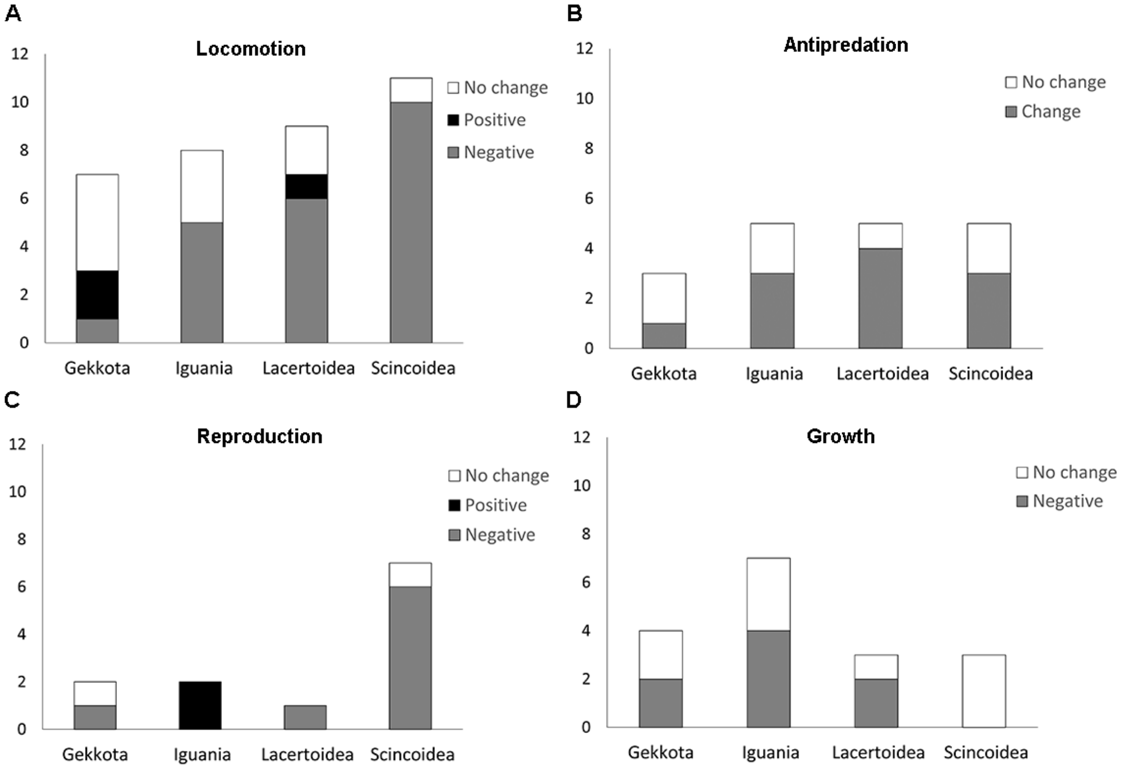


Figure 7.2. Effects of caudal autotomy (**A, B**) and regeneration (**C, D**) on the locomotor capacities, antipredator behaviour, reproductive investment and growth rates among geckos, iguanians, lacertoids and skinks. Values represent number of species.

Geckos often accumulate a great number of reserves in the tail and have consequently thick and heavy tails that are dragged during running, provoking a friction force against the substrate that hampers locomotion over horizontal surfaces (Medger *et al.* 2008). Thus, the loss of friction force after tail autotomy may explain why lacking the tail tends to improve burst speed in geckos. Surprisingly, improvement of burst speed after tail loss, despite being more frequent in geckos, was also reported for one iguanian (*Holbrookia maculata*, McElroy & Bergmann 2013) and for some lacertids (*Lacerta agilis*, Ekner-Grzyb *et al.* 2013; *P. muralis*, Brown *et al.* 1995, although in this case tail loss markedly decreased arboreal locomotor performance). This indicates that the positive effects of tail loss for running speed are not only restricted to lizards with heavy tails but can also occur in lizards with slender tails, but the underlying mechanisms remain rather misunderstood.

It is worth noting that all the reported positive effects of tail autotomy on locomotion (Table 7.1) referred to an increase in sprint speed, but no improvement of other aspects of locomotion, like climbing or jumping abilities, were reported. This may be explained because lizards' tails hold important roles for climbing or jumping, acting as a prop against the surface or as a counterpoise (Jusufi *et al.* 2008; Gillis *et al.* 2009), so its loss alters the body's biomechanics. Only one species per clade has been experimentally evaluated for the effects of tail loss on their climbing and/or jumpling ability, so no comparisons are possible between clades. Besides, the effects were variable even within species: tail loss had both negative and neutral effects on jumping and climbing of the iguanian *Anolis carolinensis* (Gillis *et al.* 2009; 2013) and the gecko *Lygodactylus capensis* (Fleming *et al.* 2009; Jusufi *et al.* 2008; Medger *et al.* 2008; Fleming & Bateman 2012). On the other hand, tail autotomy did not affect the climbing ability of the skink *Carinascincus metallicus* (Chapple & Swain 2002), while the lacertid *P. muralis* showed diminished jumping skills after tail loss (Fernández-Rodríguez & Braña 2020).

Shifts in antipredator behaviour after tail autotomy

Regarding antipredator behaviour, no differences among clades were found in the frequency of behavioural adjustments after tail loss ($X^2 = 1.729$, $p = 0.630$). Adopting behavioural changes that exacerbate lizards' predator-avoidance mechanisms after tail loss seems to be frequent among clades (Figure 7.2B), and the most common behavioural change observed by researchers was to increase the flight distance (i.e., escape from a larger distance from an approaching predator), a strategy adopted by lizards apparently regardless of their clade (e.g., Martín & Avery 1998; Downes & Shine 2001; Kelt *et al.* 2002; Capizzi *et al.* 2007). Other behavioural changes observed were remaining closer to the refuge (reported for 2 iguanian species, Cooper 2003; 2007) and increasing wariness by becoming less explorative (reported for 3 lacertoids, Martín & Salvador 1997; Michelangeli *et al.* 2020; Chapter 2).

Moreover, there is information about the effects of tail loss on locomotion for 6 out of the 11 species of lizards that exhibited an altered antipredator behaviour after caudal autotomy. All these species (2 skinks, 1 iguanian and 3 lacertoids) suffered from a diminished locomotor performance shortly after tail autotomy (Formanowicz *et al.* 1990; Martín & Avery 1998; Cooper *et al.* 2009; Cromie & Chapple 2012; Fernández-Rodríguez & Braña 2020), which reinforces the idea that these exacerbated predator-avoidance mechanisms might have arisen to counter the higher predation risk due to locomotor impairment, until tail regeneration is completed.

The costs of tail regeneration for reproduction

The studies that focused on the costs of caudal regeneration on females' reproductive investment have been focused predominantly on skinks, for which 6 out of the 7 studied species reported a reduction in females' reproductive output during regeneration.

Regarding iguanians, lacertoids and geckos, there is information available of only one or two species per clade (Figure 7.2C), which precludes further comparisons between clades with statistical analyses. Besides, only two studies have focused specifically on the costs of regeneration (Chapter 5, for *P. muralis*; Beatty *et al.* 2021, for *Anolis sagrei*), despite being crucial to understand the costs and implications of the regenerative capacities. It is worth noting the studies of Fox & McCoy (2000) and Beatty *et al.* (2021), in which they found partially positive results in some components of the reproductive investment of females during tail regeneration. In both cases there were no differences in clutch size or mass, but tailless females produced slightly larger hatchlings, which the authors explain by a potential difference in the quality of the eggs or a possible increase in the energy efficiency during regeneration.

As it has been explained in detail in Chapter 5, previous authors, such as Bernardo & Agosta (2005), concluded from the available information of lizards and salamanders that the presence of abdominal fat bodies is a key trait for the severity of the costs of caudal autotomy and regeneration for reproductive investment. Lizards or salamanders with reduced or lacking abdominal fat bodies tend to suffer greater reproduction costs after tail loss, as autotomy causes the loss of the caudal fat reserves that are necessary for clutch development. Further studies on geckos would be of high interest regarding this issue, as in these lizards the tail holds an important role for reserves storage (Bustard 1967).

The costs of caudal regeneration for body growth

Finally, there are no significant differences among clades in the effects of the allocation costs of caudal regeneration on growth rates (Figure 7.2D; $X^2 = 3.429$, $p = 0.330$). However, the available information about these effects (Table 7.1, Figure 76.2D) leads to confusion and does not allow solid conclusions about the real consequences of the demands of regeneration during early growth among different lizard species. As explained in Chapter 4, food availability seems to be crucial for the occurrence of a trade-off between tail regeneration and body growth, as it seems that regeneration only has a negative effect on growth rates under low food availability (Lynn *et al.* 2013; Chapter 4). Negative correlations between two traits may evidence a trade-off due to the dominance of resources allocation over acquisition, but an absence of a negative correlation does not necessarily imply that there is not a competence for the resources. Positive or no correlations, like in these cases, may also occur during a trade-off when there is a predominance of resource acquisition over allocation or when there is a high variability among individuals in the acquisition of resources (van Noordwicjk & de Jong 1986; Reznick *et al.* 2000).

Most studies that evaluated the effects of tail regeneration on body growth under laboratory conditions subjected the animals to an *ad libitum* food regimen. This probably masked the negative effects that the energy demands of regeneration could have had under a resource limitation (even when this is not severe but likely close to what animals may face in natural populations). For instance, it seems remarkable that any of the studied skink species in Table 7.1 showed a reduction in growth rates during tail regeneration (Figure 7.2D), which could lead to wrong conclusions if it is not taken into consideration that in all those studies the lizards were fed *ad libitum*. Further studies that include food treatments that impose a restriction of resources and, specially, experimental field studies or studies carried out under conditions similar to those experienced by natural populations are needed to reach solid conclusions about the differences among clades in the effects of regeneration on body growth.

