



'Tail' autotomy and consequent stinger loss decrease predation success in scorpions

Solimary García-Hernández^{a,*}, Glauco Machado^b

^a Programa de Pós-graduação em Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, São Paulo, SP, Brazil

^b LAGE do Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, São Paulo, SP, Brazil

ARTICLE INFO

Article history:

Received 15 April 2020

Initial acceptance 17 July 2020

Final acceptance 10 August 2020

Available online 26 September 2020

MS. number: A20-00258

Keywords:

compensatory mechanism
defence

foraging success

handling time

prey capture

prey profitability

prey size

stinger use

subduing success

Predation success depends on factors such as hunger, prey size, prey availability and intensity of competition. A neglected factor that may also influence predation success is the proper function of morphological traits related to prey search, capture and manipulation. Injuries that compromise the functionality of these morphological traits may reduce predation success. In many invertebrates, autotomy can compromise predation success because the detached body part may be crucial for hunting. However, empirical evidence linking autotomy and predation success is relatively scarce. We filled this gap using the scorpion *Ananteris balzani*, which autotomizes the last abdominal segments, known as the 'tail'. This is a unique form of autotomy as 'tail' autotomy implies the loss of the stinger, an organ used for venom inoculation, which is the main form of large prey subjugation. Using a paired experimental design, we found that for both small and large prey, subduing success was higher when individuals were intact than when they were autotomized. After autotomy, subduing success of male scorpions decreased from 90% to 17% for small prey and from 47% to 1% for large prey. Subduing success of female scorpions after autotomy decreased from 98% to 93% for small prey and from 97% to 70% for large prey. Autotomized individuals took longer than intact individuals to subdue both small and large prey, but the effect size was higher for large prey. Considering that the tail does not regenerate, autotomized individuals (especially males) will experience a lifelong reduction in trophic niche breadth because their diet will be mostly composed of small prey. Moreover, autotomized individuals probably move more to enhance the likelihood of finding small prey, which may increase their exposure to predators and consequently the costs related to tail loss.

© 2020 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Among predators, predation success depends on extrinsic factors, such as habitat structure, prey availability and intensity of competition for prey, as well as intrinsic factors, such as hunger, learned experiences and nutrient requirements (reviewed in [Perry & Pianka, 1997](#)). A neglected factor that may also influence predation success is the proper function of morphological traits related to the search, detection, capture, manipulation and ingestion of prey. Because predators need to be in good physical condition to subdue their prey, injuries caused during predator–prey interactions may compromise the functionality of the morphological traits used for hunting, thus reducing predation success (reviewed in [Mukherjee & Heithaus, 2013](#)). Among crabs, for instance, individuals with worn claw teeth take longer to crack clams, while individuals with a

broken claw are simply unable to crack them ([Juanes & Hartwick, 1990](#)). In a similar way, broken teeth, a common injury associated with hunting large or dangerous prey, may severely reduce predation success of injured individuals among carnivorous mammals (reviewed in [Van Valkenburgh, 1988](#)).

Injuries associated with manipulating large, hard or dangerous prey are not the only way to compromise the functionality of the morphological traits used for hunting. At least one defensive strategy against predation, known as autotomy, can also injure morphological traits used for hunting because the body part that is voluntarily detached in response to the predatory attack may be important in the foraging process (reviewed in [Emberts, Escalante, & Bateman, 2019](#); [Fleming, Muller, & Bateman, 2007](#); [Maginnis, 2006](#)). In arachnids, for instance, the loss of one or more ambulatory legs may reduce the locomotor performance, sensory perception and foraging ability of the autotomized individuals (e.g. [Brueseke, Rypstra, Walker, & Persons, 2001](#); [Guffey, 1999](#); [Riechert, 1988](#); [Steffenson, Formanowicz, & Brown, 2014](#)). In some crabs,

* Correspondence: S. García-Hernández, Programa de Pós-graduação em Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav. 14, no. 321, São Paulo, SP, 05508-090, Brazil.

E-mail address: solimarygarcia@gmail.com (S. García-Hernández).

individuals missing a claw tend to feed mostly on smaller or soft items because they show a reduction in the maximum crushing force when compared with intact individuals (e.g. Davis et al., 2005; Flynn, Mellish, Pickering, & Quijón, 2015; Juanes & Smith, 1995; Patterson, Dick, & Elwood, 2008; Smith & Hines, 1991; Wasson, Lyon, & Knope, 2002). Finally, the loss of one or two arms in sea stars makes the individuals unable to open and consume mussels as effectively as intact individuals, constraining automatized individuals to feed mostly on smaller prey (e.g. Lawrence et al., 1986; Ramsay, Kaiser, & Richardson, 2001). Thus, autotomy has many negative effects on different phases of the foraging process, especially prey capture and manipulation.

