

Short- and long-term effects of an extreme case of autotomy: does “tail” loss and subsequent constipation decrease the locomotor performance of male and female scorpions?

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Abstract

In many taxa, individuals voluntarily detach a body part as a form to increase their chances of escaping predation. This defense mechanism, known as autotomy, has several consequences, such as changes in locomotor performance that may affect fitness. Scorpions of the genus *Ananteris* autotomize the “tail”, which in fact corresponds to the last abdominal segments. After autotomy, individuals lose nearly 25% of their body mass and the last portion of the digestive tract, including the anus, which prevents defecation and leads to constipation, because regeneration does not occur. Here, we experimentally investigated the short- and long-term effects of tail loss on the locomotor performance of *Ananteris balzani*. In a short-term experiment, the maximum running speed (MRS) of males and females did not change after autotomy. Moreover, the relative mass of the lost tail did not affect the change in MRS after autotomy. In a long-term experiment, autotomy had a negative effect on the MRS of males, but not of females. Autotomized over-fed individuals suffered from severe constipation but were not slower than autotomized normally fed individuals. In conclusion, tail loss has no immediate effect on the locomotor performance of scorpions. The long-term decrease in the locomotor performance of autotomized males may impair mate searching. However, because death by constipation takes several months, males have a long time to find mates and reproduce. Thus, the prolonged period between autotomy and death by constipation is crucial for understanding the evolution of one of the most extreme cases of autotomy in nature.

Key words: fitness costs, maximum running speed, sex differences, tail loss, weight loss

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INTRODUCTION

One of the most extreme forms of defense against predation is to voluntarily detach a body part. This defense, known as autotomy, has been reported for numerous animal groups, including vertebrates and invertebrates (reviewed in Fleming *et al.* 2007 and Emberts *et al.* 2019). The immediate benefit of autotomy to the individuals under attack is survival of the predation attempt. In fact,

there is evidence that tail autotomy in lizards and salamanders, as well as limb autotomy in different arthropod taxa, increases the chances of escaping predatory attacks (e.g. Congdon *et al.* 1974; Beneski 1989; Wasson *et al.* 2002). However, autotomy may have additional consequences, and some of them may decrease the fitness of the autotomized individuals. For instance, leg loss in insects and spiders may reduce foraging efficiency, increase susceptibility to future predation events, and even increase exposure to infection (see examples in Fleming *et al.* 2007 and Emberts *et al.* 2019).

A widespread consequence of losing a body part is a change in locomotor performance (Fleming *et al.* 2007; Bateman & Fleming 2009; Emberts *et al.* 2019). The specific consequence depends on the function of the autotomized appendage in locomotion. Individuals that lose an appendage with a direct locomotor function, such as an ambulatory leg, usually show a decrease in locomotor performance. For instance, the running speed of many terrestrial arthropods decreases after leg loss (Fleming *et al.* 2007). However, if individuals lose an appendage that has no direct locomotor function, the effects of autotomy are controversial. This is the case for tail autotomy in lizards, which may have both positive and negative effects on the locomotor performance (Bateman & Fleming 2009; McElroy & Bergmann 2013). If the tail assists locomotion by balancing the body during movement, autotomy may decrease locomotor performance (e.g. Punzo 1982; Jagnandan & Higham 2017). In turn, if the tail represents a great portion of the total body mass and does not assist locomotion, tail loss may increase locomotor performance (e.g. Daniels 1983). Enhanced locomotor performance has also been reported for other taxa after the loss of large body parts, such as a pedipalp in male spiders (Ramos *et al.* 2004) or a major claw in male crabs (Gerald & Thiesen 2014).

Recently, the first cases of autotomy in scorpions were reported for several species of the genus *Ananteris* (Buthidae) (Lira *et al.* 2014; Mattoni *et al.* 2015). Unlike other arthropods that autotomize appendages, such as legs, pedipalps, forceps, cerci, or antennae, scorpions detach the metasoma, which corresponds to the last abdominal segments. The metasoma, commonly known as the “tail”, contains the posterior part of the nervous, circulatory, and digestive systems and the telson, which bears the stinger and venom gland (Hjelle 1990). When the tail is autotomized, the individual loses the anus because scar tissue completely blocks the posterior end of the digestive system (Mattoni *et al.* 2015; Fig. 1a). Because there is no regeneration of the tail, the scar tissue does not allow defecation, so autotomized individuals accumulate feces over

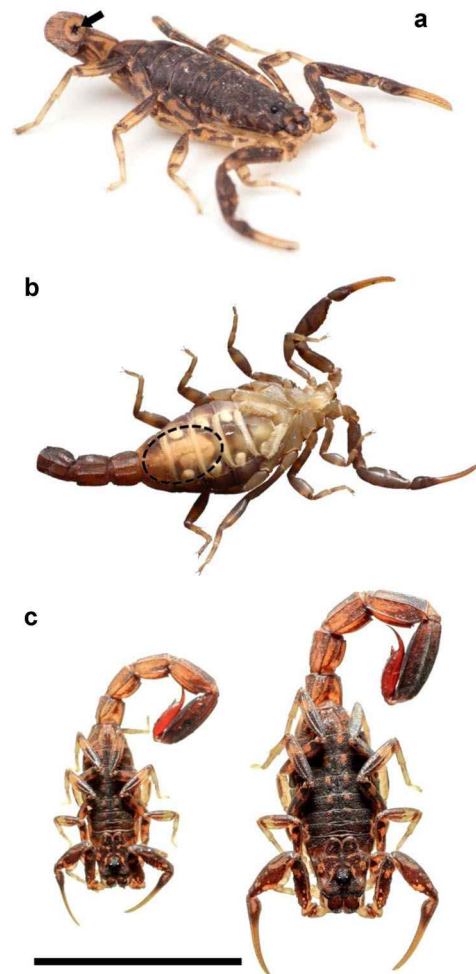


Figure 1 Individuals of the scorpion *Ananteris balzani*. (a) After tail autotomy, dark scar tissue (arrow) blocks the posterior end of the digestive tract, preventing defecation and causing constipation. (b) Ventral view of a male 50 days after autotomy. The white patch inside the dashed ellipsis is caused by the accumulation of feces in the midgut. (c) Dorsal view of a male (left) and a female (right) showing the well-marked sexual size dimorphism in the species (scale bar = 1 cm). Note that the tail is relatively longer in the male than in the female. (Photos: John Uribe.)

time and eventually die of constipation (Mattoni *et al.* 2015; Fig. 1b). Recently, it has been shown that tail autotomy impairs prey capture of both males and females of *Ananteris balzani* (García-Hernández & Machado 2020). Given that the stinger is used to inject venom and subdue large prey, tail loss decreases the subduing success and increases the handling time. These negative effects are more pronounced in males than in females because even the largest males are smaller than the smallest females (García-Hernández & Machado 2020; Fig. 1c). Moreover,

Table 1 Predictions of the weight loss hypothesis for the short- and long-term effects of tail autotomy on the locomotor performance of scorpions. The hypothesis states that weight has a negative effect on the locomotor performance, so that heavier individuals should be slower than lighter individuals

	Predictions
Short-term effects of autotomy	
1. Effect of tail autotomy	The locomotor performance of the individuals will increase after tail autotomy because they will lose weight
2. Effect of relative mass of the lost tail	The greater the relative mass of the lost tail, the greater the increase in locomotor performance
3. Sexual difference	Males will have a better locomotor performance than females after autotomy as the tail of the males is relatively heavier
Long-term effects of autotomy	
4. General tendency	Individuals will gradually decrease locomotor performance after autotomy due to an increase in body mass caused by constipation
5. Effect of diet [†]	Normally fed individuals will have a better locomotor performance than over fed individuals, which will be heavier

[†] Given the amount of food provided in our experiment, we are confident that both normally and over fed individuals have enough energy for the locomotion performance tests.

tail autotomy decreases female fecundity but does not affect male mating success (García-Hernández & Machado 2021).