References

Althoff, D.M. & Thompson, J.N. (1994). The effects of tail autotomy on survivorship and body growth of Uta stansburiana under conditions of high mortality. *Oecologia*, 100: 250–255.

Argaez, V.; Solano-Zavaleta, I. & Zúñiga-Vega, J.J. (2018). Another potential cost of tail autotomy: tail loss may result in high ectoparasite loads in *Sceloporus* lizards. *Amphibia-Reptilia*, 39(2): 191–202.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Arnold, E.N. (1998). Structural niche, limb morphology and locomotion in lacertid lizards (Squamata, Lacertidae): a preliminary analysis. *Bulletin of the British Museum (Natural History) Zoology*, 64: 63–89.

Ballinger, R.E. & Tinkle, D.W. (1979). On the cost of tail regeneration to body growth in lizards. *Journal of Herpetology*, 13: 374–375.

Ballinger, R.E.; Nietfeldt, J.W. & Krupa, J.J. (1979). An experimental analysis of the role of the tail in attaining high running speed *in Cnemidophorus sexlineatus* (Reptilia: Squamata: Lacertilia). *Herpetologica*, 35: 114–116.

Bauwens, D. (1981). Survival during hibernation in the European common lizard, *Lacerta vivipara. Copeia*, 1981: 741–744.

Beatty, A.E.; Mote, D.M. & Schwartz, T.S. (2021). Tail of reproduction: Regeneration leads to increased reproductive investment. *Journal of Experimental Zoology Part A Comparative Experimental Biology*, 335(5): 522–528.

Bellairs, A. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bely, A.E. & Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170.

Bergmann, P.J. & Irschick, D.J. (2012). Vertebral evolution and diversification in squamate reptiles. *Evolution*, 66: 1044–1058.

Bernardo, J. & Agosta, S.J. (2005). Evolutionary implications of hierarchical impacts of nonlethal injury on reproduction, including maternal effects. *Biological Journal of the Linnean Society*, 86: 309–331.

Bonvini, L.A. (2007). Jumping behavior and the effects of caudal autotomy on performance in Anolis carolinensis. Honors Thesis, Mount Holyoke College, South Hadley, USA.

Braña, F. (1981). Sobre la alimentación de *Podarcis muralis* (Reptilia: Lacertidae) en Asturias: espectro taxonómico y tamaño de las presas. *Resúmenes de Comunicaciones de la V Reunión Bienal de la Real Sociedad Española de Historia Natural*. Oviedo: 179.

Braña, F. (2003). Morphological correlates of burst speed and field movement patterns: the behavioral adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society*, 80(1): 135–146.

Brownikowski, A.M. & Arnold, S.J. (1999). The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. *Ecology*, 80: 2314–2325.

Brown, R.M., Taylor, D.H. & Gist, D.H. (1995). Effect of caudal autotomy on locomotor performance of wall lizards (*Podarcis muralis*). *Journal of Herpetology*, 29: 98–105.

Bustard, H.R. (1967). Gekkonid lizards adapt fat storage to desert environments. *Science*, 158(3805): 1197–1198.

Capizzi, D., Luiselli, L. & Vignoli, L. (2007). Flight initiation distance in relation to substratum type, sex, reproductive status and tail condition in two lacertids with contrasting habits. *Amphibia-Reptilia*, 28: 403–407.

Castilla, A.; Gosá, A.; Galán, P. & Pérez-Mellado, V. (1999). Green tails in lizards of the genus *Podarcis*: do they influence the intensity of predation? *Herpetologica*, 55(4): 530–537.

Chapple, D.G. & Swain, R. (2002). Effect of caudal autotomy on locomotor performance in a viviparous skink, *Niveoscincus metallicus*. *Functional Ecology*, 16: 817–825.

Chapple, D.G. & Swain, R. (2004). Caudal autotomy does not influence thermoregulatory characteristics in the metallic skink, *Niveoscincus metallicus*. *Amphibia-Reptilia*, 25: 326–333.

Chapple, D.G.; McCoull, C.J. & Swain, R. (2002). Changes in reproductive investment following caudal autotomy in viviparous skinks (*Niveoscincus metallicus*): lipid depletion or energetic diversion? *Journal of Herpetology*, 36: 480–486.

Chapple, D.G.; McCoull, C.J. & Swain, R. (2004). Effect of tail loss on sprint speed and growth in newborn skinks, *Niveoscincus metallicus*. *Journal of Herpetology*, 38: 137–140.

Civantos, E.; Salvador, A. & Veiga, J.P. (1999). Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia*, 1999: 1112–1117.

Clause, A.R. & Capaldi, E.A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology*, 305A: 965–973.

Congdon, J.D.; Vitt, L.J. & King, W.W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science*, 184: 1379–1380.

Cooper W.E., Jr.; Wilson, D.S. & Smith, G.R. (2009). Sex, reproductive status, and cost of tail autotomy via decreased running speed in lizards. *Ethology*, 115: 7–13.

Cooper, W.E. & Smith, C.S. (2009). Costs and economy of autotomy for tail movement and running speed in the skink *Trachylepis maculilabris*. *Canadian Journal of Zoology*, 87: 400–406.

Cooper, W.E. Jr. & Vitt, L.J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift für Tierpsychologie*, 70: 265–276.

Cooper, W.E., Jr (2003). Shifted balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (*Holbrookia propinqua*). *Behavioral Ecology and Sociobiology*, 54: 179–187.

Cooper, W.E., Jr (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., Jr; Pérez-Mellado, V. & Vitt, L.J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoology*, 262: 243–255.

Cromie, G.L. & Chapple, D.G. (2012). Impact of tail loss on the behaviour and locomotor performance of two sympatric *Lampropholis* skink species. *PLoS ONE,* 7(4): e34732.

Daniels, C.B. (1983). Running: an escape strategy enhanced by autotomy. *Herpetologica*, 39: 162–165.

Daniels, C.B. (1985). The effect of tail autotomy on the exercise capacity of the water skink, *Sphenomorphus quoyii*. *Copeia*, 1985: 1074–1077.

Dial, B.E. & Fitzpatrick, L.C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia*, 51: 310–317.

Dial, B.E. & Fitzpatrick, L.C. (1983). Lizard tail autotomy: Function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science,* 219: 391–393.

Ding, G.; Fu, T.; Zhou, Z. & Ji, X. (2012). Tail autotomy does not increase locomotor costs in the oriental leaf-toed gecko *Hemidactylus bowringii*. *Asian Herpetological Research*, 3: 141–146.

Domínguez-López, M.E.; Ortega-León, A.M. & Zamora-Abrego, G.C. (2015). Tail autotomy effects on the escape behavior of the lizard *Gonatodes albogularis* (Squamata: Sphaerodactylidae), from Córdoba, Colombia. *Revista Chilena de Historia Natural*, 88: 1.

Doughty, P. & Shine, R. (1997). Detecting life-history trade-offs: measuring energy stores in ‘capital’ breeders reveals costs of reproduction. *Oecologia*, 110: 508–513.

Doughty, P. & Shine, R. (1998). Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology*, 79: 1073–1083.

Downes, S.J. & Shine, R. (2001). Why does tail loss increase a lizard’s later vulnerability to snake predators? *Ecology*, 82: 1293–1303.

Ekner-Grzyb, A.; Sajkowska, Z.; Dudek, K.; Gawalek, M.; Skórka, P. & Tryjanowski, P. (2013). Locomotor performance of sand lizards (*Lacerta agilis*): effects of predatory pressure and parasite load. *Acta Ethologica*, 16(3): 173–179.

Emberts, Z.; Escalante, I. & Bateman, P.W. (2019). The ecology and evolution of autotomy. *Biological Reviews*, 94(6): 1881–1896.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15(6): 511–521.

Fernández-Rodríguez, I.; Barroso, F.M. & Carretero, M.A. (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.

Fleming, P.A. & Bateman, P.W. (2012). Autotomy, tail regeneration and jumping ability in Cape dwarf geckos (*Lygodactylus capensis*) (Gekkonidae). *African Zoology,* 47(1): 55–59.

Fleming, P.A.; Verburgt, L.; Scantlebury, M.; Medger, K. & Bateman, P.W. (2009). Jettisoning ballast or fuel? caudal autotomy and locomotory energetics of the Cape dwarf gecko, *Lygodactylus capensis* (Gekkonidae). *Physiological and Biochemical Zoology*, 82: 756–765.

Formanowicz, D.R.; Brodie, E.D. & Bradley, P.J. (1990). Behavioural compensation for tail loss in the ground skink, *Scincella lateralis*. *Animal Behaviour*, 40: 782–784.

Fox, S.F. & McCoy, J.K. (2000). The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia*, 122: 327–334.

Fox, S.F. & Rostker, M.A. (1982). Social cost of tail loss in *Uta stansburiana*. *Science*, 218: 692–693.

Fox, S.F.; Heger, N.A. & Delay, L.S. (1990). Social cost of tail loss in *Uta stansburiana*: lizard tails as status-signalling badges. *Animal Behaviour*, 39: 549–554.

Fox, S.F.; Rose, E. & Myers, R. (1981). Dominance and the acquisition of superior home ranges in the lizard *Uta stansburiana*. *Ecology*, 62: 888–893.

García-Porta, J.; Irisarri, I.; Kirchner, M.; *et al*. (2019). Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature Communications*, 10: 4077.

Garland, T. & Losos, J.B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P.C. & Reilly S.M. (Eds.), *Ecological morphology.* University of Chicago Press, Chicago, pp. 240–302.

Gillis, G.B.; Bonvini, L.A. & Irschick, D.J. (2009). Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis. Journal of Experimental Biology,* 212: 604–609.

Gillis, G.B.; Kuo, C.-Y. & Irschick, D.J. (2013). The impact of tail loss on stability during jumping in green anoles (*Anolis carolinensis*). *Physiological and Biochemical Zoology*, 86: 680–689.