An interesting animal group to investigate the costs of autotomy on foraging behaviour are scorpions of the genus *Ananteris* (Lira, Sarinho, De Souza, & Albuquerque, 2014; Mattoni et al., 2015). Unlike other arachnids, such as spiders, whip-spiders and harvestmen, in which individuals autotomize legs (reviewed in Roth & Roth, 1984), scorpions autotomize the metasoma, the last abdominal segments, commonly known as the 'tail' (Mattoni et al., 2015). The tail contains the last part of the nervous, circulatory and digestive systems, as well as the telson, which contains a pair of venom glands and the stinger organ (Hjelle, 1990). The autotomized segments never regenerate and, because the individuals lose the anus, they are unable to defecate and die from constipation some months after autotomy. Here, we experimentally tested how metasomal autotomy may affect the predation success of males and females in the scorpion *Ananteris balzani* to understand one of the possible costs of this extreme form of defence.

In scorpions, the probability of using the stinger to subdue prey seems to be related to prey size. Scorpions can subdue small prey with their pedipalps and do not use their stinger to inject venom (Rein, 1993). However, inoculation of venom is crucial to subdue large prey, especially for small-bodied scorpion species with slender pedipalps (Edmunds & Sibly, 2010; Rein, 1993), as is the case for all species in the genus *Ananteris* (Botero-Trujillo & Florez, 2011; Fig. 1). Autotomized individuals of *Ananteris solimariae* can capture small prey using only their pedipalps (Mattoni et al., 2015), which suggests that venom inoculation is not a necessary condition for prey capture. However, the effect of metasoma loss on the capture of large prey has not been investigated previously. Moreover, considering that species of the genus *Ananteris* show marked sexual size dimorphism, with males being considerably smaller than females in total body size (Botero-Trujillo & Florez, 2011; Fig. 1), the negative effects of metasoma loss on predation success should be sex dependent. For small prey, we expected that metasomal autotomy should have similarly low negative effects on both males and females, which are likely to subdue prey using only their slender pedipalps. For large prey, however, metasomal autotomy should be more detrimental for males than for females because even the largest males are smaller than the smallest females. Therefore, males should be unable to subdue large prey using only their pedipalps.

METHODS

Collection and Maintenance

We collected 53 adult males and 49 adult females of *A. balzani* during October 2017 and January 2018 in a savanna area at Santa Bárbara Ecological Station (24°48'S, 49°13'W), state of São Paulo, southeastern Brazil. The animals were found mainly along dirt roads, where the substrate consisted of sand with occasional grass, leaf litter and stones. We brought the individuals to the laboratory, where they were maintained during 2 months in individual plastic containers with a piece of wet cotton to provide water and moisture

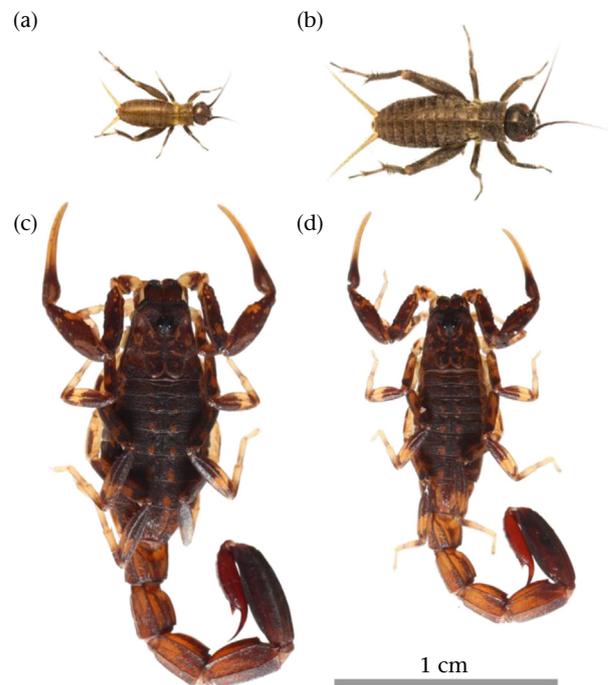


Figure 1. Nymphs of the cricket *Acheta* sp. offered as prey for individuals of the scorpion *Ananteris balzani* (photos by John Uribe). (a) Small prey, 4–5 mm total body length. (b) Large prey, 8–9 mm total body length. Dorsal view of (c) a female and (d) a male *A. balzani* showing the well-marked sexual size dimorphism in the study species. Note that the relative sizes of both small and large prey are larger for male *A. balzani* than for female *A. balzani*.

and a dry leaf for use as a diurnal shelter. Because many individuals collected in the field are parasitized by a nematomorph that makes them lethargic and can kill the host upon emergence, the long quarantine period was necessary to eliminate parasitized individuals from our experiment. Also, because some individuals collected in the field have the tip of the stinger (i.e. the venom-injecting barb) broken, we only included individuals with intact stingers in our experiment.