As a next step to understand the effects of tail autotomy for males and females in scorpions, we investigate here how tail loss affects the locomotor performance of *A. balzani*. More specifically, we tested the *weight loss* hypothesis (reviewed in Jagnandan & Higham 2018), according to which weight has a negative effect on the locomotor performance, so that heavier individuals should be slower than lighter individuals. In the scorpion *Centruroides vittatus* (Buthidae), for instance, running speed of females decreases when they are carrying offspring on their dorsum but increases after the nymphs disperse (Shaffer & Formanowicz 1996). Similarly, total body mass in *A. balzani* is considerably reduced after tail autotomy, which could increase running speed (prediction 1 in Table 1). Following this rationale, the higher the mass of the lost tail, the greater the positive effect on the running speed should be (prediction 2 in Table 1). Because males lose a relatively large percentage of their total body mass after tail loss (Fig. 1c; see also “Study species” below), the positive effect of autotomy on the running speed should be higher in males than in females (prediction 3 in Table 1). Moreover, given that autotomized individuals are unable to eliminate feces, constipation will increase body mass over time. Thus, in the long-term, tail loss should reduce running speed in comparison with the period immediately after autotomy (prediction 4 in Table 1).

Finally, the weight loss hypothesis predicts that the long-term negative effect of tail autotomy should be more pronounced in over fed than in normally fed individuals of both sexes, because the former will accumulate more feces over time and then will be heavier and consequently slower (prediction 5 in Table 1).

MATERIALS AND METHODS

Study species

Ananteris balzani occurs in areas of savannah in central and southeastern Brazil (Giupponi *et al.* 2009). Individuals are active exclusively at night, when they are found walking or running on sand soil or leaf litter (see video in Supporting Information S1). As other scorpion species, *A. balzani* shows marked sexual size dimorphism (Fig. 1c), with males much smaller than females in total body size (García-Hernández & Machado 2020; see also Fig. S1, Supporting Information S2). Mean (\pm SD) metasoma/carapace length ratio in males is 5.05 ± 0.10 ($N = 34$) and in females it is 4.79 ± 0.16 ($N = 18$), indicating that the length of the tail is relatively longer in males (ANOVA: $F_{1,50} = 63.41$, $P < 0.001$).

Adult individuals of both sexes autotomize the tail only when it is grabbed, but females are clearly more reluctant than males to shed their tail. In an experiment performed with another species of *Ananteris*, 88% of the males released their tail when grabbed by forceps whereas only

20% of the females did so (Mattoni *et al.* 2015). This sexual difference in the willingness to autotomize the tail may explain why the frequency of autotomized males in natural populations is higher than that of females (Mattoni *et al.* 2015; García-Hernández & Machado 2021). Moreover, the mean (\pm SD) percentage of the total body mass represented by the lost tail in males is $22.0\% \pm 3.3\%$ ($N = 29$) and in females it is $19.5\% \pm 3.9\%$ ($N = 20$), indicating that males lose relatively more mass after autotomy (ANOVA: $F_{1,47} = 6.44$, $P = 0.015$). As occurs with some lizards, the tail of both males and females twitches automatically for as much as 50 seconds after autotomy (see video in Supporting Information S1).

Collection and maintenance

We collected 154 adult individuals (52 females, 102 males) from October 2016 to April 2017 at Santa Bárbara Ecological Station ($24^{\circ}48'S$, $49^{\circ}13'W$), state of São Paulo, southeastern Brazil. The specimens were maintained under quarantine conditions for 2 months in the laboratory inside individual plastic containers with a piece of wet cotton and a dry leaf to provide shelter. The long quarantine was necessary to eliminate individuals parasitized by nematomorphs that make them lethargic. Moreover, the metabolic rate of scorpions shows a decrease after some weeks in captivity (Terblanche *et al.* 2007). Thus, the long quarantine also ensured that all individuals used in the experiments were equally acclimated to the laboratory conditions. During the quarantine period, we fed the individuals every 10 days with cricket nymphs (*Acheta* sp.). Because the total body mass of females is larger than that of males (Fig. S1, Supporting Information S2), females received twice as much food as males, which means that females received 0.0052 g (approx. 6% of their total body mass) and males received 0.0026 g (approx. 5% of their total body mass) every 10 days. During the entire study period, we kept the individuals under a photoperiod of 12:12 h and conducted the experiments at room temperature with minimal variation (range: 24.5 – $26.5^{\circ}C$).

We induced tail autotomy by grasping and pulling the last metasomal segment of the individuals with forceps. If after 2 min, the individual did not release its tail, we repeated the protocol 2 h later. Before autotomy, each individual was weighed in a digital balance (precision = 0.0001 g). After autotomy, we weighed and froze the autotomized tail, and individuals were allowed to rest for 3 days to complete healing of the fracture point (Fig. 1a). To avoid possible behavioral biases due to differences in the way individuals from the 2 experimental groups (in-

tact and autotomized, see below) were manipulated, we used the same protocol to induce tail autotomy in individuals assigned to the intact group. In this case, however, we grasped the first metasomal segment, where tail autotomy is impossible. Given that scorpions are viviparous, we excluded females bearing embryos ($N = 5$) from the experiment to avoid the possible confounding effects that the extra load caused by the embryos could have on their locomotor performance.

Locomotor performance trials

At the beginning of each trial, we placed an individual inside a transparent plastic vial at one end of a 1 m-long racetrack (3 cm wide, 5 cm high) with sand as the substrate. After 5 min, the vial was removed, and the individual was stimulated to run by repeated taps on its dorsum with forceps (see video in Supporting Information S1). Cardboard was placed over the other end of the racetrack to provide shelter. When the individual was positioned inside the shelter, it was covered with a plastic vial for 10 min. After that, the individual was stimulated to run again in the other direction on the racetrack. The sand was changed after 2 trials with the same individual to remove possible chemical cues left on the substrate. We repeated this procedure 24 h later to complete 4 trials for each individual. All trials were conducted at night (1800–2300 hours) under dim red illumination and were always performed by the same person (SGH). We filmed the trials with a digital camcorder (Sony Handycam HDR-CX405) and, based on the footage, recorded the maximum running speed (MRS), which is a commonly used proxy of locomotor performance in studies about the effects of autotomy in terrestrial invertebrates (e.g. Amaya *et al.* 2001; Apontes & Brown 2005; Brown & Formanowicz 2012) and vertebrates (e.g. McElroy & Bergmann 2013; Fernández-Rodríguez & Braña 2020). The MRS was measured as the highest speed value over a 10 cm-interval of the racetrack, considering the 4 trials conducted with each individual. The measurements of MRS were highly consistent between individuals in all phases of the experiment (Table S1, Supporting Information S2), which indicates that there is little within-individual variation in the trials we conducted in the laboratory.

