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

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Abstract

Autotomy is the ability to shed a body part when an animal receives an attack by a predator. It is often followed by regeneration of the lost parts to counteract dysfunctions related to limb amputation. Caudal autotomy is frequently used in lizards to avoid predation and is followed by tail regeneration. Regeneration is a costly process that can limit the resources available for somatic growth, accumulation of reserves and reproduction. We carried out two experiments to evaluate the short-term and long-term effects of regeneration following caudal autotomy 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 development of the second clutch (built under an income breeding strategy). In the long-term experiment, we studied the investment made in the first spring clutch by intact and regenerating females, to assess the effects of regeneration that started prior to wintering, likely affecting the lipid storages required to develop that clutch (built under a capital breeding strategy). Regenerating females of both experiments presented a reduction in clutch mass relative to snout-vent length, but greater in the short-term than in the long-term experiment. Furthermore, 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.

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 moult, accumulation of toxins, or predatory attacks (Bely & Nyberg, 2010; Maginnis, 2006). 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 (Díaz-Guisado et al., 2006; Kuo et al., 2013; Medger et al., 2008; Salvador et al., 1995). 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 (Barrios et al., 2008; Fox & Rostker, 1982).

Caudal autotomy is commonly used as an antipredator strategy in lizards and occurs in 13 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% (Bateman & Fleming, 2009; Bellairs & Bryant, 1985; Pafilis et al., 2009). The tail of lizards is important for locomotion, energy storage and social interaction (Bellairs & Bryant, 1985; Gillis et al., 2013; Peters et al., 2007), so its

loss may decrease the performance of ecologically relevant tasks (for a review, see Bateman & 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; Fernández-Rodríguez & Braña, 2020; Zamora-Camacho et al., 2016), thus increasing their long-term survival and fitness (Goss, 1969; Lin et al., 2017). 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 (Bateman & Fleming, 2009; Hoso, 2012; Lawrence, 2010; Maginnis, 2006; 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; Morgan & Jangoux, 2004; Pomory & Lawrence, 1999; 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 (Dial & Fitzpatrick, 1981; Fox, 1978) 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 (Messina & Fox, 2001; Roff, 1992; Stearns, 1992), 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 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* (Laurenti, 1768). 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 have used adult common lizards, *Podarcis muralis*, captured 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. The common wall lizard is a small lacertid lizard (Reptilia: Lacertidae) that occurs in central and southern Europe inhabiting rocky environments of natural and urban areas from 0 to 2400 m of elevation (Salvador, 2014). 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 1 month, being the first clutch larger than the other two (Ji & Braña, 2000; Saint Girons & Duguy, 1970). 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 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 (Ji & Braña, 2000; Weiss, 2002; 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 (for the females collected in the field had advanced oviductal eggs) or immediately after laying their first clutch (for 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 and Braña (2020). The grasping point was approximately the same for all animals, leaving an average tail stub of 0.802 ± 0.169 cm (mean $\pm$ SD).

Feeding rate was measured every 6 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 2 h, 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 base 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: tail volume = (π x tail length x (tail base width/2)^2)/3.

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 and 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 1 or 2 months prior to winter brumation. According to previous studies, wall lizards have highly developed abdominal fat bodies in this phase of the cycle (Braña, 1984; Saint Girons & Duguy, 1970). 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', $N = 19$). 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 and leaving a tail stub of 0.816 ± 0.124 cm (mean $\pm$ 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 7 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 separately 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 pseudoreplication, 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 $\log_{10}$ 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, that is, 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 1), and this difference tended to increase with females' SVL (Fig. 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 (Fig. 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; Fig. 3). The decrease in clutch mass from the first to the second clutch was significantly greater in tailless females than in tailed ones (Fig. 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 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

Table 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

	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 (n° 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)