During the quarantine, we fed individuals every 10 days with cricket nymphs (*Acheta* sp.). Given that female body mass (mean \pm SE = 51.73 \pm 2.78; $N = 21$) is two times greater than male body mass (mean \pm SE = 119.61 \pm 3.15; $N = 27$), at each feeding event males received two cricket nymphs with 3 mm of total length, whereas females received four cricket nymphs of the same size. During the entire period of maintenance in the laboratory, we kept the individuals on a 12:12 h light:dark cycle, which is similar to the natural habitat. Moreover, we conducted the experiment described below in an environment with minimal temperature variation (mean = 25 °C, range 24.5–26.5 °C).

Experimental Design

First, we randomly split the individuals of both sexes into two experimental groups: control, containing 28 males and 27 females; and treatment, containing 25 males and 22 females. In the pre-manipulation phase of the experiment, individuals of both experimental groups were intact (i.e. nonautotomized). In the postmanipulation phase of the experiment, we induced metasomal autotomy in each individual belonging to the treatment group by repeatedly touching the body of the individual with forceps for 1 min and then grasping and pulling its last metasomal segment with the same forceps. If the individual did not release its metasoma after 2 min, we repeated the protocol after 2 h of resting. For

individuals assigned to the control group, we used the same protocol to induce autotomy, but we grasped the first metasomal segment, where autotomy is impossible.

To increase the hunger level of the scorpions and their motivation to hunt, 2 weeks before the beginning of the experiment (i.e. the premanipulation phase), we fed both males and females once per week with half of the number of cricket nymphs they received during the quarantine. Thus, at the beginning of the trials, individuals had 7 days of fasting. On the day before the beginning of the experiment, we placed each scorpion inside a plastic box (20 × 15 cm and 15 cm tall) with sand as substrate, a piece of wet cotton to provide water and moisture and a dry leaf for use as a diurnal shelter. The next day, we removed the wet cotton and the dry leaf 1 h before the beginning of the trial because these objects could provide shelter for the prey we offered to the scorpions, thus interfering with the foraging process. All foraging trials were conducted at night (1800–2300 hours) under dim red illumination, which does not disturb scorpions (Machan, 1968).

The prey we offered to the scorpions was the same cricket species we used to feed them before the experiment. We selected two prey sizes from our stock population: (1) small, which corresponds to crickets with 4–5 mm of total body length and 4.5 mg of total body mass; (2) large, which corresponds to crickets with 8–9 mm of total body length and 45 mg of total body mass (Fig. 1). Small and large cricket nymphs have similar general morphology and behaviour, so the main difference between them is size. The prey/predator mass ratio for small prey was 0.086 for intact males and 0.038 for intact females. Moreover, the total length of small prey was 1.8 and 1.4 times larger than the prosoma length of males and females, respectively (Fig. 1). The prey/predator mass ratio for large prey was 0.865 for intact males and 0.375 for intact females. Finally, the total length of large prey was 3.4 and 2.7 times larger than the prosoma length of males and females, respectively (Fig. 1). For both males and females, the prey/predator mass ratio for large prey was 10 times larger than that for small prey and, when compared with prosoma length of scorpions, the relative size of large prey was two times larger than that of small prey.

To estimate the predation success of males and females according to prey size, we performed two trials per individual in the premanipulation phase: one with a small prey and another with a large prey. The two trials were separated by a 3 h interval, and the order in which each scorpion received the small and the large prey was randomized among the individuals. We followed each interaction for up to 10 min after the first physical contact between the hunting scorpion and the cricket. If the prey was not subdued within this 10 min interval, we considered the scorpion to be unsuccessful. To maintain similar hunger levels between trials, we did not allow the scorpion to consume the cricket after it was completely subdued. Thus, when the scorpion effectively captured the prey, we pulled the cricket out with forceps to induce the scorpion to release it. Moreover, we removed the cricket from the experimental box when it was not captured. After the first trial, we let the scorpion rest for 3 h inside the experimental box. In the first 2 h of resting, we put the wet cotton and the dry leaf back into the box to provide a water source and shelter for the individuals. In the last hour, we removed the wet cotton and the dry leaf from the box, following the same procedure used in the first trial. Then, we conducted the second trial repeating the same procedure explained above but using prey of a different size from the prey used in the first trial. We also did not allow the scorpion to consume the prey in the second trial.

Immediately after the end of the second trial of the premanipulation phase, we fed each individual the same number of cricket nymphs it had received during the 2-week interval before the beginning of the experiment. The next day, we started the

postmanipulation phase, where individuals of the control group were maintained intact, whereas individuals of the treatment group were autotomized. After manipulation, individuals of both experimental groups were allowed to rest for 6 days, which is sufficient for autotomized individuals to complete healing of the fracture point. During this 6-day period individuals were not fed. One week after the end of the premanipulation phase, we estimated individuals' predation success using the same procedure outlined above. Thus, we obtained repeated measures of each individual's predation success for small and large prey in two phases (i.e. pre- and postmanipulation of the metasoma).