Short-term effects of tail autotomy

First, we measured the *premanipulation* MRS of 113 intact individuals, including females ($N = 38$) and males ($N = 75$). After the 4 running trials described above, we

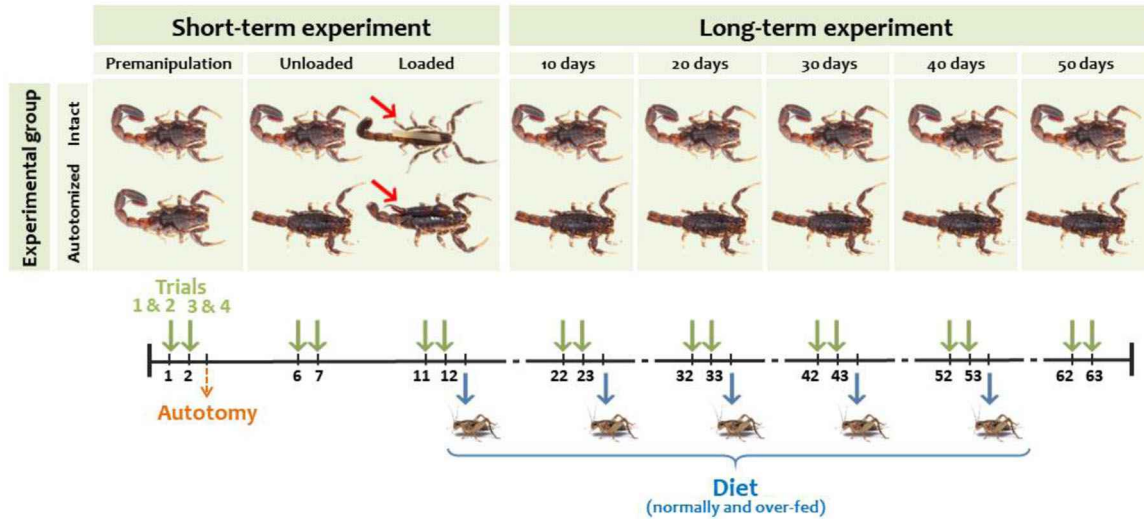


Figure 2 Scheme of the experimental setup to test the effect of tail autotomy on the locomotor performance of the scorpion *Ananteris balzani*. During the short-term experiment, we conducted 3 measurements of maximum running speed (MRS) on days 1–2, 6–7, and 11–12. Each measurement was conducted in 2 consecutive days (2 trials/day totaling 4 trials), which are indicated with green arrows. First, we estimated the premanipulation MRS of individuals that would be later assigned to the intact and autotomized groups (days 1–2). Then, we induced autotomy in individuals of the autotomized group and manipulated the individuals of the intact group without inducing autotomy (orange dashed arrow). It was given 3 days for the autotomized individuals to complete the healing process. After that, we performed other 2 measurements of MRS. In the first one (days 6–7), half of the individuals of both experimental groups were unloaded and the other half was carrying a load. Intact individuals were loaded with a piece of raw spaghetti and autotomized with the same piece of tail they lost (red arrows). In the second one (days 11–12), individuals that were previously carrying a load were tested unloaded, whereas individuals that were previously unloaded were tested carrying a load. During the 12 days of the short-term experiment, individuals were not fed to avoid marked changes in their body mass. On day 13, we began the long-term experiment, which was performed with all individuals unloaded. For the long-term experiment, we split intact and autotomized individuals into 2 diet treatments: normally fed, which received 10% of their original body mass in the form of cricket nymphs every 10 days since day 13, and over fed, which received 20% of their original body mass in the form of cricket nymphs every 10 days since day 13 (blue arrows, corresponding to days 13, 24, 34, 44, and 54). During the long-term experiment, we performed 5 measurements of MRS: days 22–23 (i.e. 10 days after the beginning of the long-term experiment), 32–33 (i.e. 20 days), 42–43 (i.e. 30 days), 52–53 (i.e. 40 days), and 62–63 (i.e. 50 days). As in the short-term experiment, each measurement was conducted in 2 consecutive days (2 trials/day to complete 4 trials), which are indicated with green arrows. The long-term experiment ended on day 64.

randomly assigned each individual to 1 of 2 experimental groups. The *autotomized* group included 25 females and 46 males for which we induced tail autotomy. The *intact* group included 13 females and 29 males that were left intact. We assigned more individuals to the autotomized group because individual mortality was higher than in the intact group during the long-term experiment (see “Probability of mortality” in Supporting Information S2). The premanipulation MRS did not differ between individuals in the 2 experimental groups (Table S2, Supporting Information S2).

We measured the MRS of all individuals belonging to both experimental groups 3 days after the measurement of the premanipulation MRS, when the healing process of the autotomized individuals was completed. To evaluate the effect of tail mass on the MRS, we submitted half

of the individuals in each experimental group to 2 loading treatments: *loaded* and *unloaded*. To load the autotomized individuals, we glued the same piece of the tail they autotomized, which was previously unfrozen for 2 h, onto their dorsum (Fig. 2). To load intact individuals, we glued onto their dorsum a cylinder (females: length = 9 mm, diameter = 1.8 mm; males: length = 7 mm, diameter = 1.2 mm) made of raw spaghetti (Fig. 2), weighing 20% and 22% of the total body mass of females and males, respectively. These values correspond to the mean relative mass of the lost tail in individuals of each sex (see “Study species” above). Although tail loss probably promotes a forward displacement of the center of mass (see Jagnandan *et al.* 2014 for an example with lizards), by gluing the tail or the cylinder along the central axis of the mesosoma (Fig. 2), we tried to avoid further shifts on

the center of mass of the individuals. For unloaded individuals of the intact and autotomized groups, we applied only a glue drop onto their dorsum. Finally, 3 days after the second round of running trials, we inverted the loading treatment (loaded and unloaded) of each individual in each experimental group (intact and autotomized) and measured again the MRS.

The short-term experiment lasted 12 days, during which individuals were not fed to prevent great mass changes that could influence their locomotor performance (see scheme of the experimental design in Fig. 2). At the end of the short-term experiment, we had 12 repeated measurements of MRS for each individual of both experimental groups: 4 in the premanipulation trials, 4 in the unloaded trials, and 4 in the loaded trials. For each set of 4 measurements, we extracted 1 single value of MRS, which corresponds to the highest value of MRS recorded in the running trials (i.e. the maximum value of MRS).

Long-term effects of tail autotomy

After the short-term experiment, we used a subset of intact (13 females, 29 males) and autotomized individuals (24 females, 43 males) to evaluate the long-term effects of autotomy on the MRS (always with unloaded individuals). Half of the males and half of the females in each experimental group (intact and autotomized) were randomly assigned to 2 diet treatments: *normally fed*, in which individuals received 10% of their original body mass (i.e. before autotomy) in the form of cricket nymphs every 10 days, and *over fed*, in which individuals received 20% of their original body mass in the form of cricket nymphs every 10 days. The individuals assigned to both experimental groups always entirely consumed all cricket nymphs offered to them over the course of the long-term experiment.

The diet treatments started on day 13, one day after the end of the short-term experiment, but the first measures of MRS for the long-term experiment were obtained only 10 days later (see scheme of the experimental design in Fig. 2). We called the first trials of the long-term experiment *day 10*. The following trials were conducted at 10-day intervals and were referred to *days 20, 30, 40, and 50*. On all these days, the MRS was measured using the same protocol as in the short-term experiment. At the end of the 5 rounds of trials (days 10 to 50), we had 20 repeated measurements of MRS for each individual (Fig. 2). We also measured the body mass of each individual at the beginning (day 10) and at the end (day 50) of the long-term experiment. Based on these 2 measurements, we estimated the effect of the diet treatment on the body mass of the

individuals. Due to the long duration of this experiment, some individuals died (see “Probability of mortality” in Supporting Information S2), and thus we had a complete dataset for 13 intact females, 15 autotomized females, 27 intact males, and 26 autotomized males.