Goodman, R.M. (2006). Effects of tail loss on growth and sprint speed of juvenile *Eumeces fasciatus* (Scincidae). *Journal of Herpetology*, 40: 99–102.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Harris, D.J. & Sá-Sousa, P. (2002). Molecular Phylogenetics of Iberian Wall Lizards (*Podarcis*): Is *Podarcis hispanica* a Species Complex? *Molecular Phylogenetics and Evolution*, 23(1): 75–81.

Hedges, S.B. (2014). The high-level classification of skinks (Reptilia, Squamata, Scincomorpha). *Zootaxa*, 3765(4): 317–338.

Henle, K. (1990a). Population ecology and life history of the arboreal gecko *Gehyra variegata* in arid Australia. *Herpetological Monographs*, 4: 30–60.

Henle, K. (1990b). Population ecology and life history of three terrestrial geckos in arid Australia. *Copeia*, 1990(3): 759–781.

Herczeg, G.; Kovács, T.; Tóth, T.; Török, J.; Korsós, Z. & Merilä, J. (2004). Tail loss and thermoregulation in the common lizard *Zootoca vivipara*. *Naturwissenschaften*, 91: 485–488.

Higham, T.E. & Russell, A.P. (2010). Flip, flop and fly: modulated motor control and highly variable movement patterns of autotomized gecko tails. *Biology Letters,* 6: 70–73.

Hsieh, S.-T.T. (2016). Tail loss and narrow surfaces decrease locomotor stability in the arboreal green anole lizard (*Anolis carolinensis*). *Journal of Experimental Biology*, 219: 364–373.

Huey, R.B.; Dunham, A.E.; Overall, K.L. & Newman, R.A. (1990). Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. *Physiological Zoology*, 63: 845–872.

Hughes, A. & New, D. (1959). Tail regeneration in the geckonid lizard, *Sphaeridactylus. Journal of Embryology and experimental Morphology,* 7: 281–302.

Husak, J.F.; Fox, S.F.; Lovern, M.B. & Van Den Bussche, R.A. (2006). Faster lizards sire more offspring: Sexual selection on whole-animal performance. *Evolution*, 60(10), 2122–2130.

Iraeta, P.; Salvador, A. & Díaz, J.A. (2012). Effects of caudal autotomy on postnatal growth rates of hatchling *Psammodromus algirus. Journal of Herpetology*, 46(3): 342–345.

Jagnandan, K.; Russell, A.P. & Higham, T.E. (2014). Tail autotomy and subsequent regeneration alter the mechanics of locomotion in lizards. *Journal of Experimental Biology*, 217: 3891–3897.

Ji, X. & Braña, F. (2000). Among clutch variation in reproductive output and egg size in the wall lizard (*Podarcis muralis*) from a lowland population of Northern Spain. *Journal of Herpetology*, 34(1): 54–60.

Jusufi, A.; Goldman, D.I.; Revzen, S. & Full, R.J. (2008). Active tails enhance arboreal acrobatics in geckos. *Proceedings of the National Academy of Sciences of the United States of America,* 105: 4215–4219.

Kaiser, B.W. & Mushinsky, H.R. (1994). Tail loss and dominance in captive adult male *Anolis sagrei*. *Journal of Herpetology*, 28: 342–346.

Kelehear, C. & Webb, J.K. (2006). Effects of tail autotomy on anti-predator behavior and locomotor performance in a nocturnal gecko. *Copeia*, 2006: 803–809.

Kelt, D.A.; Nabors, L.K. & Forister, M.L. (2002). Size specific differences in tail loss and escape behavior in *Liolaemus nigromaculatus*. *Journal of Herpetology*, 36: 322–325.

King, R.B.; Stanford, K.M.; Jones, P.C. & Bekker, K. (2016). Size matters: Individual variation in ectotherm growth and asymptotic size. *PLoS ONE*, 11(1): e0146299.

Langkilde, T.; Alford, R.A. & Schwarzkopf, L. (2005). No behavioural compensation for fitness costs of autotomy in a lizard. *Austral Ecology*, 30: 713–718.

Lin, J.-W.; Chen, Y.-R.; Wang, Y.-H.; Hung, K.-C. & Lin, S.-M. (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(1847): 20162538.

Lin, Z.-H. & Ji, X. (2005). Partial tail loss has no severe effects on energy stores and locomotor performance in a lacertid lizard, *Takydromus septentrionalis*. *Journal of Comparative Physiology B*, 175; 567–573.

Lin, Z.-H., Qu, Y.-F. & Ji, X. (2006). Energetic and locomotor costs of tail loss in the Chinese skink, *Eumeces chinensis*. *Comparative Biochemistry and Physiology Part A*, 143: 508–513.

Lin, Z.-H.; Fan, X.; Lu, H.-L.; Luo, L.-G. & Ji, X. (2010). Effect of tail loss on energy stores and locomotor performance in the blue-tailed skink, *Eumeces elegans*. *Acta Ecologica Sinica*, 30(10): 2541–2548.

Liu, P.; Liu, L.-L.; Liu, Z.-T.; Xia, Y.-G.; Ji, X. & Zhao, W.-G. (2011). Effects of tail loss on locomotor ability and selected body temperature of common lizard *Lacerta vivipara*. *Chinese Journal of Ecology*, 30(9):2034–2038.

Lorenzon, P.; Clobert, J.; Oppliger, A. & John-Adler, H. (1999). Effect of water constraint on growth rate, activity and body temperature of yearling common lizard. *Oecologia*, 118: 423–430.

Lu, H.-L.; Ji, X. & Du, W.-G. (2013). Tail loss reduces locomotor ability but not metabolic rate in a viviparous skink, *Sphenomorphus indicus*. *Animal Biology*, 63: 369–380.

Lynn, S.E.; Borkovic, B.P. & Russell, A.P. (2013). Relative apportioning of resources to the body and regenerating tail in juvenile leopard geckos (*Eublepharis macularius*) maintained on different dietary rations. *Physiological and Biochemical Zoology*, 86(6): 659–668.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Martín, J. & Avery, R.A. (1997). Tail loss affects prey capture 'decisions' in the lizard *Psammodromus algirus*. *Journal of Herpetology*, 31: 292–295.

Martín, J. & Avery, R.A. (1998). Effects of tail loss on the movement patterns of the lizard, *Psammodromus algirus*. *Functional Ecology*, 12: 794–802.

Martín, J. & Salvador, A. (1992). Tail loss consequences on habitat use by the Iberian rock lizard, *Lacerta monticola*. *Oikos*, 65: 328–333.

Martín, J. & Salvador, A. (1993a). Tail loss and foraging tactics of the Iberian rock-lizard, *Lacerta monticola*. Oikos, 66: 318–324.

Martín, J. & Salvador, A. (1993b). Tail loss reduces mating success in the Iberian rock-lizard, *Lacerta monticola*. *Behavioral Ecology and Sociobiology*, 32: 185–189.

Martín, J. & Salvador, A. (1993c). Thermoregulatory behaviour of rock lizards in response to tail loss. *Behaviour*, 124: 123–136.

Martín, J. & Salvador, A. (1995). Effects of tail loss on activity patterns of rock-lizards, *Lacerta monticola*. *Copeia*, 1995(4): 984–988.

Martín, J. & Salvador, A. (1997). Effects of tail loss on the time-budgets, movements, and spacing patterns of Iberian rock lizards, *Lacerta monticola*. *Herpetologica,* 53(1): 117–125.

McConnachie, S. & Whiting, M. (2003). Costs associated with tail autotomy in an ambush foraging lizard, *Cordylus melanotus melanotus*. *African Zoology*, 38: 57–65.

McElroy, E.J. & Bergmann, P.J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology*, 86: 669–679.

Medger, K.; Verburgt, L. & Bateman, P.W. (2008). The influence of tail autotomy on the escape response of the Cape dwarf gecko, *Lygodactylus capensis*. *Ethology*, 114: 42–52.

Michelangeli, M.; Melki-Wegner, B.; Laskowski, K.; Wong, B.B.M. & Chapple, D.G. (2020). Impacts of caudal autotomy on personality. *Animal Behaviour*, 162: 67–78.

Niewiarowski, P.H.; Congdon, J.D.; Dunham, A.E.; Vitt, L.J. & Tinkle, D.W. (1997). Tales of lizard tails: effects of tail autotomy on subsequent survival and growth of free-ranging hatchling *Uta stansburiana*. *Canadian Journal of Zoology*, 75: 542–548.

Peters, R.H. (1983). *The ecological implications of body size*. Cambridge University Press, Cambridge.

Pond, C.M. (1978). The effects of tail loss on rapid running in *Dipsosaurus dorsalis*. *American Zoologist*, 18: 612.

Punzo, F. (1982). Tail autotomy and running speed in the lizards *Cophosaurus texanus* and *Uma notata*. *Journal of Herpetology*, 16: 329–331.

Putman, B.J.; Coss, R.G. & Clark, R.W. (2015). The ontogeny of antipredator behavior: age differences in California ground squirrels (*Otospermophilus beecheyi*) at multiple stages of rattlesnake encounters. *Behavioral Ecology and Sociobiology,* 69: 1447–1457.

Pyron, R.A.; Burbrink, F.T. & Wiens, J.J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13: 93.

Reznick, D.N.; Nunney, L. & Tessier, A. (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution*, 15: 421–425.

Saint Girons, H. & Duguy, R. (1970). Le cycle sexuel de *Lacerta muralis* L. en plaine et en montagne. *Bulletin du Muséum national d'histoire naturelle*, 42: 609–625.

Salvador, A. & Veiga, J.P. (2005). Activity, tail loss, growth and survivorship of male *Psammodromus algirus. Amphibia-Reptilia,* 26: 583–585.