We filmed the trials in both phases of the experiment and, based on the footage, we estimated predation success using two proxies. The first one was 'subduing success', i.e. whether the scorpion immobilized the prey. There are two behavioural sequences that clearly indicate when the prey has been immobilized: either (1) the scorpion releases one or both pedipalpal chelae, then grasps the prey only with its chelicerae and begins walking around with the prey hanging on its mouthparts, or (2) while still grasping the prey with its pedipalpal chelae, the scorpion touches the prey with its chelicerae and starts feeding on it. The second proxy was 'handling time', which began when the scorpion first grasped the cricket with one or both pedipalpal chelae and ended the moment the prey was subdued. In each trial of the premanipulation phase in which the scorpion subdued the prey, we also recorded the 'stinger use', i.e. whether the scorpion used its stinger to inject venom and immobilize the prey. The stinger can be easily observed in the footage because the scorpion bends its metasoma forward (which is a very conspicuous behaviour) and it is usually possible to see the stinger penetrating the prey's exoskeleton (Fig. 2a).

Data Analysis

To test for the effect of metasomal autotomy on the probability of subduing a prey (i.e. subduing success), we adjusted a Bayesian generalized linear mixed model (GLMM), which is indicated for use with data sets in which the variance of some combinations of fixed effects is close to zero (the so-called 'singular fit'), and thus it is difficult to compute confidence intervals (Gelman & Hill, 2006). The Bayesian approach also gives estimates and credible intervals for all parameters (Gelman & Hill, 2006). The response variable of our model was subduing success (no = 0 and yes = 1), modelled as having a binomial error distribution and using a logit link function. The predictor variables were sex (male and female), prey size (small and large), experimental group (control and treatment) and phase of the experiment (pre- and postmanipulation). Because we obtained repeated measures from the same scorpions, we used individual identity as a random factor. Moreover, because it was not possible to perform the experiment on all individuals on the same day, we separated the trials into six temporal blocks. To control for any possible temporal difference in the behaviour of the individuals, we thus included the temporal blocks as an additional random factor in the model.

To explore the importance of the metasoma on subduing success of small and large prey by females and males, we tested whether the probability of using the stinger depends on the sex of the scorpion and prey size. Because the data on subduing success also showed singular fit, we adjusted again a Bayesian GLMM. The response variable was stinger use (no = 0 and yes = 1), modelled as having a binomial error distribution and using a logit link function. The predictor variables were sex and prey size. We did not include experimental group and phase of the experiment in this analysis because we used only intact individuals (i.e. those with a stinger) that captured prey in the premanipulation trials ($N = 44$ males and

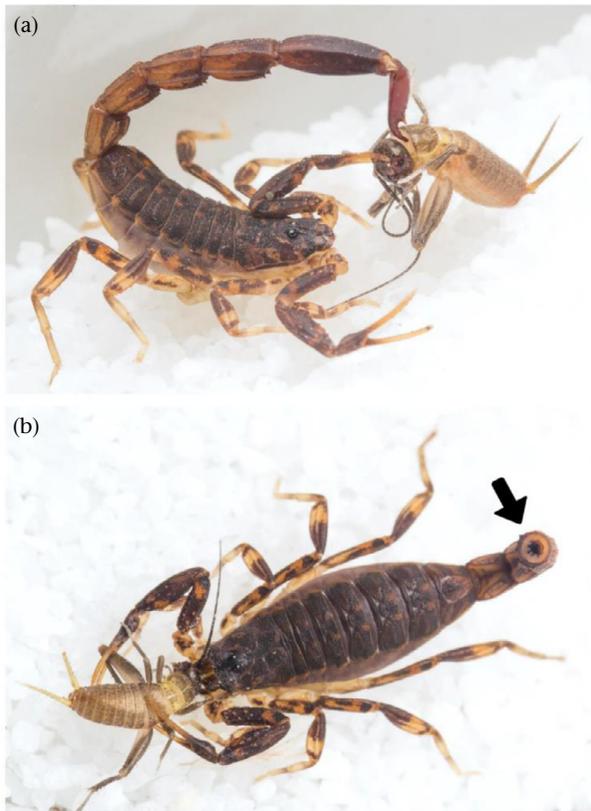


Figure 2. Adults of the scorpion *Ananteris balzani* feeding on cricket nymphs (photos by John Uribe). (a) Adult male with intact 'tail' stinging a large cricket. Note that the stinger is inserted in the exoskeleton of the cricket. (b) Adult male with autotomized tail grasping a small cricket using only his pedipalps. Note the dark scar tissue (arrow) at the end of the autotomized tail. This scar tissue blocks the posterior end of the digestive tract, preventing defecation and causing constipation.

39 females). Again, we used individual identity and temporal block as random factors.

To test for the effect of metasomal autotomy on handling time of captured prey, we adjusted a Bayesian GLMM because the data showed singular fit. The response variable was the data from handling time (ln transformed), modelled as having a Gaussian error distribution. The predictor variables were sex, prey size, experimental group and phase of the experiment. As in the previous analyses, we used individual identity and temporal block as random factors. The data set used here included only individuals that captured prey during the experiment (small prey: 70 captures for males, 75 captures for females; large prey: 48 captures for males, 68 captures for females).