Data analysis: short-term effects

We adjusted a linear mixed-effect model (LMM) for the MRS (response variable) in which we included an interaction between sex (females and males), experimental group (intact and autotomized), and loading treatment (premanipulation, loaded, and unloaded) as categorical predictor variables (factors). Body length and body mass were not included as predictor variables because they are highly correlated with sex (Fig. S1, Supporting Information S2). Individual identity was included as a random factor to control for repeated measurements in 3 moments: premanipulation, loaded trials, and unloaded trials. Based on our model, which includes interactions between all factor levels, we were able to estimate the marginal means to calculate the contrasts necessary to test predictions 1–3 (Table 1).

To test for the effect of the relative mass of the lost tail on the MRS, we only used data from individuals belonging to the autotomized group and adjusted a linear model (LM). The predictor variables were sex and relative weight loss after autotomy, estimated as the mass of the lost tail divided by total body mass before autotomy. The response variable was the change in the MRS after autotomy, estimated as the difference in MRS before and after autotomy for unloaded individuals. Negative values indicate a decrease and positive values, an increase in the MRS after autotomy.

Data analysis: long-term effects

To estimate the change in body mass of the individuals between the beginning (10 days) and the end (50 days) of the long-term experiment, we used only data from individuals that were alive at the end of the experiment. We performed paired *t*-tests using the *t.test* function of the package *stats* (R Core Team 2020) to compare the body mass of each individual on days 10 and 50. This comparison was performed separately for each combination of the levels of the categorical variables, that is, sex (females and males), experimental groups (intact and autotomized), and diet treatment (normally and over fed). We also calculated the Cohen's *d*, which is an effect size that indicates the magnitude and direction of the difference in body mass. The calculation of Cohen's *d* is given by

$(\text{mean}_{\text{day } 50} - \text{mean}_{\text{day } 10})/\text{standard deviation}_{\text{pooled}}$, which is the standardized difference between 2 means. Positive values indicate that the body mass of the individuals increased over time, while negative values indicate that the body mass of the individuals decreased over time.

To test for the long-term effects of tail autotomy on the MRS (response variable), we adjusted an LMM. We included an interaction between sex, experimental group, and diet treatment as categorical predictor variables (factors) and time (10 to 50 days) as a continuous predictor variable. Individual identity was included as a random factor to control for repeated measurements over time. We used the package *nlme* (Pinheiro *et al.* 2020) to test for temporal autocorrelation of the data on MRS over the course of the long-term experiment. After comparing models with and without temporal autocorrelation, the best model was the one without the auto-correlation structure (Table S3, Supporting Information S2). This result indicates that, after controlling for repeated measurements of the individuals, the data on MRS collected over time are not correlated with each other. Based on our model (without temporal autocorrelation), which includes interactions between all factor levels, we obtained the slopes of the LMM and were able to estimate the marginal means to calculate the contrasts necessary to test predictions 4–5 (Table 1).

The LM and the LMMs were performed using the package *lme4* (Bates *et al.* 2015). The *mvt* contrasts between estimated marginal means and slopes were performed using the package *emmeans* (Lenth 2021). All analyses were performed in the software R v.4.0.2 (R Core Team 2020). The measures of variance presented in the results always refer to standard error unless otherwise indicated.

Ethical note

Autotomy is a natural defense mechanism in *A. balzani* and the frequency of autotomized individuals collected in the study site was 3.6% for males and 1.1% for females. All trials of induced autotomy were performed carefully, stimulating the voluntary tail loss and minimizing the stress of the individuals. The experiments reported here were conducted in accordance with ASAB/ABS Guidelines for the ethical treatment of animals (Buchanan *et al.* 2012). Both the collection and maintenance of the scorpions in the laboratory were conducted with permits from the Brazilian Government (SISBIO/ICMBio, Permit 56 081).

RESULTS

Short-term experiment

In premanipulation trials, when all individuals were intact, the MRS did not differ between individuals that would be later manipulated to keep their tail or to have their tail autotomized (females_{Intact - Autotomized}: -2.408 ± 1.486 cm/s, 95% CI: -7.251 to 2.435 ; males_{Intact - Autotomized}: 0.754 ± 1.025 cm/s, 95% CI: -2.594 to 4.102). As expected, the MRS of intact females and males was similar between the premanipulation trials and the unloaded trials (Fig. 3a,b: contrasts 1 and 4). However, the MRS of intact females was also similar between the premanipulation trials and the loaded trials, when there was an experimental increase in their body mass (Fig. 3a,b: contrast 2). In the case of intact males, the MRS in the premanipulation trials was higher than in the loaded trials (Fig. 3a,b: contrast 5). The MRS of intact females did not differ between the 2 loading treatments, even though individuals were heavier in the loaded trials (Fig. 3a,b: contrast 3). Again, the pattern found for intact males was different from females: the MRS was lower in the loaded trials when compared with the unloaded trials (Fig. 3a,b: contrast 6). Finally, no sexual difference was found when we compared the MRS of intact individuals in the premanipulation trials (Fig. 3a,b: contrast 7), in the unloaded trials (Fig. 3a,b: contrast 8), and in the loaded trials (Fig. 3a,b: contrast 9).

The MRS of autotomized females and males was similar when we compared the premanipulation trials with the unloaded trials, even though individuals were lighter in the latter (Fig. 3c,d: contrasts 1 and 4). However, the MRS of autotomized females and males was similar when we compared the premanipulation trials with the loaded trials (Fig. 3c,d: contrasts 2 and 5). The MRS of females and males was similar when we compared the 2 loading treatments, even though individuals were heavier in the loaded trials (Fig. 3c,d, contrasts 3 and 6). Finally, no sexual difference was found when we compared the MRS of autotomized individuals in the premanipulation trials (Fig. 3c,d: contrast 7), in the unloaded trials (Fig. 3c,d: contrast 8), and in the loaded trials (Fig. 3c,d: contrast 9). Estimates of repeatability showed that the MRS was consistent between individuals during the short-term experiment (Table S1, Supporting Information S2).

The relative mass of the lost tail ranged from 11.8% to 28.8% (mean \pm SD = $21.2\% \pm 4.3\%$, $N = 42$) in males and from 12.5% to 26.6% in females ($19.3\% \pm 3.9\%$, $N = 22$). There was a marginally significant negative effect of the relative mass of the lost tail on the change in