Salvador, A., Martín, J. & López, P. (1995). Tail loss reduces home range size and access to females in male lizards, *Psammodromus algirus*. *Behavioural Ecology*, 6: 382–387.

Sannolo, M. & Carretero, M.A. (2019). Dehydration constrains thermoregulation and space use in lizards. *PLoS ONE*, 14(7): e0220384.

Sannolo, M.; Barroso, F.M. & Carretero, A.M. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, 126: 58–64.

Shine, R. (2003). Locomotor speeds of gravid lizards: placing 'costs of reproduction' within an ecological context. *Functional Ecology*, 17: 526–533.

Smith, G.R. (1996). Tail loss in the striped plateau lizard, *Sceloporus virgatus*. *Journal of Herpetology*, 30: 552–555.

Smyth, M. (1974). Changes in the fat stores of the skinks Morethia boulengeri and *Hemiergis peronii* (Lacertilia). *Australian Journal of Zoology*, 22: 135–145.

Starostová, Z.; Gvoždíkb, L. & Kratochvíl, L. (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.

Sun, Y.-Y.; Yang, J. & Ji, X. (2009). Many-lined sun skinks (*Mabuya multifasciata*) do not compensate for the costs of tail loss by increasing feeding rate or digestive efficiency. *Journal of Experimental Zoology*, 311A: 125–133.

Taylor, J.A. (1984). Ecology of the lizard, *Ctenotus taeniolatus*; interaction of life history, energy storage and tail autotomy. Ph.D. Thesis, University of New England, Armidale, Australia.

van Noordwicjk, A.J. & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, 128(1): 137–142.

Van Sluys, M. (1998). Growth and body condition of the saxicolous lizard *Tropidurus itambere* in southeastern Brazil. *Journal of Herpetoly*, 32: 359–365.

Vitt, L.J. & Cooper, W.E., Jr (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.

Wang, Y.; Zeng, Z.G.; Li, S.R.; Bi, J.H. & Du, W.G. (2016). Low precipitation aggravates the impact of extreme high temperatures on lizard reproduction. *Oecologia*, 182: 961–971.

Webb, J.K. (2006). Effects of tail autotomy on survival, growth and territory occupation in free-ranging juvenile geckos (*Oedura lesueurii*). *Austral Ecology*, 31: 432–440.

Willey, J.S.; Biknevicius, A.R.; Reilly, S.M. & Earls, K.D. (2004). The tale of the tail: limb function and locomotor mechanics in *Alligator mississippiensis*. *Journal of Experimental Biology*, 207: 553–563.

Wilson, B.S. (1992). Tail injuries increases the risk of mortality in free-living lizards (Uta stansburiana). *Oecologia*, 92: 145–152.

Wilson, R.S. & Booth, D.T. (1998). The effect of tail loss on reproductive output and its ecological significance in the skink *Eulamprus quoyii*. *Journal of Herpetology*, 32: 128–131.

Wilson, R.S. (1994). *Physiological responses to caudal autotomy in* Eulamprus quoyii. Honours Thesis, University of Queensland, Brisbane, Australia.

Zajitschek, F. & Connallon, T. (2017). Partitioning of resources: the evolutionary genetics of sexual conflict over resource acquisition and allocation. *Journal of Evolutionary Biology*, 30: 826–838.

Zamora-Camacho, F.J.; Reguera, S. & Moreno-Rueda, G. (2015). Does tail autotomy affect thermoregulation in an accurately thermoregulating lizard? Lessons from a 2200-m elevational gradient. *Journal of Zoology*, 297(3): 204–210.

Zamora-Camacho, F.J.; Rubiño-Hispán, M.V.; Reguera, S. & Moreno-Rueda, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid *Psammodromus algirus*. *Herpetological Journal*, 26: 213–218.

Zheng, Y. & Wiens, J.J. (2016). Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Molecular Phylogenetics and Evolution*, 94: 537–547.

VIII. CONCLUSIONS

CONCLUSIONS

1. Tail loss impairs some aspects of the locomotor performance of adult wall lizards in complex spaces with interspersed obstacles, mainly by decreasing the effectiveness of jumping dynamics. On the contrary, tail loss does not affect sprint speed over horizontal, unhindered surfaces, likely due to a compensation between a negative effect of tail loss on the stride of front limbs and a positive effect of losing mass and friction force after autotomy. The impaired locomotor capacities are fully restored after tail regeneration, which increases the efficiency of autotomy by minimizing the costs associated to tail loss.

2. Autotomized tails perform intense and complex movements and flips that are stronger immediately after detachment and experience an exponential decrease in strength with time. This forceful initial response determines the antipredator value of autotomy, as it ensures predator's distraction and the escape success. Tails with regenerated portions perform weaker movements after autotomy than intact ones, probably due to structural differences, like an unsegmented cartilaginous axis, that limit the mobility of regenerated tails. Therefore, the antipredator value of tails, as indicated by the intensity of post-autotomic movements, is only partially retrieved after regeneration.

3. After losing their tails, wall lizards modify their behaviour by spending more time performing activities with antipredatory rather than exploratory significance, which probably increases the effectiveness of their cryptic behaviour. These differences were even more important in the presence of chemical cues of specific predators (*Coronella austriaca*). These behavioural shifts imply an intensification of their

early-stage predator-avoidance mechanisms, reducing the probability of detection by predators.

4. Tail loss does not affect the thermal preferences of Bocage's wall lizards, and their dehydration rates are also not affected after tail loss, suggesting a negligible increase of water loss through the injury and no specific ecophysiological responses after autotomy regarding hydroregulation. This implies that the shifts observed in autotomized lizards in the field are to be considered primarily behavioural rather than physiological. These shifts could be caused by alterations in the thermoregulatory behaviour to diminish predation risk after autotomy.

5. The investment in tail regeneration provokes a decrease in body growth rates of juvenile wall lizards when they face a situation of low food availability, but not when food is available *ad libitum*. Therefore, food availability is a key factor for the occurrence of trade-offs between regeneration and other growth processes, so that environmental conditions would be determinant for the severity of the costs of regeneration.

6. Regenerating hatchlings increase their ingestion rates compared to intact ones during the period of greatest tail growth, which reveals a cost of tail regeneration. Besides, hatchlings invest more in tail regeneration in relation to body growth when they have few resources, rather than delay regeneration to give priority to body growth. This suggests that tail regeneration in juveniles could be prioritized even at the expenses of body growth, to restore the lost functionality as soon as possible and increase survivorship.

7. When regeneration initiates at the end of the breeding season, there is a reduction of the reproductive investment in the first clutch of the next reproductive season. This is likely because regeneration may interfere with the accumulation of reserves, which are essential for the development of the first annual clutch, built under a capital breeding strategy.

8. Caudal regeneration provokes a significant reduction of the reproductive investment of adult females when it occurs concurrently with the vitellogenesis of the second annual clutch, built under an income breeding strategy. Furthermore, the investment in tail regeneration was negatively correlated with the investment in clutch development, which evidences a clear trade-off between these two processes.

9. The reduction of reproductive investment due to tail regeneration is more severe when regeneration occurs concurrently with clutch development under an income breeding strategy, which suggests that capital breeding may buffer the costs of regeneration on reproduction.

10. The relative tail regrowth rate is much greater in juveniles in terms of length, being almost twice than that of adults, while adults regenerate almost twice than juveniles the volume of tail per day in relation to their body size. Tail regeneration may be more costly in adult than in young lizards, both in terms of time (to reach a minimum length that enables functionality) and of demands of energy and resources.

IX. RESUMEN Y CONCLUSIONES

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Introducción

Algunos de los factores más influyentes en los ciclos de vida de los organismos son la fenología de ciertos eventos clave y la cantidad de recursos destinados a ellos. En otras palabras: dado que los recursos en la vida son siempre limitados, las decisiones sobre cuándo y a dónde destinarlos definirán los ciclos de vida de los individuos (Roff 1992; van der Meer 2019). En este escenario, la inversión en el desarrollo de un rasgo implicará una menor cantidad de recursos disponibles para otros rasgos, desencadenando un conflicto de asignación de recursos (también conocido como *trade-offs* de asignación de recursos; Stearns 1992). Los *trade-offs* son muy comunes en los organismos y tienen consecuencias tanto a nivel fisiológico como evolutivo, constituyendo incluso la base de varias teorías de ciclos de vida (Stearns 1992). Los conflictos de asignación varían dependiendo del factor limitante: pueden surgir cuando la dedicación de tiempo a una actividad implica el abandono de otras, cuando el desarrollo de un rasgo funcional compromete el desarrollo o rendimiento de otros (*trade-off* funcional), o cuando el factor limitante son la energía y los recursos disponibles para el desarrollo de varios rasgos (*trade-off* de asignación de recursos). Algunos ejemplos clásicos de *trade-offs* de asignación de recursos en animales son la inversión en la reproducción actual frente a la reproducción futura, en el crecimiento frente a la reproducción (que, en organismos con crecimiento indeterminado, implica la decisión de cuándo alcanzar la madurez sexual), en el tamaño de huevo frente al tamaño de la puesta, etc (Elliott 1994; Roff 2002).

Autotomía significa, literalmente, "auto-amputación", y fue definido originalmente por Fredericq (1892) como la pérdida voluntaria y/o programada (es decir, no traumática) de una parte del cuerpo, como un acto reflejo cuando el animal se siente amenazado. De acuerdo con Emberts *et al.* (2019), la autotomía ha evolucionado independientemente al menos nueve veces en distintos grupos animales, tanto invertebrados como vertebrados. Los grupos animales capaces de autotomizar partes del cuerpo son: cnidarios, anélidos, moluscos (gasterópodos, bivalvos y cefalópodos), platelmintos, nemátodos, forónidos, artrópodos (arácnidos, decápodos e insectos), equinodermos, hemicordados y vertebrados (anfibios, reptiles y mamíferos). La autotomía suele ocurrir a través de planos de fractura predefinidos, que facilitan la ruptura y minimizan los costes asociados a la herida mediante la reducción de la pérdida de fluidos, la disminución de la probabilidad de contraer infecciones y la aceleración de la curación de la herida (Maginnis 2006). El beneficio más común de la autotomía es sobrevivir a ataques subletales de depredadores (Fleming *et al.* 2007; Emberts *et al.* 2019), pero para algunos animales, la autotomía también ofrece otros beneficios, como solucionar complicaciones de la muda (Maginnis 2008), eliminar toxinas de una parte del cuerpo (Moore *et al.* 1989) o sobrevivir a daños físicos causados por factores abióticos (Wulff 2006).