To each fitted model, we calculated the relevant contrasts between the levels of the fixed effects. We expected to find lower subduing success and longer handling time for individuals of the treatment group when they were in the postmanipulation phase (i.e. autotomized) than when they were in the premanipulation phase (i.e. intact). The contrasts in this case were calculated as the premanipulation estimates minus the postmanipulation estimates and, according to our prediction, we expected the values to be positive for the subduing success analysis and negative for the handling time analysis. Moreover, we expected the negative effect of autotomy to be more pronounced when the prey was large than when the prey was small. The contrasts in this case were calculated as the estimate for small prey minus the estimate for large prey and, according to our prediction, we expected the values to be positive for the subduing success analysis and negative for the handling

time analysis. Finally, we expected that the negative effect of autotomy would be more pronounced in males than in females. The contrast to test for sex differences was calculated as the estimate of males minus the estimate of females and, according to our prediction, we expected the values to be negative for the subduing success analysis and positive for the handling time analysis.

All Bayesian GLMMs were performed in the 'MCMCglmm' package (Hadfield, 2010), using the software R v.3.5.2 (R Core Team, 2018). The parameters of all priors used in each model are presented in the Appendix, Table A1. The contrasts were performed in the 'emmeans' package (Lenth, 2019).

Ethical Note

Our experiments were conducted in accordance with ASAB/ABS Guidelines for the ethical treatment of animals (ASAB/ABS, 2012). Induced autotomy was necessary to perform the experiments. However, autotomy is a natural process (Mattoni et al., 2015), and induced autotomy was done carefully, stimulating the voluntary metasomal autotomy with minimum stress to the individuals. Both the collection and maintenance of individuals in captivity were conducted with proper permits of the Brazilian Government (SIS-BIO/ICMBio, permit 56081).

RESULTS

General Description of the Hunting Behaviour

Typically, after a cricket was placed inside the plastic box, the focal scorpion started moving its pedipalps while reorienting its body and walking towards the potential prey. When the scorpion was near the cricket (ca. 1 cm), it attempted to grasp the cricket with one or both pedipalpal chelae. Once the cricket was firmly grasped, the scorpion quickly stung it (Fig. 2a). In some cases, however, after touching the cricket for the first time, the scorpion was unable to grasp it, and the cricket escaped capture. On some of these occasions, the scorpion chased the cricket that had escaped during the first capture attempt. The hunting behaviour of autotomized scorpions was similar to that of intact individuals, except that they were unable to sting the cricket. However, similar to intact scorpions, autotomized individuals bent the first segments of their remaining metasoma forward, in an attempt to sting the captured cricket with a 'ghost stinger' (females: 61%; males: 40%) (Fig. 2b).

Subduing Success

In the premanipulation phase, when all individuals were intact, subduing success of control and treatment groups was qualitatively similar (Fig. 3a, contrasts 1–4 in Fig. 3b; see also Appendix, Tables A2–A3). In the postmanipulation phase, subduing success differed between the control (i.e. intact) and the treatment (i.e. autotomized) groups (Fig. 3a, contrasts 5–8 in Fig. 3b; see also Appendix, Tables A2–A3). As expected, subduing success of intact individuals in the control group during the postmanipulation phase was qualitatively similar to that observed during the premanipulation phase, regardless of prey size (contrasts 5–8 in Fig. 3b). For autotomized individuals in the treatment group, however, subduing success was greater when the prey was small than when it was large (contrasts 5–6 in Fig. 3b). Moreover, subduing success of males was lower than that of females, regardless of prey size (contrasts 7–8 in Fig. 3b).

When comparing the pre- and postmanipulation phases, subduing success in the control group tended to be slightly higher in the premanipulation phase (contrasts 9–12 in the Fig. 3b). In the