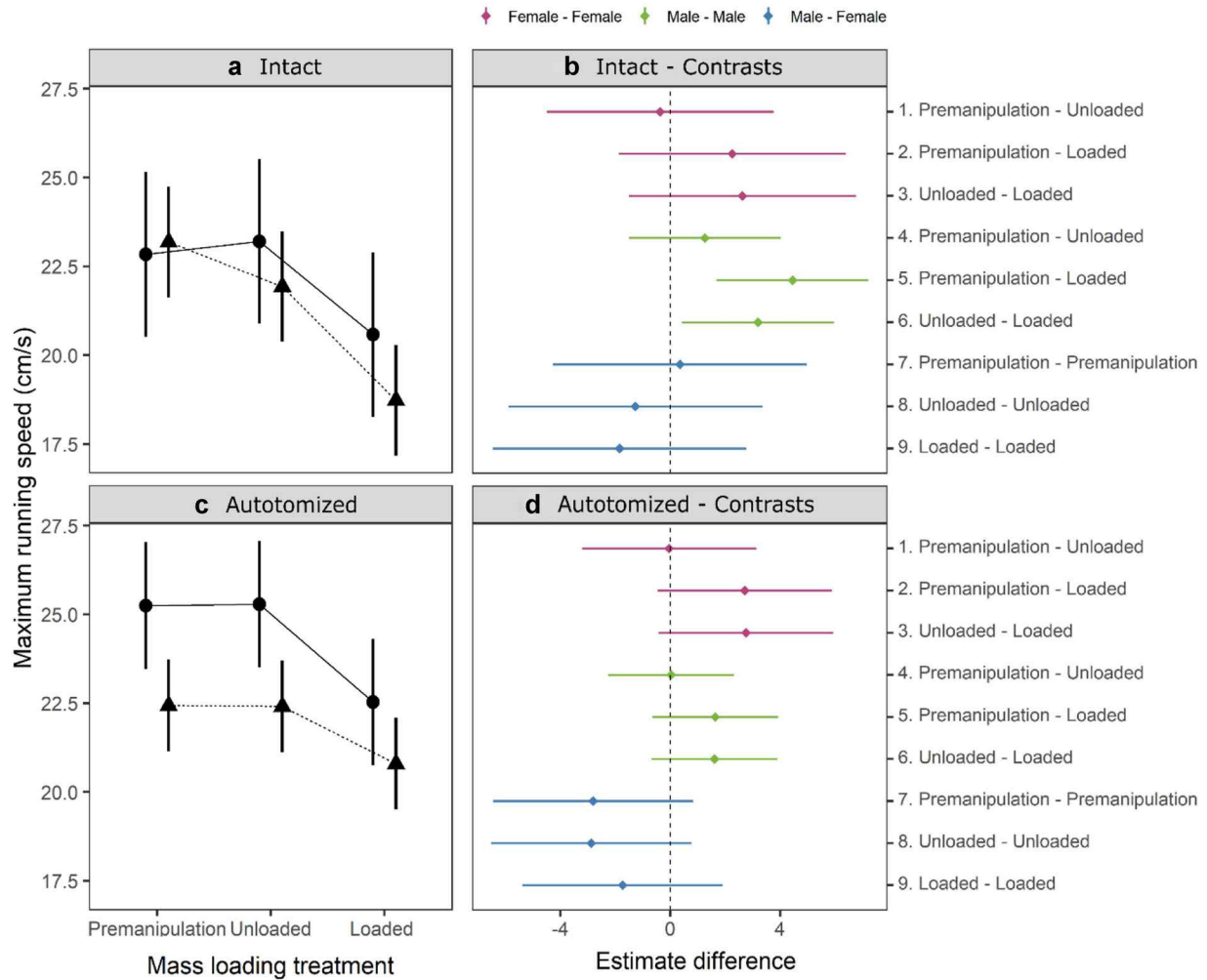


Figure 3 Short-term effects of tail autotomy on the maximum running speeds (MRS) of the scorpion *Ananteris balzani*. Estimates of MRS of females (triangle) and males (circle) were performed before the beginning of the experiment (i.e. premanipulation) and after that for 2 loading treatment (unloaded and loaded). This procedure was performed separately for individuals belonging to the (a) intact and (c) autotomized groups. Contrasts of the estimated MRS for each combination of sex, loading treatment, and experimental group are presented in the panels (b) and (d). A positive value of contrast indicates that the estimated MRS for the first term of the contrast was higher than the second term, whereas a negative value indicates the opposite. Symbols indicate mean values (a,c) or mean differences (b,d). In all panels, bars indicate 95% confidence intervals. Values of all estimates and contrasts illustrated here are presented in Table S4, Supporting Information S2.

MRS of males (slope = -0.28 ± 0.14 ; $F_{1,40} = 4073.0$, $P = 0.050$, $R^2 = 0.092$). For females, there was no significant effect of the relative mass of the lost tail on the change in MRS (slope = 0.54 ± 0.32 ; $F_{1,20} = 2813.0$, $P = 0.191$, $R^2 = 0.123$).

Long-term experiment

The body mass of intact and autotomized normally fed females did not show a significant increase over time

(Table 2). In turn, the body mass of intact and autotomized over fed females showed a significant increase over time, and the magnitude of this increase was higher for autotomized females, as would be expected by the effect of constipation (Table 2). The body mass of intact and autotomized normally fed males did not show a significant increase over time (Table 2). Contrary to what happened with females, the body mass of intact over fed males did not show a significant increase over time (Table 2). However, the body mass of autotomized

Table 2 Results of paired *t*-tests comparing the mean (\pm SE) body mass of individuals of the scorpion *Ananteris balzani* between the beginning (day 10) and the end (day 50) of the long-term experiment

Factors	Body mass (g)		Statistics	Cohen's d
	10 days	50 days		
Females				
Intact—Normally fed (<i>N</i> = 6)	0.125 \pm 0.019	0.120 \pm 0.021	<i>t</i> = 1.124; df = 5; <i>P</i> = 0.312	−0.228
Autotomized—Normally fed (<i>N</i> = 8)	0.109 \pm 0.031	0.120 \pm 0.016	<i>t</i> = −0.884; df = 7; <i>P</i> = 0.4061	0.459
Intact—Over fed (<i>N</i> = 7)	0.121 \pm 0.031	0.133 \pm 0.035	<i>t</i> = −3.926; df = 6; <i>P</i> = 0.008	0.352
Autotomized—Over fed (<i>N</i> = 7)	0.110 \pm 0.018	0.143 \pm 0.023	<i>t</i> = −2.983; df = 6; <i>P</i> = 0.025	1.555
Males				
Intact—Normally fed (<i>N</i> = 13)	0.055 \pm 0.007	0.058 \pm 0.023	<i>t</i> = −0.413; df = 12; <i>P</i> = 0.689	0.174
Autotomized—Normally fed (<i>N</i> = 13)	0.046 \pm 0.007	0.050 \pm 0.007	<i>t</i> = −1.978; df = 12; <i>P</i> = 0.071	0.567
Intact—Over fed (<i>N</i> = 14)	0.058 \pm 0.007	0.057 \pm 0.007	<i>t</i> = 0.586; df = 13; <i>P</i> = 0.137	−0.134
Autotomized—Over fed (<i>N</i> = 13)	0.048 \pm 0.007	0.062 \pm 0.007	<i>t</i> = −7.546; df = 12; <i>P</i> < 0.001	1.932

Significant differences in body mass over time are highlighted in bold. Cohen's *d* is an effect size that indicates the magnitude and direction of the difference in body mass. *Positive* values indicate that the body mass *increased* over time, while *negative* values indicate that the body mass *decreased* over time.

over fed males showed a significant increase over time (Table 2).

The MSR of intact normally fed males and females showed no significant increase over time (Tables 2–3; Fig. 4a). The same happened with the MSR of intact over fed males (Table 3; Fig. 4a), which did not experience a significant increase in body mass (Table 2). However, the MRS of intact over fed females, which experienced a significant increase in body mass (Table 2), decreased over time (Table 3; Fig. 4a). The MRS of autotomized males and females decreased over time, regardless of the diet treatment (Table 3; Fig. 4b). Although the MRS of intact and normally fed females tended to remain constant over time, whereas the MRS of autotomized and normally fed females showed a decrease over time, we found no significant difference between them (Fig. 4c: contrast 1). In the case of intact and autotomized over fed females, the decrease of MRS over time was also similar (Fig. 4c: contrast 2). The reduction of MRS over time was higher in autotomized males than in intact males. Again, this pattern was the same for both diet treatments (Fig. 4c: con-

trasts 5–6), even though over fed males experienced a greater increase of body mass than normally fed males (Table 2).