Entre los vertebrados, la autotomía es particularmente frecuente en reptiles y especialmente en saurios, en los que está realmente extendida, estando presente en 13 de las 20 familias de saurios (Clause & Capaldi 2006). La autotomía caudal en saurios ocurre a través de planos de fractura presentes en las vértebras postpigales, que son con mucha mayor frecuencia intravertebrales (e.g., en lacértidos, escíncidos, geckos, cordílidos o teíidos) que intervertebrales (e.g., en algunas especies de agámidos). Los planos de fractura intravertebrales atraviesan cada vértebra transversalmente, pasando a través del centro y del arco neural (ver Figura 1.2). Las características y mecanismos de la autotomía caudal en saurios, y particularmente en lacértidos, han sido ampliamente revisadas por Bellairs & Bryant (1985).

En saurios, la autotomía caudal se utiliza frecuentemente como una estrategia antidepredación, y tiene una gran incidencia en poblaciones naturales de algunas especies, en las que más de la mitad de la población adulta ha autotomizado la cola al menos una vez en su vida (Arnold 1988; Downes & Shine 2001; Pafilis *et al.* 2008 para lacértidos). La autotomía caudal no sólo permite al lagarto zafarse del depredador cuando es agarrado por la cola, sino que además permite una distracción efectiva del depredador que facilita la huida, ya que la cola realiza movimientos y saltos vigorosos y complejos inmediatamente tras su desprendimiento (Dial & Fitzpatrick 1983; Arnold 1988; Fernández-Rodríguez & Braña 2020). Sin embargo, en muchas especies de saurios la cola juega un papel importante en la locomoción, en la señalización social y en la acumulación de reservas, de manera que su pérdida puede tener consecuencias negativas a corto plazo, pudiendo inducir cambios comportamentales o disminuir la eficiencia de otras funciones como la alimentación, el emparejamiento o el patrulleo.

La regeneración es la restauración, parcial o completa, de una parte del cuerpo perdida mediante una herida traumática o voluntaria (Maginnis 2006), y permite el reemplazo de las partes perdidas que no son vitales pero que sí incrementan la probabilidad de supervivencia o la capacidad de inversión reproductora (Goss 1969). La regeneración se originó probablemente en los primeros animales con el desarrollo de la multicelularidad (Bely & Nyberg 2010), posiblemente como un efecto secundario de un acceso continuado a los programas de desarrollo, que podrían ser reactivados cada vez que se amputaba una parte del cuerpo (hipótesis del epifenómeno, Goss 1992; Mani & Tlusty 2021). En la actualidad, la capacidad de regenerar partes del cuerpo varía considerablemente entre animales. Por ejemplo, todos los linajes de metazoos basales (placozoos, poríferos, ctenóforos y cnidarios), junto con algunos lofotrocozoos y deuteróstomos no craneados, son capaces de regenerar cualquier parte del cuerpo, mientras que esa capacidad de regeneración completa está ausente en ecdisozoos y craneados (para una revisión completa, ver Bely & Nyberg 2010).

En saurios, la regeneración completa de la cola requiere en torno a tres meses en la mayoría de las especies (Bellairs & Bryant 1985), y es un proceso epimórfico (es decir, implica proliferación celular y la formación de un blastema, Goss 1969). La regeneración ocurre en tres fases: i) recubrimiento y curación de la herida, ii) desdiferenciación celular y formación del blastema, y iii) proliferación celular y formación de la cola (Bryant *et al.* 2002; Gilbert *et al.* 2013). Las dos primeras fases tienen una duración corta y se consideran como un periodo latente, en el que no hay crecimiento evidente de la cola. Inmediatamente tras la pérdida de la cola, los restos de haces musculares y tejidos epiteliales se contraen para cerrar la herida, y durante las horas siguientes se secarán y coagularán junto con los restos de sangre para formar una postilla que protegerá la herida durante la curación y formación del blastema (McLean & Vickaryous 2011). Durante estos primeros días ocurre una importante migración y desdiferenciación celular bajo la postilla para formar el blastema, que es una masa de células desdiferenciadas derivadas del tejido preexistente del muñón (Bellairs & Bryant 1985; Lozito & Tuan 2016). Al cabo de aproximadamente una semana, la postilla cae y comienza el periodo de crecimiento y elongación (McLean & Vickaryous 2011; Fernández-Rodríguez & Braña 2020); durante esta fase inicial la porción regenerada tiene un aspecto violáceo oscuro, y la queratinización, formación de escamas y pigmentación no ocurrirán hasta pasadas aproximadamente tres semanas tras la autotomía (Bellairs & Bryant 1985).

La cola regenerada no es una réplica perfectamente fiel, ya que presenta varias diferencias estructurales. La mayor diferencia es el eje esquelético, ya que en la cola regenerada este eje es un tubo continuo de cartílago, en lugar de vértebras óseas articuladas (Hughes & New 1959; ver Figura 1.3). Esta diferencia tiene importantes implicaciones funcionales, ya que el eje continuo de cartílago no sólo limita la movilidad y flexibilidad de la cola regenerada, sino que además impide una nueva autotomía a través de la parte regenerada, ya que el eje de cartílago carece de planos de fractura. Por tanto, una nueva autotomía sólo será posible a través de las porciones anteriores de cola aún intacta. Además, la disposición de los paquetes musculares en la cola regenerada es irregular, son variables en número y se unen de manera irregular al eje de cartílago, mientras que los músculos de la cola intacta se agrupan regularmente en cuatro cuadrantes fijados a la columna vertebral y participan tanto en la movilidad de cada vértebra como en la fractura en un punto específico durante la autotomía (Fischer *et al.* 2012). A pesar de estas diferencias morfológicas, la regeneración permite la restauración de varias de las funcionalidades perdidas tras la autotomía caudal, proporcionando varios beneficios que incrementan la probabilidad de supervivencia y la *fitness* del lagarto (Bateman & Fleming 2009). Sin embargo, la regeneración implica una importante demanda de energía y materiales, y esto puede interferir con otros procesos con alta demanda como el crecimiento o la reproducción, con posibles consecuencias para la supervivencia y la *fitness* (Maginnis 2006).

Objetivos

La autotomía y la regeneración de partes del cuerpo, pese a sus múltiples beneficios, pueden provocar varios *trade-offs* funcionales y de asignación de recursos cuya intensidad puede variar en función del sexo, la fase del ciclo vital o las condiciones ambientales (Maginnis 2006). El balance entre los costes y beneficios de la autotomía y la regeneración moldea su evolución y es determinante para la presencia o ausencia de estas dos adaptaciones en los animales. Por tanto, el estudio de este balance, incluyendo el análisis de sus implicaciones fisiológicas, funcionales y ecológicas, es un enfoque apropiado para entender la evolución de la autotomía y la regeneración en los distintos grupos animales.

En este contexto, los principales objetivos de esta Tesis Doctoral son el estudio de los efectos de la autotomía y la regeneración sobre varios de los principales aspectos de los ciclos de vida y funciones vitales de las lagartijas. Para la consecución de dichos objetivos generales, se definieron los siguientes objetivos específicos:

1) Estudiar los costes de la autotomía caudal en varios aspectos funcionales de la biología de las lagartijas, cuya alteración probablemente influencie el comportamiento y el rendimiento de funciones ecológicas con una relevancia potencial para la *fitness*. En relación con este objetivo, se han estudiado los siguientes aspectos: rendimiento en locomoción (**Capítulo 1**), comportamiento antidepredación (**Capítulo 2**), termorregulación e hidrorregulación (**Capítulo 3**).
2) Evaluar los costes de la regeneración caudal y estudiar, bajo el marco teórico de los *trade-offs* de asignación de recursos, cómo afecta a los dos principales compartimentos en los que se destina el crecimiento en diferentes etapas del ciclo de vida. En este contexto, se estudió el efecto de la regeneración caudal en el crecimiento corporal de juveniles sexualmente inmaduros (**Capítulo 4**) y en la inversión reproductora (tamaño de huevo, tamaño de puesta, masa de puesta y frecuencia entre puestas) de hembras adultas (**Capítulo 5**).

Resultados principales y discusión

Los **Capítulos 1 y 2** se centraron en el estudio de los efectos de la autotomía caudal tomando como modelo de estudio la lagartija roquera (*Podarcis muralis*), mientras que los **Capítulos 3 y 4** se enfocaron en el estudio de los costes de la regeneración de la cola en esta especie. Adicionalmente, en el **Capítulo 3** se estudiaron los efectos de la pérdida de la cola en las preferencias térmicas y las tasas de pérdida de agua en la lagartija de Bocage (*P. bocagei*), ya que este estudio se realizó durante una estancia internacional en el centro de investigación CIBIO-InBIO, en el norte de Portugal, donde no hay *P. muralis.* Estas dos especies están próximamente relacionadas, ocupan hábitats similares y tienen requerimientos ecológicos similares (Harris & Sá-Sousa 2002; García-Porta *et al.* 2019). Los aspectos descritos anteriormente fueron elegidos como objeto de estudio porque representan algunos de los principales componentes de los ciclos de vida y funciones vitales con una gran relevancia ecológica, con repercusión en todo el organismo y que están, por tanto, sometidos a selección natural. De esta manera, los estudios llevados a cabo en esta tesis doctoral pretenden trazar una visión bastante amplia de la evolución de la autotomía y la regeneración en lagartijas roqueras.