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.
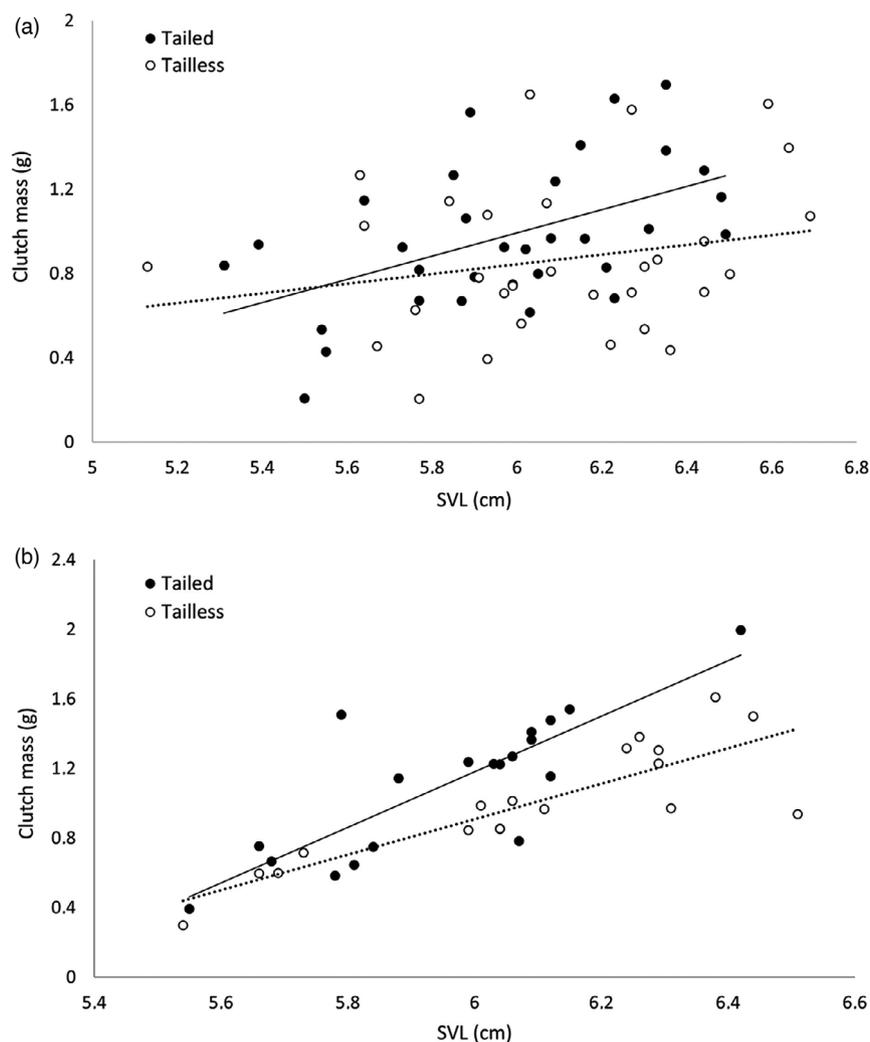

Figure 1 Clutch mass of tailed (black) and tailless (white) females in relation to their size (SVL: snout-vent length). (a) Short-term experiment: caudal autotomy was induced a few days before/after laying the first clutch in the spring, and regeneration occurred simultaneously with the development of 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 8 months later.
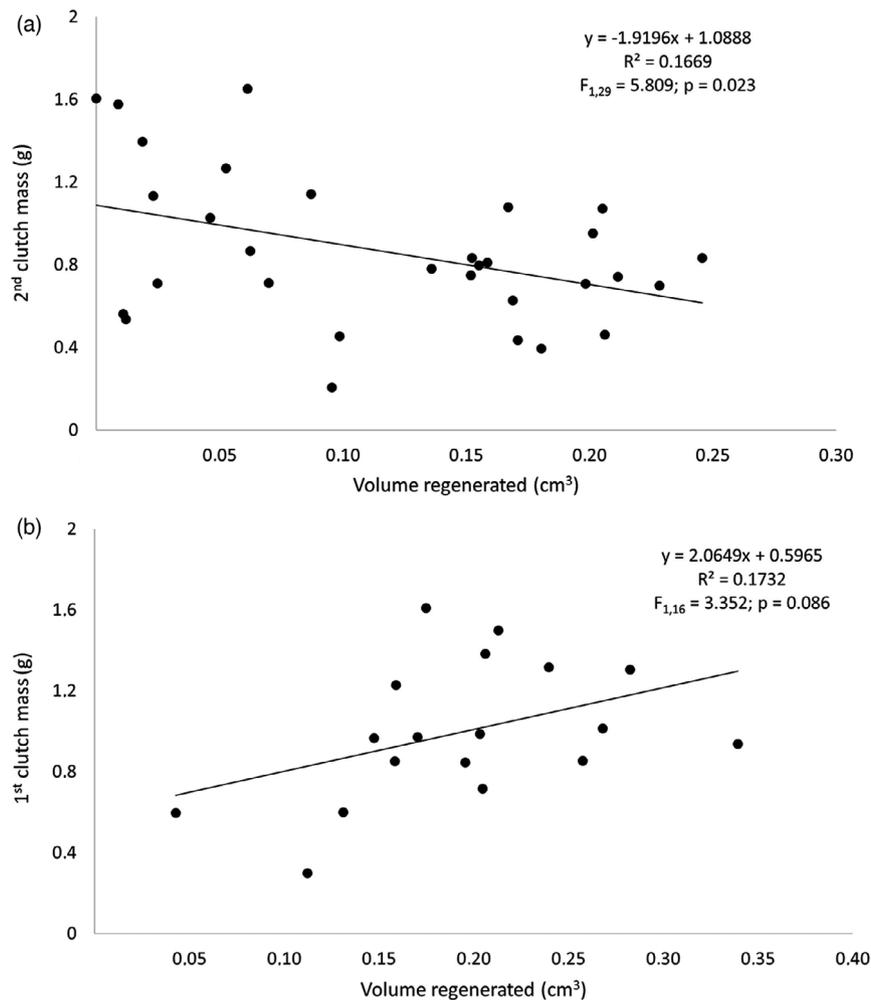
Figure 2 Volume of regenerated tail against total clutch mass of tailless females. (a) Short-term experiment, in which caudal autotomy was induced a few days before/after laying the first clutch, and regeneration occurred simultaneously to the development of the second clutch. (b) Long-term experiment, 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.