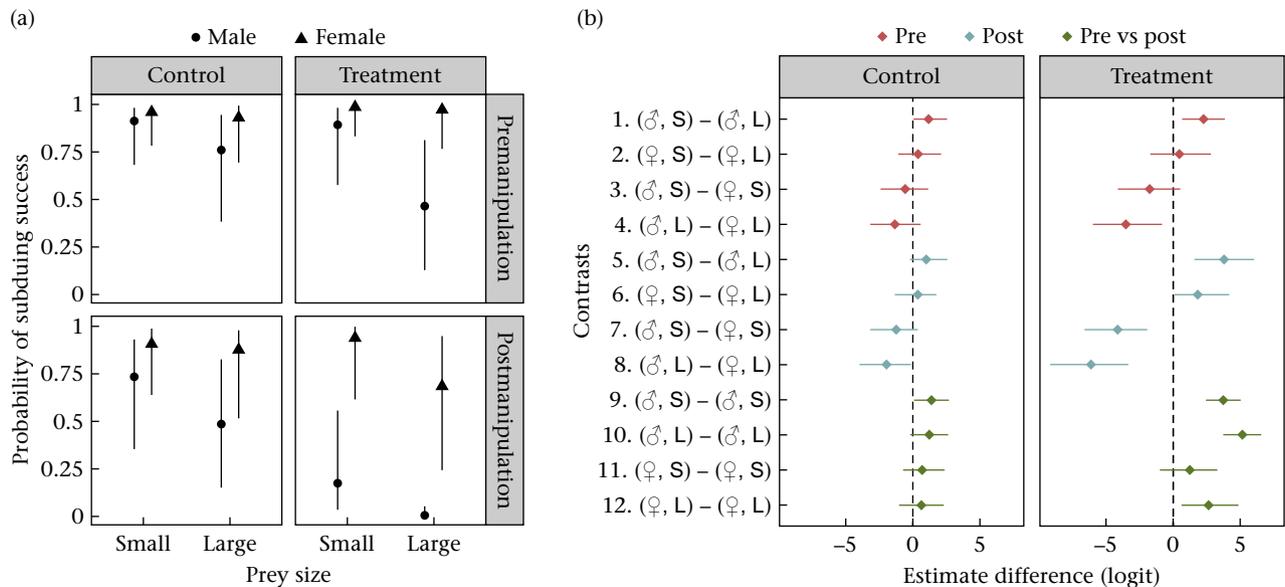


Figure 3. Effects of ‘tail’ autotomy on subduing success of the scorpion *Ananteris balzani*. (a) Estimated subduing success (backtransformed from logit) of males and females depending on prey size (small or large), experimental group (control or treatment) and phase of the experiment (pre- or postmanipulation of the tail). (b) Contrasts of subduing success (logit scale) for combinations of sex (δ : male; ♀ : female) and prey size (S: small; L: large) within and between phases of the experiment. Each contrast value is calculated as ((sex, prey size) minus (sex, prey size)), so that a positive value indicates that the estimated parameter for the first term was higher than the second term, whereas a negative value indicates the opposite. Symbols indicate mean values (a) or mean differences (b). In both panels, bars indicate 95% confidence intervals. Values of all estimated contrasts in (b) are presented in [Tables A3 and A6](#).

treatment group, subduing success in the premanipulation phase (i.e. when individuals were intact) was higher than in the postmanipulation phase (i.e. when individuals were autotomized) for both small and large prey (contrasts 9–12 in [Fig. 3b](#)). This difference was smaller for females when the prey was small (contrast 11 in [Fig. 3b](#)). For males in both control and experimental groups, subduing success of small prey was higher in the premanipulation phase than in the postmanipulation phase (contrast 9 in [Fig. 3b](#)). However, the magnitude of this difference was 2.6 times greater in the treatment group than in the control group ([Appendix, Table A3](#)), indicating that autotomized individuals showed lower subduing success than intact individuals.

Stinger Use

When the prey was small, 98% of the males and 82% of the females used their stinger in the subjugation process ([Fig. 4](#)). When the prey was large, all males and females used their stinger in the subjugation process ([Fig. 4](#)). A within-sex comparison revealed that the probability of stinger use was lower when the prey was small, but this pattern was only evident for females (contrasts 1–2 in [Fig. 5b](#); see also [Appendix, Tables A4–A5](#)). A between-sex comparison revealed that the probability of stinger use was higher for males than for females when the prey was small (contrast 3 in [Fig. 5b](#); see also [Appendix, Tables A4–A5](#)). However, when the prey was large, there was no difference between the sexes in the probability of stinger use (contrast 4 in [Fig. 5b](#); see also [Appendix, Table A4–A5](#)).

Handling Time

In the premanipulation phase, when all individuals were intact, handling time of control and treatment groups was qualitatively similar ([Fig. 6a](#), contrasts 1–4 in [Fig. 6b](#); see also [Appendix, Tables A2–A6](#)). In the postmanipulation phase, handling time differed between the control (i.e. intact) and the treatment (i.e.

autotomized) groups ([Fig. 6](#)). As expected, intact individuals in the control group showed the same pattern as observed in the premanipulation phase (contrasts 5–8 in [Fig. 6b](#)). For autotomized individuals in the treatment group, however, handling time was shorter for small prey than for large prey (contrasts 5–6 in [Fig. 6b](#)). Although this pattern was qualitatively similar to that reported for the premanipulation phase, the magnitude of the difference was 2.9 times greater for males and 3.7 times greater for females ([Appendix, Table A6](#)), indicating that autotomized individuals took much longer to subdue large prey. When the prey was small, autotomized males took much longer to subdue it than autotomized females (contrast 7 in [Fig. 6b](#)). When the prey was large, there was no sex difference in handling time (contrast 8 in [Fig. 6b](#)).