Efectos de la autotomía caudal

La autotomía caudal es común en el lacértido *Podarcis muralis*, en el que más del 50% de los individuos de la población estudiada en esta Tesis Doctoral muestran signos de haber autotomizado la cola al menos una vez en su vida (**Capítulo 1**; Fernández-Rodríguez & Braña 2020). La autotomía caudal proporciona un beneficio inmediato para la supervivencia al facilitar el escape de la lagartija distrayendo al depredador (Arnold 1988; Higham & Russell 2010). Tras sobrevivir al ataque del depredador mediante el desprendimiento de la cola, la primera consecuencia inmediata de la autotomía está relacionada con la capacidad de locomoción del animal (ver Figura 7.1). La cola juega un papel importante en la locomoción de los saurios actuando como contrapeso, ayudando a mantener la estabilidad mientras corren o trepan, e influenciando las dinámicas del salto (Arnold 1988; Jusufi *et al.* 2008), de manera que su pérdida puede alterar el centro de masas del animal y la biomecánica de su locomoción. En particular, la autotomía caudal perjudica algunos aspectos del rendimiento locomotor de las lagartijas roqueras adultas, principalmente reduciendo la efectividad de las dinámicas del salto (**Capítulo 1**; Fernández-Rodríguez & Braña 2020). Este deterioro de la capacidad de locomoción afectará probablemente a actividades rutinarias de las lagartijas que dependen de la movilidad (como el patrulleo, la obtención de alimento, el emparejamiento, etc; Garland & Losos 1994; Braña 2003; Husak 2006) y, por supuesto, dificultarán el escape de nuevos ataques de depredadores. En dichas circunstancias, las lagartijas probablemente se enfrentan a un mayor riesgo de depredación tras la autotomía, incrementado por la imposibilidad de recurrir a la autotomía caudal de nuevo en futuros encuentros con depredadores.

Sin embargo, las lagartijas sin cola frecuentemente recurren a comportamientos compensatorios, que reducen el riesgo de depredación. De acuerdo con los datos y análisis presentados en el **Capítulo 2**, algunas de estas alteraciones comportamentales incluyen la intensificación de las fases tempranas de los mecanismos de evasión de depredadores (ver Putman *et al.* 2015), para reducir la probabilidad de detección por depredadores y disminuir la frecuencia de ataques. Estos cambios comportamentales, como reducir la movilidad, incrementar el uso del refugio o disminuir la exploración (Fox *et al.* 1981; Martín & Salvador 1993b; Michelangeli *et al.* 2020), probablemente incrementan la probabilidad de supervivencia inmediata de las lagartijas sin cola, pero a costa de reducir el rendimiento de otras actividades relevantes, como la obtención de alimento o el emparejamiento (Martín & Salvador 1993a; Martín & Avery 1997). La disminución del rendimiento de estas actividades relevantes podría secundariamente afectar a las tasas de crecimiento de los juveniles o al éxito reproductor de los adultos, con un potencial efecto global en la *fitness* del individuo (ver Figura 7.1).

Otros aspectos comportamentales y/o fisiológicos, como las preferencias térmicas y la selección de temperaturas corporales, no estuvieron aparentemente afectadas por la pérdida de la cola en *P. bocagei* (**Capítulo 3**; Fernández-Rodríguez *et al.* 2021). El nivel de hidratación es un factor clave para la termorregulación, ya que la deshidratación aumenta el riesgo de sobrecalentamiento y compromete los niveles de actividad, el crecimiento o la reproducción (Lorenzon *et al.* 1999; Wang *et al.* 2016; Sannolo & Carretero 2019). Seleccionar temperaturas más altas tras la autotomía podría resultar en tasas de desecación más rápidas, siendo incluso más severo en regiones con periodos de sequía estival cortos o relativamente largos (Sannolo *et al.* 2018). Por otro lado, disminuir la temperatura corporal ralentizaría el metabolismo y tendría un impacto negativo en el rendimiento de las lagartijas. Por tanto, parece plausible que la opción más beneficiosa tras la autotomía, en cuanto a mantener la homeostasis interna, sea mantener la temperatura corporal preferida. Otro factor importante a tener en cuenta es que este estudio se centró sólo en la temperatura corporal, cuando hay otros factores comportamentales y fisiológicos de la termorregulación de los saurios que podrían verse afectados tras la pérdida de la cola. Algunos de estos aspectos podrían ser el tiempo y la frecuencia de los episodios de termorregulación (que determinan la precisión termorreguladora), los microhábitats seleccionados para la termorregulación, o las tasas de enfriamiento y calentamiento (dado que la pérdida de la cola implica un cambio drástico en morfología, en masa y en relación superficie/volumen). Las tasas de deshidratación en *P. bocagei* tampoco se vieron afectadas tras la pérdida de la cola

(**Capítulo 3**; Fernández-Rodríguez *et al.* 2021), aunque de nuevo estos resultados aportan una visión preliminar (y novedosa) de los potenciales efectos de la autotomía caudal en el balance hídrico de las lagartijas. Para alcanzar una mejor comprensión de los efectos a corto plazo de la pérdida de la cola en los balances térmico e hídrico, deben tenerse en cuenta más factores, como diferentes niveles de hidratación y sus consecuencias para la termorregulación tras la autotomía.

Efectos de la regeneración de la cola

Los costes funcionales y comportamentales a corto-medio plazo de la autotomía caudal anteriormente descritos son con una gran probabilidad meramente temporales, de manera que desaparezcan progresivamente a medida que progresa la regeneración de la cola. En *Podarcis muralis* la elongación de la cola se completa tras aproximadamente 2-3 meses, y la diferenciación externa de las escamas se completa tras 3-4 meses (observación personal de la autora). La regeneración tras la autotomía permite la restauración de las funcionalidades perdidas con la parte desprendida (Goss 1969; Clause & Capaldi 2006), como se demostró en *P. muralis* para la capacidad de locomoción (**Capítulo 1**; Fernández-Rodríguez & Braña 2020). Por tanto, la regeneración incrementaría la probabilidad de supervivencia mediante la restauración de ciertas capacidades funcionales del animal (Lin *et al.* 2017). Sin embargo, la cola regenerada mantiene pobremente su valor protector en futuros encuentros con depredadores en los que se necesitaría recurrir de nuevo a la autotomía. En primer lugar, porque la capacidad de desprenderse de la cola queda restringida a la porción anterior de la cola que aún está intacta, ya que la ausencia de planos de fractura en la cola regenerada impiden que se pueda romper a través de ella. Y, en segundo lugar, porque algunas características esenciales que aumentan la eficacia de la autotomía (i.e., incrementan su beneficio, Emberts *et al.* 2019) son los violentos movimientos y saltos realizados por la cola desprendida, especialmente intensos durante los primeros segundos tras el desprendimiento (Dial & Fitzpatrick 1983; Fernández-Rodríguez & Braña 2020). Estos fuertes movimientos iniciales refuerzan sustancialmente la distracción del depredador, incrementando las posibilidades de la lagartija de sobrevivir al ataque y escapar (Bellairs & Bryant 1985; Higham & Russell 2010). Como se demostró en el **Capítulo 1**, las colas regeneradas se mueven significativamente menos y durante menos tiempo que las intactas, de manera que su valor antidepredador es sin duda considerablemente inferior. Este hecho está muy probablemente relacionado con las diferencias estructurales entre las colas intactas y regeneradas, que restringen los movimientos y las flexiones de las regeneradas (Hughes & New 1959). En conclusión, la regeneración caudal incrementa la eficiencia de la autotomía mediante la reducción de los costes (a corto-medio plazo) de la pérdida de la cola, pero no restaura por completo la eficacia de futuras autotomías caudales.

La regeneración de la cola implica una gran demanda de energía y recursos que puede desencadenar un conflicto de asignación de recursos entre invertir en regeneración o en otros procesos que también demandan grandes cantidades de recursos, como el crecimiento la reproducción (ver Figura 7.1). En el **Capítulo 4** se ha demostrado que la inversión en regeneración caudal provoca una disminución de las tasas de crecimiento en crías de lagartija roquera cuando afrontan una situación de baja disponibilidad de alimento. Es probable que las crías y los juveniles afronten con relativa frecuencia dicha limitación en la disponibilidad de recursos en condiciones naturales, si consideramos que: i) las crías no conocen el territorio y no han perfeccionado aún las habilidades para identificar, abordar y capturar a las presas (Iraeta *et al.* 2012); ii) las lagartijas jóvenes disponen de un abanico menos amplio de presas potenciales, restringidas tanto en tamaño como en espectro taxonómico (ver Braña 1981 para *P. muralis*); iii) las crías y los juveniles aún no tienen desarrolladas por completo sus capacidades de locomoción (Iraeta *et al.* 2012), y esta limitación es aún mayor para los individuos sin cola (Fernández-Rodríguez & Braña 2020), que puede que tengan por tanto una eficiencia de obtención de alimento menor.

Sin duda, el valor adaptativo de la autotomía y regeneración depende del balance entre sus costes y beneficios, el cual determinará en gran medida su selección y mantenimiento (Arnold 1988). Alcanzar un tamaño corporal grande durante los primeros meses de vida es crucial en varios aspectos: incrementa el espectro de presas potenciales, reduce el riesgo de depredación y permite afrontar el primer periodo de brumación invernal en una buena condición física en términos de tamaño y reservas energéticas. Esto incrementa la probabilidad de supervivencia de las lagartijas juveniles y reduce el tiempo necesario para alcanzar la madurez sexual (Bauwens 1981; Peters 1983; Brownikowski & Arnold 1999). Por tanto, invertir en crecimiento corporal parece prioritario, pero aún a costa de reducir las tasas de crecimiento corporal, parece plausible que la restauración plena de las capacidades perdidas proporciona grandes beneficios a largo plazo, dado que dichas capacidades funcionales son esenciales también durante la edad adulta.