covariate: $F_{1,21} = 0.119$, $P = 0.733$) nor between the incubation time of eggs from tailed and tailless females (Table 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 of 125 eggs laid by tailed females, against 79 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 1), and this difference increased with females' SVL (Fig. 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 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 (Fig. 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 1; one-way ANCOVA with egg mass as covariate:
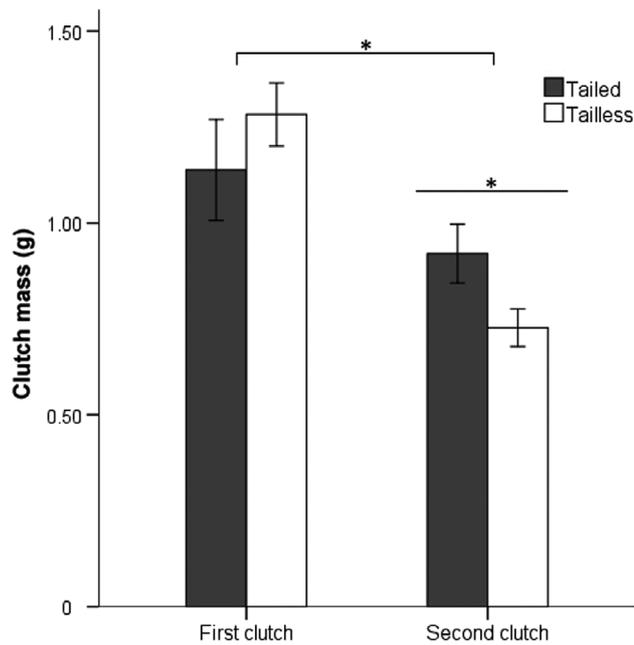
Figure 3 Total mass of the first and second clutches of tailed (grey) and tailless (white) females. Tailless (i.e. regenerating) females were induced caudal autotomy a few days before/after laying the first clutch, and regeneration occurred simultaneously to the development of the second clutch (short-term 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; *$P < 0.05$.

$F_{1,24} < 0.001$, $P = 0.999$), 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 1; one-way ANOVA: $F_{1,24} = 1.083$, $P = 0.308$). Incubation failure (i.e. eggs incubated that did not hatch) was not different for eggs laid by tailless or tailed females (34 failed eggs of 76 eggs from tailed females, against 36 failed eggs of 66 from tailless ones: $X^2_1 = 1,359$, $P = 0.244$).

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 ($F_{1,34} = 7.747$, $P = 0.009$).

Discussion

Caudal autotomy is a useful antipredator strategy that has not only obvious benefits for immediate survival but also some

negative effects, and it is known that tail regeneration largely restores the functions lost after autotomy (Fernández-Rodríguez & Braña, 2020; Lin et al., 2017; Zamora-Camacho et al., 2016). 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; Lynn et al., 2013; 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% (Bernardo & Agosta, 2005; Chapple et al., 2002; Dial & Fitzpatrick, 1981; Smyth, 1974; Taylor, 1984; Wilson & Booth, 1998), 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 (Beatty et al., 2021; Fox & McCoy, 2000). However, the valuable field experiment by Fox and McCoy (2000) reported not only 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 aforementioned studies, Bernardo and Agosta (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 (Dial & Fitzpatrick, 1981; Doughty et al., 2003; Maiorana, 1977; Smyth, 1974). 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 (Braña, 1983; Braña et al., 1991; Saint Girons & Duguy, 1970) 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 heavier 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 and Licht (1991) and Bernardo and Agosta (2005). Dial and 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 oviposition in female wall lizards occurs when embryos reached stages 25–29, according to the Dufaure and Hubert (1961) development table (Braña et al., 1991), and that the incubation time at 29°C 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 a 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); however, 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 (Braña, 1983; Saint Girons & Duguy, 1970). 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 (Derickson, 1976; Hahn & Tinkle, 1965). Previous studies on the same population of wall lizards as 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 invertebrates living in markedly seasonal environments (Bonnet et al., 1998; Sainmont et al., 2014; Varpe et al., 2009). 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 and 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: first, 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); second, 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 and 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 8 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 (Reznick et al., 2000; van Noordwicjk & de Jong, 1986). 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 (Pianka, 1976; Williams, 1966). Thus, animals may adjust the amount of energy allocated to each trait in a way that maximizes its lifetime fitness, that is, 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 (Dial & Fitzpatrick, 1981; Maiorana, 1977; Vitt et al., 1977). 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 Barbault & Mou, 1988; Castanet & Roche, 1981; 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.

Acknowledgements

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