The decrease of MRS over time in intact individuals (males and females) was similar between normally and over fed individuals (Fig. 4c: contrasts 3 and 7). A similar pattern was found for autotomized females (Fig. 4c: contrast 4) and males (Fig. 4c: contrast 8), even though the increase in body mass over time was much higher for over fed than for normally fed females (Table 2). Estimates of repeatability show that the MRS was consistent between individuals during the long-term experiment (Table S1, Supporting Information S2).

DISCUSSION

Here, we investigated the short- and long-term effects of an extreme form of autotomy that includes abdomen loss and permanent constipation in a scorpion species. We tested 5 predictions of the weight loss hypotheses (Table 1), which states that weight has a negative

Table 3 Results of the linear mixed-effect model for the long-term effects of tail autotomy on the maximum running speed (MRS) of the scorpion *Ananteris balzani*

Diet treatment	Experimental group	
	Intact	Autotomized
	Slope \pm SE (95% CI)	
Normally fed		
Females ($N_{\text{int}} = 6$; $N_{\text{auto}} = 10$)	-0.061 ± 0.040 (-0.140 to 0.018)	-0.183 ± 0.034 (-0.250 to -0.115)
Males ($N_{\text{int}} = 15$; $N_{\text{auto}} = 20$)	-0.047 ± 0.027 (-0.100 to 0.006)	-0.181 ± 0.026 (-0.231 to -0.130)
Over fed		
Females ($N_{\text{int}} = 7$; $N_{\text{auto}} = 10$)	-0.096 ± 0.037 (-0.169 to -0.023)	-0.083 ± 0.035 (-0.153 to -0.013)
Males ($N_{\text{int}} = 14$; $N_{\text{auto}} = 19$)	-0.019 ± 0.026 (-0.071 to 0.032)	-0.155 ± 0.025 (-0.204 to -0.105)

We present estimated slopes for the relationship between time (days) and MRS for females and males belonging to each experimental group (intact and autotomized) and diet treatment (normally and over fed). *Negative* values of slopes indicate that the MRS *decreased* over time. When the 95% confidence interval (95% CI) of the slope does not overlap zero, we consider it as statistically significant and highlight the value in bold. N_{int} , sample size for intact individuals; N_{auto} , sample size for autotomized individuals.

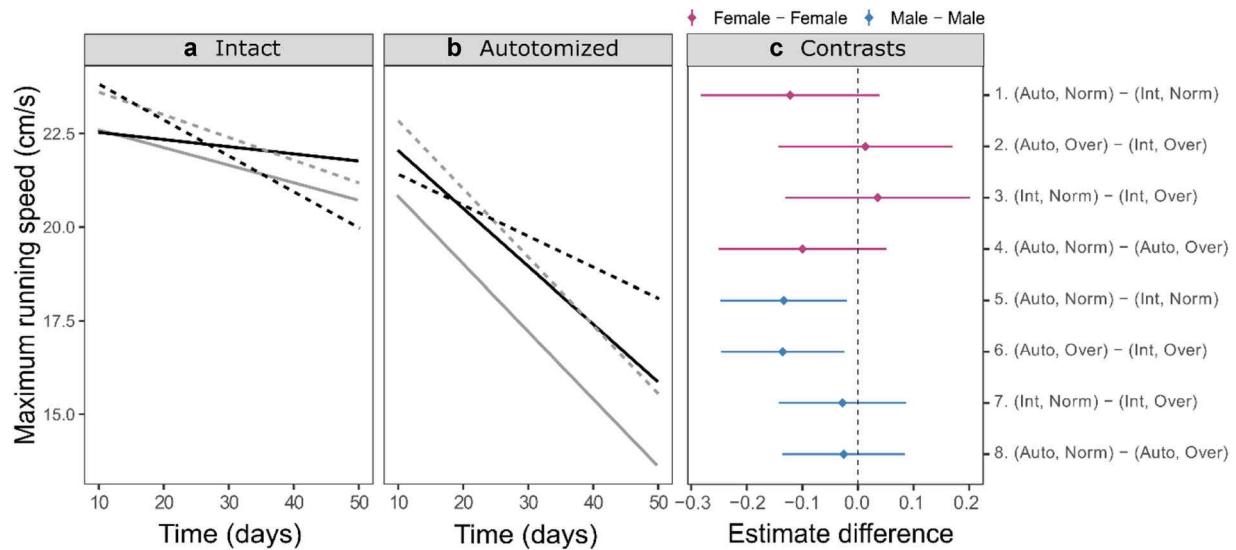


Figure 4 Long-term effects of tail autotomy on the maximum running speed (MRS) of the scorpion *Ananteris balzani*. (a,b) Regressions between MRS and time are shown for intact (a) and autotomized (b) individuals belonging to 2 diet treatments: normally fed (gray) and over fed (black). Females are represented by the dashed lines and males by the solid lines. (c) We estimated the difference (contrast) between the slopes of MRS for relevant combinations of sex, diet treatment, and experimental group (intact and autotomized). A positive value of contrast indicates that the estimated MRS for the first term of the contrast was higher than the second term, whereas a negative value indicates the opposite. Symbols indicate mean differences and bars indicate 95% confidence intervals. Abbreviations: Int, intact; Auto, autotomized; Norm, normally fed; Over, over fed. Values of all estimates and contrasts illustrated here are presented in Table S5, Supporting Information S2.

effect on the locomotor performance, so that heavier individuals should be slower than lighter individuals (reviewed in Jagnandan & Higham 2018). In what follows, we contrast our findings with the predictions of this hypothesis and compare the patterns found here with tail autotomy in ectotherm vertebrates, especially lizards. We understand that the morphology and physiology of scorpions and ectotherm vertebrates are markedly different, so comparisons between these taxa require caution. However, the loss of an elongated abdomen (tail) in scorpions is a unique defensive behavior, so that tail loss in ectotherm vertebrates offers the best benchmark for possible comparisons.

Short-term effects on locomotor performance

The results of the short-term experiment refute the predictions of the weight loss hypothesis tested here. Prediction 1 states that tail autotomy should increase locomotor performance due to weight loss (Table 1), but our findings show that tail autotomy does not have any detectable effect on the running speed of the individuals. The only difference occurred in the *intact* group, in which overloaded males decreased their locomotor performance. Under natural conditions, there are 2 situations in which scorpions carry a load on their dorsum. The first is when females and males transport dead prey (Ojanguren-Affilastro *et al.* 2016), a behavior recorded for *A. balzani* (pers. obs.). In this case, our findings suggest that males, but not females, may have their locomotor performance decreased when they are transporting prey. Considering that the load we used was proportional to the total body mass of females and males, the sexual difference in locomotor performance of loaded individuals requires a biological explanation. We argue that females are better than males at coping with an extra load because they naturally experience great increase in total body mass during pregnancy (Warburg 2011). The second situation is when females are carrying offspring, a widespread maternal behavior in scorpions (Polis & Sissom 1990). Carrying offspring decreases locomotor performance of *Centruroides vittatus* females (Shaffer & Formanowicz 1996) and also the foraging success of *C. sculpturatus* females (Webber & Rodríguez-Robles 2013). Contrary to these studies, the locomotor performance of intact females of *A. balzani* carrying an extra load was not decreased. However, while the extra load we used represents 22% of the females' body mass, the relative load represented by the offspring in the species is 47% (García-Hernández & Machado 2021). Thus, we suggest that there is a threshold of weight above which the locomotor performance of the females

starts to be negatively affected. In the future, an experimental study can be designed to test this hypothesis and determine precisely the value of this possible threshold.