When comparing the pre- and postmanipulation phases, the handling time of small prey was similar for both males and females in the control group (contrasts 9 and 11 in [Fig. 6b](#)). For large prey, females in the control group showed similar handling times in the pre- and postmanipulation phases (contrast 12 in [Fig. 6b](#)). For males in the control group, however, handling time of large prey was slightly longer in the premanipulation phase (contrast 10 in [Fig. 6b](#)). In the treatment group, handling time of small prey in the premanipulation phase (i.e. when individuals were intact) was similar to that in the postmanipulation phase (i.e. when individuals were autotomized) for both males and females (contrasts 9 and 11 in [Fig. 6b](#)). Handling time of large prey was shorter when males and females were intact than when they were autotomized (contrasts 10 and 12 in [Fig. 6b](#)).

DISCUSSION

We investigated how metasomal autotomy and the consequent loss of the organ used for venom inoculation (i.e. the stinger) may affect predation success in a scorpion species. We found that the permanent loss of the stinger negatively affected predation success by decreasing subduing success and increasing prey handling time. For both small and large prey, subduing success was higher when

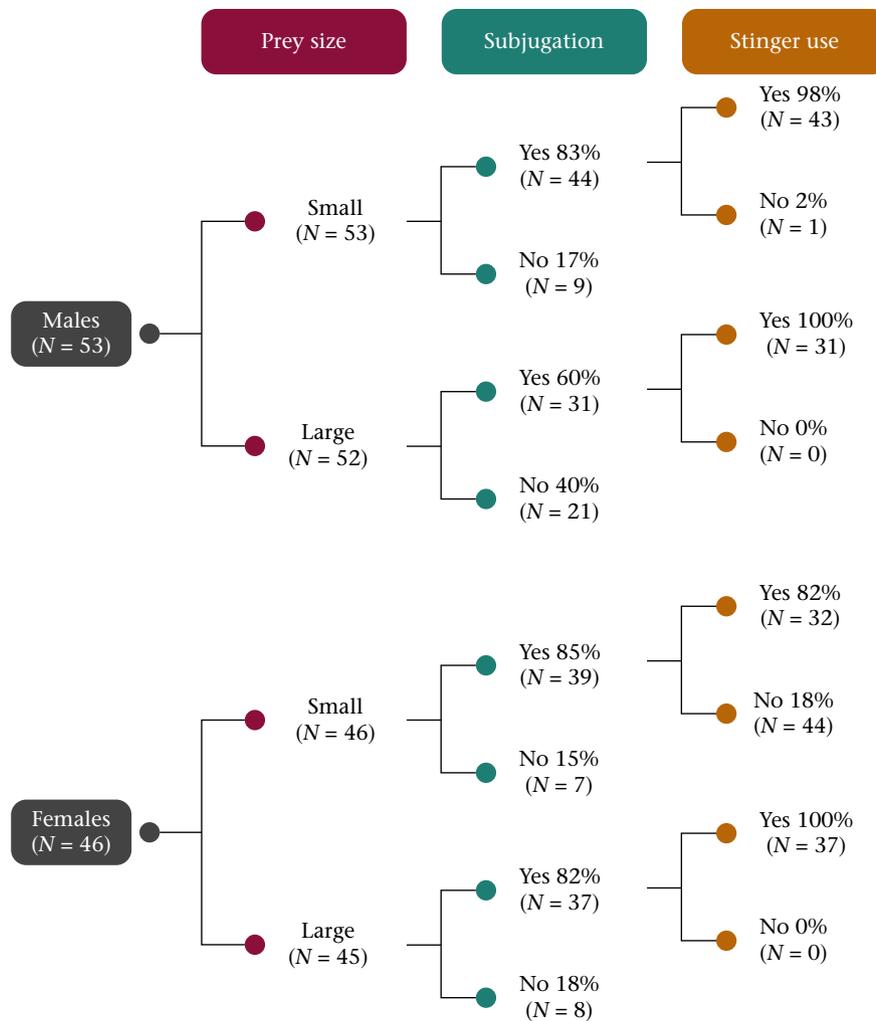


Figure 4. Percentage of trials during the premanipulation phase of the experiment in which intact males and females of the scorpion *Ananteris balzani* subdued small and large prey and used their stinger to inject venom and immobilize the prey. Given that all scorpions were intact in the premanipulation phase, data from the control and treatment groups were pooled.

scorpions were intact than when they were autotomized. In accordance with our prediction, metasomal autotomy had more pronounced negative effects on subduing success when the prey was large and when the scorpion was male, which is the smaller sex. In terms of handling time, large prey demanded more time to be completely subdued, but autotomized males and females had similar performances for both types of prey, a result that contrasts with our prediction.