En relación a los costes de la regeneración en etapas adultas, se ha demostrado que la regeneración tiene efectos negativos en la inversión reproductora de las hembras adultas tanto a corto como a largo plazo, provocando una reducción en el peso de la puesta de huevos (**Capítulo 5**). Además, esta reducción es más severa cuando la regeneración ocurre a la vez que el desarrollo de los huevos bajo una estrategia *income breeding*. Pese a que los costes en *P. muralis* no son tan extremos como en otras especies (como *Ctenotus taeniolatus*, que omite la formación de la puesta durante la regeneración, Taylor 1984), estos costes no son para nada triviales. Por tanto, para entender el balance de los costes y beneficios de la regeneración durante etapas adultas, es necesario considerar su impacto tanto en la reproducción actual como futura. Esto significa que, pese a que la regeneración de la cola implique una reducción en la inversión simultánea en reproducción, incrementará la probabilidad de supervivencia (como se explicó anteriormente en el **Capítulo 1**) y la reproducción futura, lo que en último término se traduce en una mayor *fitness* total del individuo.

El crecimiento corporal es uno de los procesos que más energía y recursos demanda durante etapas juveniles, mientras que durante la estación reproductora las hembras adultas invierten la mayor parte de sus recursos principalmente en el desarrollo de los huevos, por lo que puede considerarse que la reproducción es el proceso que más energía demanda en las hembras adultas durante esos meses. Merece la pena destacar las diferencias entre crías y hembras adultas en el impacto de la regeneración, dado que las consecuencias parecen ser más severas para la inversión reproductora de las hembras (**Capítulo 5**) que para las tasas de crecimiento de las crías (**Capítulo 4**). De hecho, los juveniles sólo ralentizan las tasas de crecimiento durante la regeneración cuando disponen de pocos recursos, mientras que las hembras redujeron su inversión reproductora incluso aun disponiendo de alimento *ad libitum*. Las hembras de lagartija roquera desarrollan hasta tres puestas de huevos al año con un intervalo de un mes entre puestas, y cada una representa el 30-40 % de su masa corporal (Saint-Girons & Duguy 1970; Ji & Braña 2000), lo que implica una inversión de recursos muy intensa en un periodo corto de tiempo. Sin embargo, el crecimiento de las crías, pese a ser rápido, es un proceso más continuo en el que la inversión está más extendida en el tiempo, por lo que probablemente la inversión en crecimiento sea más gradual y menos intensa que en la formación de la puesta. La alta demanda energética de la reproducción, y especialmente su concentración en un periodo corto de tiempo, puede explicar parcialmente el hecho de que las hembras adultas, al contrario que las crías, fuesen incapaces de satisfacer los requerimientos energéticos de la reproducción y la regeneración simultáneamente, incluso con una gran disponibilidad de alimento.

Las diferencias entre juveniles y adultos en la velocidad de la regeneración caudal también pueden aportar información sobre cómo la importancia de este fenómeno puede cambiar a lo largo del ciclo de vida del animal. Si consideramos el valor absoluto del incremento en longitud de la cola regenerada en crías y adultos (ambos alimentados *ad libitum*), las tasas de regeneración en longitud son similares en ambos grupos de edad. Sin embargo, si comparamos la longitud de cola regenerada por día, en relación al tamaño corporal del animal (medido en longitud hocico-cloacal), el crecimiento relativo fue mucho mayor en juveniles, casi el doble que el de los adultos. Además, considerando el volumen de biomasa regenerada, la misma longitud de cola regenerada implica una tasa más rápida en adultos que en juveniles, como apoyaron los resultados de esta tesis doctoral. Esto es esperable, ya que la anchura del muñón de cola es mucho mayor en adultos que en juveniles. Curiosamente, al comparar el volumen de cola regenerada al día en relación al tamaño corporal, los adultos regeneran al día casi el doble de volumen relativo que las crías. Estos resultados tienen dos implicaciones importantes: en primer lugar, dado que la capacidad de locomoción está estrechamente relacionada con la longitud de la cola (Zamora-Camacho *et al.* 2016; **Capítulo 1** y Fernández-Rodríguez & Braña 2020 para *P. muralis*), los juveniles restaurarían las capacidades perdidas en un periodo de tiempo más corto que los adultos. En segundo lugar, como señalaron Bellairs & Bryant (1985), dados dos saurios de diferente tamaño pero igual proporción, que autotomizaran la misma proporción lineal de cola, la pérdida absoluta de longitud y volumen de cola sería mayor en el lagarto de mayor tamaño. Aunque la elongación relativa de la cola es más lenta en adultos que en crías, regeneran más volumen que las crías al día para alcanzar una longitud determinada. Estas dos implicaciones significan que la regeneración de la cola debería ser más costosa en lagartijas adultas que en crías, tanto en términos de tiempo (para regenerar un tamaño mínimo funcional) como de demanda de energía y recursos. Esto puede contribuir también a explicar el mayor impacto de la regeneración caudal observado en la inversión reproductora de hembras adultas que en las tasas de crecimiento corporal de crías.

Conclusiones

1. La pérdida de la cola tiene efectos negativos sobre algunos aspectos de la capacidad de locomoción de las lagartijas roqueras adultas en espacios complejos con obstáculos, principalmente reduciendo la eficiencia de las dinámicas del salto. Por el contrario, la pérdida de la cola no afecta a la velocidad de carrera en superficies horizontales sin obstáculos, probablemente debido a una compensación entre el efecto negativo de perder la cola sobre la zancada de las patas delanteras, y el efecto positivo de perder masa y fuerza de fricción tras la autotomía. Las capacidades de locomoción perdidas son completamente restauradas tras la regeneración de la cola, lo cual aumenta la eficiencia de la autotomía mediante la reducción de los costes asociados a la pérdida de la cola.

2. Las colas autotomizadas realizan movimientos y saltos intensos y complejos, en especial durante los segundos inmediatos tras la autotomía, que sufren un decrecimiento exponencial en intensidad con el tiempo. Esta respuesta inicial tan fuerte determina el valor antidepredador de la autotomía, ya que asegura la distracción del depredador y el éxito del escape. Las colas con porciones regeneradas realizan movimientos más débiles que las intactas tras la autotomía, probablemente debido a diferencias estructurales como el eje continuo de cartílago que tienen las

colas regeneradas en lugar de vértebras articuladas, lo que limita su movilidad. Por tanto, el valor antidepredador de las colas en cuanto a la intensidad de sus movimientos tras la autotomía, se recupera sólo parcialmente tras la regeneración.

3. Las lagartijas roqueras modifican su comportamiento tras perder la cola, empleando más tiempo en actividades con significado antidepredador en lugar de exploratorio, lo que probablemente incrementa la efectividad de su comportamiento críptico. Estas diferencias se manifiestan también en presencia de señales químicas de depredadores específicos (*Coronella austriaca*). Dichos cambios comportamentales implican una intensificación de las primeras etapas de los mecanismos de evasión de depredadores, reduciendo la probabilidad de ser detectadas por depredadores.

4. La pérdida de la cola no afecta a las preferencias térmicas de las lagartijas de Bocage ni a sus tasas de deshidratación, lo que sugiere que el incremento en la pérdida de agua a través de la herida es insignificante y que no hay respuestas ecofisiológicas específicas tras la autotomía en lo referente a la hidrorregulación. Esto implica que los cambios observados en condiciones naturales en animales autotomizados deberían considerarse principalmente comportamentales y no fisiológicos. Estos cambios podrían ser también provocados por alteraciones en el comportamiento termorregulador para disminuir el riesgo de depredación tras la autotomía.

5. La inversión en regeneración caudal provoca una disminución en la tasa de crecimiento corporal de las lagartijas roqueras juveniles cuando afrontan situaciones de baja disponibilidad de alimento, pero no cuando disponen de comida *ad libitum*. Por tanto, la disponibilidad de alimento es un factor clave para la manifestación de *trade-offs* entre la regeneración y otros procesos relacionados con el crecimiento, de manera que las condiciones ambientales serían determinantes para la severidad de los costes de la regeneración.

6. Las crías que regeneraron la cola incrementaron sus tasas de ingestión en comparación con las crías con colas intactas durante el periodo de mayor crecimiento de la cola, lo que revela un coste de la regeneración caudal. Además, cuando las crías disponen de pocos recursos, invierten más en la regeneración de la cola en relación al crecimiento corporal, en lugar de ralentizar la regeneración para dar prioridad al crecimiento corporal. Esto sugiere que la regeneración caudal en juveniles podría ser priorizada incluso a costa del crecimiento corporal, para restaurar las funcionalidades perdidas lo antes posible y aumentar la probabilidad de supervivencia.

7. Cuando la regeneración comienza al final del periodo reproductor, hay una reducción de la inversión reproductora en la primera puesta de huevos de la siguiente estación reproductora. Esto se debe probablemente a una interferencia de la regeneración con la acumulación de reservas, las cuales son esenciales para el desarrollo de la primera puesta anual, ya que se desarrolla en base a una estrategia *capital breeding*.

8. La regeneración caudal provoca una reducción significative de la inversión reproductora de las hembras adultas cuando ocurre a la vez que la vitelogénesis de la segunda puesta anual, construida en base a una estrategia *income breeding*. Además, la inversión en regeneración caudal está correlacionada negativamente con la inversión en el desarrollo de la puesta, lo que evidencia un claro *trade-off* entre estos dos procesos.

9. La reducción de la inversión reproductora debido a la regeneración caudal es más severa cuando la regeneración ocurre a la vez que el desarrollo de la puesta de huevos bajo una estrategia *income breeding*, lo que sugiere que la estrategia *capital breeding* podría amortiguar los costes de la regeneración en la reproducción.