Although the mean weight of the lost tail in females and males represents, respectively, 19% and 21% of the total body mass, the relative mass of the lost tail had no marked effect on the change in locomotor performance of both sexes. This finding refutes prediction 2, according to which the greater the relative mass of the lost tail, the greater the locomotor performance should be (Table 1). An experimental study with the scorpion *C. vittatus* revealed that dehydrated individuals lost on average 15% of their body mass compared with less than 0.5% of control (i.e. nondehydrated) individuals. In locomotor performance trials, dehydrated individuals increased their running speed in 7.5% whereas control individuals showed a decrease of nearly 28% (Carlson & Rowe 2009). According to the authors, the increase in locomotor performance of dehydrated individuals could be better explained by weight loss. Why a weight loss superior to 15% in autotomized individuals of *A. balzani* does not promote an increase in locomotor performance is still an open question. However, when comparing the results obtained with *C. vittatus* and *A. balzani*, it is clear that the weight loss necessary to cause an increase in locomotor performance varies among scorpion species, possibly in response to differences in body size, metabolic rates, or biomechanics of locomotion.

One aspect of tail loss that we did not explore here is how the precise fracture point affects the locomotor performance. Some lizard species also not detach the entire tail upon a predator attack, probably to minimize the costs associated with autotomy and regeneration (e.g. Werner 1968; Daniels 1985; Arnold 1988; Lin & Ji 2005; Cooper & Smith 2009). Individuals of *A. balzani* frequently detach their tail between the third and fourth metasomal segments, which corresponds to the most distal fracture point recorded so far (Mattoni *et al.* 2015). There are 2 other fracture points (between the first and second and between the second and third segments) where natural or induced autotomy is rarely recorded (Mattoni *et al.* 2015). This information suggests that the so-called "economy of autotomy" hypothesis proposed for lizards (Woodland 1920) may also apply to scorpions. The economy of autotomy also has an additional implication that may shed light on the evolution of tail autotomy in scorpions. Individuals that lose the tail between the third and fourth segments may later induce autotomy between the second and third segments to eliminate feces accumulated at the end of the digestive tract after the first autotomy event (Mattoni *et al.* 2015). By doing so, individuals may attenuate the

long-term costs of autotomy, increase their lifespan, and improve their chances of mating and giving birth before constipation causes their death.

Prediction 3 states that autotomy should increase locomotor performance of males more than females because the tail of the males is relatively larger, and consequently heavier than the tail of females (Table 1). However, our comparisons show that there is no sexual difference in locomotor performance of autotomized individuals. In many lizard species, tail loss also has no sexually dependent effects on the locomotor performance of the individuals (see examples in Bateman & Fleming 2009). One point that deserves attention, however, is that autotomized males and females may respond differently depending on the proxy of locomotor performance used in the study. In the skink *Niveoscincus metallicus*, for instance, tail autotomy affects *endurance* in females and *sprint speed* in males (Chapple & Swain 2002). In the case of *A. balzani*, we argue that the difference in the relative weight of the lost tail between males and females is too small, and this value is likely not high enough to promote sexually dependent effects on our proxy of locomotor performance, which was MRS. Given that the running speed of male and female scorpions is not affected by tail autotomy, we suggest that, in the short-term, autotomized individuals are as efficient as intact individuals in accomplishing several vital activities that are directly related to locomotor performance, such as escaping from predators and active foraging (Webber & Rodríguez-Robles 2013).

As a final remark, we stress that short-term changes in locomotor performance after tail autotomy may also be caused by a sudden shift in the center of body mass. In lizards, for instance, tail loss shifts the center of body mass anteriorly, reducing hind limb propulsive force and consequently impairing locomotor performance (e.g. Ballinger *et al.* 1979; Jagnandan *et al.* 2014; see also Gillis *et al.* 2013). However, the data obtained in our short-term experiment do not provide support to the hypothesis that a shift in the center of body mass caused by tail autotomy decreases locomotor performance of male and female scorpions. Basically, we detected no difference in running speed between intact individuals in the premanipulation trials and autotomized individuals in the unload trials (Fig. 3d: contrasts 1 and 4). A detailed study on the kinematics of scorpion locomotion revealed that individuals running in defensive posture (i.e. with the tail bended forward) experience a shift in the center of body mass when compared with individuals running in the non-defensive posture (i.e. with the tail extended backward). In response to this shift, individuals deploy a modified gait that is highly effective in stabilizing their run (Telheiro *et al.* 2021). In fact, the alternating tetrapod gait exhibited

by scorpions is known to be a particularly stable locomotion pattern because each side of the individual is always supported by at least an alternate pair of legs (first and third or second and fourth) (Bowerman 1975). This stable locomotion pattern may explain why tail loss—which probably promotes a forward displacement of the center of mass similar to that described above—has no negative short-term effect on the locomotor performance of the scorpion *A. balzani*.

Long-term effects on locomotor performance

The results of the long-term experiment are also not consistent with predictions of the weight loss hypothesis. Prediction 4 states that autotomy should decrease locomotor performance over time due to an increase in body mass caused by constipation (Table 1). Although autotomized individuals indeed showed a decrease of locomotor performance over time, this decrease cannot be attributed to changes in body weight because autotomized normally fed individuals did not increase their mass over time. Only autotomized over fed individuals experienced such increase and, if weight was the main driver of changes in locomotor performance, the decrease in the running speed should be restricted to these individuals. More importantly, when comparing intact and autotomized individuals over time, we only found differences in locomotor performance of males, which reduced their running speed after tail loss regardless of they were normally or over fed. Because the increase in body mass caused by constipation is a unique side-effect of autotomy in scorpions, it is difficult to compare our results with those obtained for other animals. Moreover, only a few studies accessed the long-term effects of tail autotomy. An example is a study with the leopard gecko *Eublepharis macularius* that showed that the running speed does not change over the 22 weeks required for tail regeneration, even though the body mass of the individuals shows a marked increase due to tail regeneration (Jagnandan *et al.* 2014). Thus, despite the difference in the mechanisms that promote an increase in body mass in *A. balzani* (constipation) and *E. macularius* (tail regeneration), the long-term consequences of tail autotomy on the locomotor performance seem to be dissociated of changes in body weight. Finally, the decrease in locomotor performance found here cannot be either attributed to a shift in the center of body mass caused by tail loss. Given that no tail regeneration occurs in *A. balzani*, any negative effect promoted by such a shift should occur in both sexes and be quantitatively similar in both diet treatments, but our results do not support these predictions.

In a previous experiment with *A. balzani*, we showed that the reproductive success of autotomized females was greatly decreased, but that the courtship behavior and spermatophore transfer of autotomized males were not impaired, indicating that tail loss has no short-term negative effect on male reproductive success (García-Hernández & Machado 2021). The data obtained here show an opposite pattern: in the long-term, tail loss has a negative effect on the locomotor performance of males, but not of females. Considering that the locomotor performance of autotomized males decreases over time when compared with intact males, tail loss may impose long-term negative effects on male reproductive success. In scorpions, males show intense surface activity during the breeding season, searching for receptive females (Polis & Sissom 1990). Thus, decreased locomotor performance may decrease the chances of autotomized males to find receptive females, either because they are slower than intact males or because autotomy induces behavioral changes, such as lower movement rates (e.g. harvestmen: Guffey 1999; lizards: Formanowicz *et al.* 1990) or increased tendency to remain in safer places (e.g. crickets: Bateman & Fleming 2006; lizards: Cooper 2003). Moreover, autotomized males show decreased predation success, constraining their diet to only small prey (García-Hernández & Machado 2020). Thus, in the long-term, autotomized males should be in poorer body condition than intact males due to lower food intake. Considering that courtship is a condition-dependent behavior in many groups, including scorpions (see Olivero *et al.* 2019 and references therein), autotomized males should have a decreased courtship performance after some months. Finally, accumulation of feces inside autotomized males may cause deformities in the hemi-spermatophores, compromising the functionality of the spermatophore. Taking all these effects together, we suggest that autotomized males pay long-term reproductive costs, which deserve future investigation.

Prediction 5 states that autotomized normally fed individuals should have a better locomotor performance than autotomized over fed individuals, because the latter are heavier (Table 1). However, we found no effect of diet treatment (normally and over fed) on the locomotor performance of the individuals, regardless of their tail condition (intact or autotomized). In the case of autotomized individuals, over fed females and males experienced an increase in body mass over time due to constipation, whereas normally fed did not. Despite this difference in body mass, the locomotor performance of the normally fed and over fed individuals showed a similar decrease over time, a result that refutes the weight loss hypothesis.

In the short-term experiment, a weight loss of 22% in females and 19% in males was not enough to affect their locomotor performance. In the long-term experiment, we found that a weight gain of 30% in females and 29% in males was also not enough to affect their locomotor performance. This finding reinforces the notion that individuals of *A. balzani* can suffer marked changes in body mass (both gains and losses) without a negative effect on their locomotor performance. Over feeding, however, may have an important long-term negative effect that was not detailedly explored here: severe constipation may increase the deleterious effects of accumulation of toxins and reduce the longevity of individuals (Mattoni *et al.* 2015). The data obtained during the long-term experiment shows that the probability of mortality was 7 times higher for autotomized than for intact individuals, but there was no difference between normally fed and over fed autotomized individuals (see “Probability of mortality” in Supporting Information S2). Thus, although tail autotomy indeed reduces longevity, this negative effect seems to be not directly related to the degree of constipation experienced by the individuals. One implication of this result is that, under natural conditions, individuals are not expected to reduce their feeding rates after tail loss.

As a final remark, we argue that the negative long-term effects of autotomy on the locomotor performance of *A. balzani* can be addressed from a perspective different than an increase in body mass over time. Tail autotomy probably has implications for water balance and accumulation of nitrogenous wastes, because the rectum is the principal site at which osmotic and ionic composition are changed in scorpions (Hadley 1990). Thus, the loss of the hindgut may promote an imbalance of body fluids and solutes that may compromise homeostasis and have negative effects for locomotor performance. This imbalance may explain why the locomotor performance of autotomized males was lower than that of intact males, although no difference was detected among feeding treatments. Moreover, the accumulation of by-products of anaerobic processes (e.g. lactate) may explain why the locomotor performance of autotomized females was not as negatively affected as males. Male scorpions are usually more vagile than females (Polis & Sissom 1990) and have proportionally longer legs than females (McLean *et al.* 2018). Thus, males have more body mass devoted to running muscles than females, which translates in more production and accumulation of lactate, and probably greater loss of running speed (Prestwich 2006). We do not know whether the accumulation of lactate is indeed affected by tail autotomy in scorpions. However, when compared with females, male scorpions have relatively smaller

hepatopancreas (Gefen 2008), an organ that produces several enzymes, including the lactate dehydrogenase, responsible for the conversion of lactate to pyruvate, a metabolite with less negative effects (Padmaja *et al.* 2010; Warburg 2012). Consequently, tail autotomy should have a more pronounced negative effect in males than in females, as we report here. In conclusion, physiological changes after tail autotomy seems to offer a better explanation than the weight loss hypothesis for the patterns found in the long-term experiment.

CONCLUSIONS

The results of the short- and long-term experiments clearly refute the weight loss hypothesis. In fact, the main conclusion of the short-term experiment is that tail loss has no effect on the locomotor performance of the scorpions. A similar pattern has also been reported for several lizard species, in which tail loss also does not impose either negative or positive effects on the locomotor performance of the individuals (reviewed in Bateman & Fleming 2009). Contrary to most studies with lizards, which focus on a single sex or lump individuals of both sexes, we compared the effects of tail loss on the locomotor performance of females and males separately. Given that the running speed of females and males was not affected by tail loss, we suggest that vital activities performed by both sexes and directly linked to locomotor performance, such as escaping from predators and active foraging, are not immediately impaired by autotomy. In the long term, there is a marked decrease in locomotor performance of males (but not of females), and this reduction is not associated with an increase in body mass caused by constipation. Given that scorpion males rely on active searching to find mating partners (Polis & Sissom 1990), a long-term decrease in locomotor performance of autotomized males may lead to a reduction in their lifetime reproductive success when compared with intact males. However, because death caused by constipation takes several months, autotomized males may have enough time to find several mating partners and sire offspring (García-Hernández & Machado 2021). Thus, the long period between tail loss and death by constipation is a key factor in understanding the evolution of one of the most extreme cases of autotomy in nature.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Video

Supporting Information S2 Additional results

Figure S1 Sexual differences between body mass and body size of the scorpion *Ananteris balzani*. (a) Total

body mass of intact males ($N = 27$) and females ($N = 21$). (b) Carapace length of intact males ($N = 27$) and females ($N = 21$). Horizontal lines represent the median, the upper and lower limits of the box represent the third and first quartiles (75th and 25th percentile), respectively, vertical lines represent the range, and the circle represents an outlier (i.e. a datapoint beyond 1.5 times the interquartile range).

Table S1 Estimates of repeatability index for the maximum running speeds in the short-term and long-term experiments. The repeatability index ranges from 0 (low repeatability, high within-individual variance) to 1 (high repeatability, low within-individual variance). Numbers in parentheses indicate the 95% confidence interval.

Table S2 Summary of the estimated maximum running speed (cm/s) of males and females of the scorpion *Ananteris balzani* belonging to both experimental groups (intact and autotomized) for the 3 phases of the short-term experiment: premanipulation, loaded trials, and unloaded trials. For each combination of factors, we present the standard error, the sample size (N) and the 95% confidence interval (95%CI).

Table S3 Summary of the model selection analysis performed to investigate whether the data on the maximum running speed obtained in the long-term experiment show temporal autocorrelation, even after controlling for individual identity. Δ AIC is the difference in the Akaike information criterion (AIC) between each model and the most plausible model. The letter k indicates the

number of parameters in each model. Weight = Akaike weight. The simplest model is highlighted in bold.

Table S4 Summary of relevant contrasts of the estimated maximum running speed (cm/s) in the short-term experiment. We contrast combinations of sex (female and male) and loading treatment (premanipulation, unloaded, and loaded) for both experimental groups (intact and autotomized). In parentheses are presented the estimated difference and the 95% confidence interval (95% CI). The direction of the difference is showed using the following symbols: > (i.e. the contrast value is positive, and the 95% CI does not overlap zero), < (i.e. the contrast value is negative, and the 95% CI does not overlap zero), and \approx (i.e. regardless of the contrast value, the 95% CI overlaps zero).

Table S5 Summary of the relevant contrasts of the estimated slope of maximum running speed (cm/s) in the long-term experiment. We contrast combinations of diet treatment (normally and over fed) and sex (female and male) for both experimental groups (intact and autotomized). In parentheses are presented the estimated difference and the 95% confidence interval (95% CI). The direction of the difference is showed using the following symbols: > (i.e. the contrast value is positive, and the 95% CI does not overlap zero), < (i.e. the contrast value is negative, and the 95%CI does not overlap zero), and \approx (i.e. regardless of the contrast value, the 95% CI overlaps zero). Abbreviations: Int = Intact, Auto = Autotomized, Norm = Normally fed and Over = Over fed.

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