10. La tasa relativa de regeneración caudal es mucho mayor en las crías en términos de longitud, siendo casi el doble que la de los adultos, mientras que los adultos regeneran al día casi el doble de volumen que las crías en relación a su tamaño corporal. La regeneración de la cola sería más costosa en lagartijas adultas que en crías, tanto en términos de tiempo (para alcanzar una longitud mínima funcional) como de demandas de energía y recursos.

Referencias

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Arnold, E.N. (1988). Caudal autotomy as a defense. In: Gans, C. & Huey, R.B. (Eds.), *Biology of the Reptilia*. Vol. 16, Ecology B, Defense and Life History. Alan R. Liss, Inc., New York, pp. 237–273.

Bateman, P.W. & Fleming, A. (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.

Bauwens, D. (1981). Survival during hibernation in the European common lizard, *Lacerta vivipara. Copeia*, 1981: 741–744.

Bellairs, A. & Bryant, S.V. (1985). Autotomy and regeneration in reptiles. In: Gans, C. Billett, F. & Maderson, P.F.A. (Eds.), *Biology of the Reptilia*. Vol. 15, Development B. John Wiley and Sons, New York, pp. 301–410.

Bely, A.E. & Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. *Trends in Ecology and Evolution*, 25(3): 161–170.

Braña, F. (1981). Sobre la alimentación de *Podarcis muralis* (Reptilia: Lacertidae) en Asturias: espectro taxonómico y tamaño de las presas. *Resúmenes de Comunicaciones de la V Reunión Bienal de la Real Sociedad Española de Historia Natural*. Oviedo: 179.

Braña, F. (2003). Morphological correlates of burst speed and field movement patterns: the behavioral adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society*, 80(1): 135–146.

Brownikowski, A.M. & Arnold, S.J. (1999). The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. *Ecology*, 80: 2314–2325.

Bryant, S.V.; Endo, T. & Gardiner, D.M. (2002). Vertebrate limb regeneration and the origin of limb stem cells. *International Journal of Developmental Biology*, 46: 887–896.

Clause, A.R. & Capaldi, E.A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology Part A Comparative Experimental Biology*, 305: 965–973.

Dial, B.E. & Fitzpatrick, L.C. (1983). Lizard tail autotomy: Function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science,* 219: 391–393.

Downes, S. & Shine, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology,* 82: 1293–1303.

Elliott, J.M. (1994). *Quantitative Ecology and the Brown Trout*. Oxford Series in Ecology and Evolution, Oxford University Press.

Emberts, Z.; Escalante, I. & Bateman, P.W. (2019). The ecology and evolution of autotomy. *Biological Reviews*, 94(6): 1881–1896.

Fernández-Rodríguez, I. & Braña, F. (2020). The movement dynamics of autotomized lizards and their tails reveal functional costs of caudal autotomy. *Integrative Zoology*, 15(6): 511–521.

Fernández-Rodríguez, I.; Barroso, F.M. & Carretero, M.A. (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.

Fischer, R.E.; Geiger, L.A.; Stroik, L.K.; Hutchins, E.D.; George, R.M.; Denardo, D.F.; Kusumi, K.; Rawls, J.A. & Wilson-Rawls, J. (2012). A histological comparison of the original and regenerated tail in the green anole, *Anolis carolinensis*. *The Anatomical Record*, 295: 1609–1619.

Fleming, P.A.; Muller, D. & Bateman, P.W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews*, 82: 481–510.

Fox, S.F.; Rose, E. & Myers, R. (1981). Dominance and the acquisition of superior home ranges in the lizard *Uta stansburiana*. *Ecology*, 62: 888–893.

Fredericq, L. (1892). Nouvelles recherches sur l'autotomie chez le crab. *Archives de Biologie*, 12: 169–197.

García-Porta, J.; Irisarri, I.; Kirchner, M.; *et al*. (2019). Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature Communications*, 10: 4077.

Garland, T. & Losos, J.B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P.C. & Reilly, S.M. (Eds.), *Ecological morphology.* University of Chicago Press, Chicago, pp. 240–302.

Gilbert, E.A.; Payne, S.L.; Vickaryous, M.K. (2013). The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological & Biochemical Zoology*, 86: 631–44.

Goss, R.J. (1969). *Principles of Regeneration*. 2nd edition. Academic Press, USA.

Goss, R.J. (1992). The evolution of regeneration: Adaptive or inherent? *Journal of Theoretical Biology*, 159: 241–260.

Harris, D.J. & Sá-Sousa, P. (2002). Molecular phylogenetics of Iberian wall lizards (*Podarcis*): Is *Podarcis hispanica* a species complex? *Molecular Phylogenetics and Evolution*, 23(1): 75–81.

Higham, T.E. & Russell, A.P. (2010). Flip, flop and fly: modulated motor control and highly variable movement patterns of autotomized gecko tails. *Biology Letters*, 6: 70–73.

Hughes, A. & New, D. (1959). Tail regeneration in the geckonid lizard, *Sphaeridactylus. Journal of Embryology and experimental Morphology,* 7: 281–302.

Husak, J.F. (2006). Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology*, 20: 174–179.

Iraeta, P.; Salvador, A. & Díaz, J.A. (2012). Effects of caudal autotomy on postnatal growth rates of hatchling *Psammodromus algirus. Journal of Herpetology*, 46(3): 342–345.

Ji, X. & Braña, F. (2000). Among clutch variation in reproductive output and egg size in the wall lizard (*Podarcis muralis*) from a lowland population of Northern Spain. *Journal of Herpetology*, 34(1): 54–60.

Jusufi, A.; Goldman, D.I.; Revzen, S. & Full, R.J. (2008). Active tails enhance arboreal acrobatics in geckos. *Proceedings of the National Academy of Sciences of the United States of America*, 105: 4215–4219.

Lin, J.-W.; Chen, Y.-R.; Wang, Y.-H.; Hung, K.-C. & Lin, S.-M. (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(1847): 20162538.

Lorenzon, P.; Clobert, J.; Oppliger, A. & John-Adler, H. (1999). Effect of water constraint on growth rate, activity and body temperature of yearling common lizard. *Oecologia*, 118: 423–430.

Maginnis, T.L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology*, 17(5): 857–872.

Maginnis, T.L. (2008). Autotomy in a stick insect (Insecta: Phasmida): predation versus molting. *Florida Entomologist*, 91: 126–127.

Mani, S. & Tlusty, T. (2021). A comprehensive survey of developmental programs reveals a dearth of tree-like lineage graphs and ubiquitous regeneration. *BMC Biology*, 19(1): 111.

Martín, J. & Avery, R.A. (1997). Tail loss affects prey capture 'decisions' in the lizard *Psammodromus algirus*. *Journal of Herpetology*, 31: 292–295.

Martín, J. & Salvador, A. (1993). Tail loss reduces mating success in the Iberian rock-lizard, *Lacerta monticola*. *Behavioral Ecology and Sociobiology*, 32: 185–189.

Martín, J. & Salvador, A. (1993). Thermoregulatory behaviour of rock lizards in response to tail loss. *Behaviour*, 124: 123–136.

McLean, K.E. & Vickaryous, M.K. (2011). A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius. BMC Developmental Biology*, 11: 50.

Michelangeli, M.; Melki-Wegner, B.; Laskowski, K.; Wong, B.B.M. & Chapple, D.G. (2020). Impacts of caudal autotomy on personality. *Animal Behaviour*, 162: 67–78.

Moore, A.; Tabashnik, B.E. & Stark, J.D. (1989). Leg autotomy: A novel mechanism of protection against insecticide poisoning in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Enthomology*, 82(5): 1295–1298.

Pafilis, P.; Pérez-Mellado, V. & Valakos, E. (2008). Postautotomy tail activity in the Balearic lizard, *Podarcis lilfordi*. *Naturwissenschaften*, 95: 217–221.

Peters, R.H. (1983). *The ecological implications of body size*. Cambridge University Press, Cambridge.

Putman, B.J.; Coss, R.G. & Clark, R.W. (2015). The ontogeny of antipredator behavior: age differences in California ground squirrels (*Otospermophilus beecheyi*) at multiple stages of rattlesnake encounters. *Behavioral Ecology and Sociobiology,* 69: 1447–1457.

Roff, D.A. (1992). *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York.

Roff, D.A. (2002). *Life History Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Saint Girons, H. & Duguy, R. (1970). Le cycle sexuel de *Lacerta muralis* L. en plaine et en montagne. *Bulletin du Muséum national d'histoire naturelle*, 42: 609–625.

Sannolo, M. & Carretero, M.A. (2019). Dehydration constrains thermoregulation and space use in lizards. *PLoS ONE*, 14(7): e0220384.

Sannolo, M.; Barroso, F.M. & Carretero, A.M. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, 126: 58–64.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford University Press, London.

Taylor, J.A. (1984). Ecology of the lizard, *Ctenotus taeniolatus*; interaction of life history, energy storage and tail autotomy. Ph.D. Thesis, University of New England, Armidale, Australia.

van der Meer, J. (2019). Metabolic theories in ecology: the dynamic energy budget theory and the metabolic theory of ecology. *Encyclopedia of Ecology (Second Edition)*, 3: 463–471.

Wang, Y.; Zeng, Z.G.; Li, S.R.; Bi, J.H. & Du, W.G. (2016). Low precipitation aggravates the impact of extreme high temperatures on lizard reproduction. *Oecologia*, 182: 961–971.

Wulff, J.L. (2006). Resistance vs. recovery: morphological strategies of coral reef sponges. *Functional Ecology*, 20: 699–708.

Zamora-Camacho, F.J.; Rubiño-Hispán, M.V.; Reguera, S. & Moreno-Rueda, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid *Psammodromus algirus*. *Herpetological Journal*, 26: 213–218.