To feed on large prey, most scorpions need to use venom because they are usually unable to subdue relatively large prey using only the pedipalps (Edmunds & Sibly, 2010; Rein, 1993). In our experiment, we used two prey sizes (small and large) but, considering the marked sexual size dimorphism in *A. balzani*, the relative size of these prey intentionally differed between males and females (Fig. 1). For males, which are much smaller than females, the prey/predator mass ratio was above 0.086 for both prey sizes, whereas for females, the prey/predator mass ratio was above this value only for large prey. Therefore, metasomal autotomy and the consequent loss of the stinger should be more detrimental to males, regardless of prey size. Indeed, after metasomal autotomy, the subduing success of small prey by males decreased from 90% to 17%, whereas the subduing success of large prey decreased from 47% to 1%. In the case of females, the subduing success of small prey

decreased from 98% to 93%, whereas the subduing success of large prey decreased from 97% to 70% (Fig. 3). Based on these results, we expect that under natural conditions, autotomized males will have a great reduction in the trophic niche because only small prey would be profitable. A reduction of prey size ranges after autotomy has already been recorded for crabs after the loss of a claw (e.g. Flynn et al., 2015; Juanes & Smith, 1995; Smith & Hines, 1991; Wasson et al., 2002) and for sea star after the loss of an arm (e.g. Lawrence et al., 1986; Ramsay et al., 2001). However, crabs and sea stars suffer from a reduction in prey size range only during a brief period of their lives, because after limb regeneration they achieve the same niche breadth they once had. Scorpions, in turn, do not regenerate the lost metasoma, and thus autotomy entails a decrease in niche breadth until the autotomized individual perishes.

Although males and females almost always used the stinger to subdue large prey (Fig. 4), after autotomy some individuals, especially females, were still able to subdue large prey using only their pedipalps. This finding shows that the injection of venom is not essential for females to subdue large prey. To use only the pedipalps to subdue large prey may be a compensatory adjustment that mitigates the negative effects of stinger loss after metasomal autotomy. An analogous compensatory adjustment

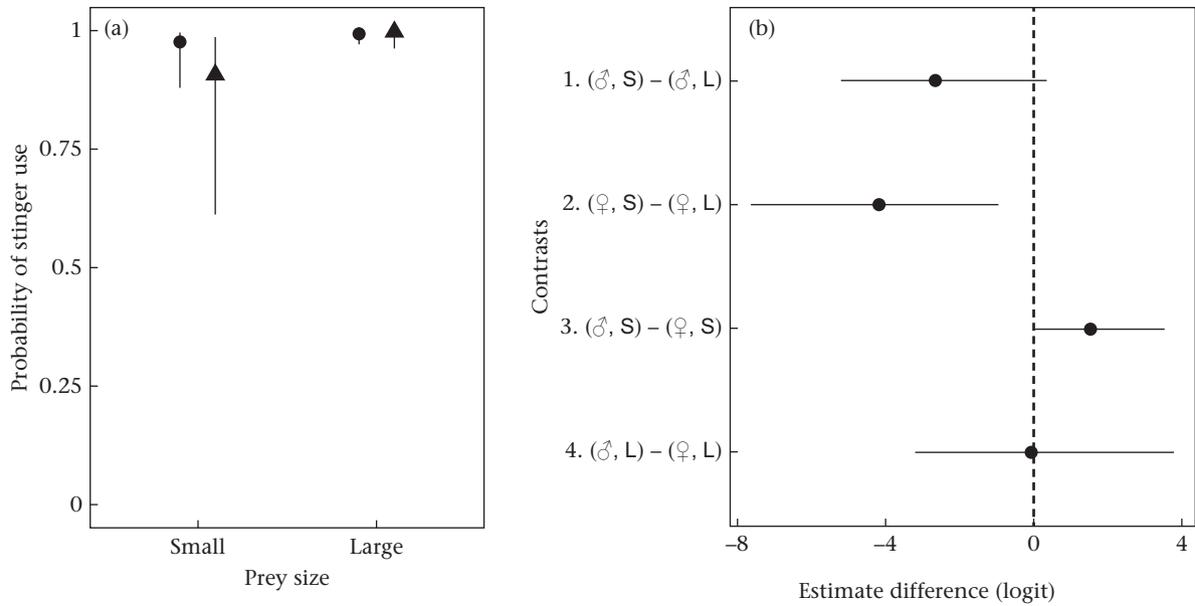


Figure 5. Stinger use for intact (i.e. nonautotomized) individuals of the scorpion *Ananteris balzani*. (a) Estimated probability (backtransformed from logit) of stinger use for males (circle) and females (triangle) that subdued a prey (small and/or large) during the premanipulation phase of the experiment (see also Appendix, Table A4). (b) Contrasts of the estimated probability of stinger use (logit scale) for combinations of sex (♂: male; ♀: female) and prey size (S: small; L: large). Each contrast value is calculated as ((sex, prey size) minus (sex, prey size)), so that a positive value indicates that the estimated parameter for the first term was higher than the second term, whereas a negative value indicates the opposite. In both panels, bars indicate 95% confidence intervals. Values of all estimated contrasts in (b) are presented in Table A5.

occurs among crabs, in which individuals use the minor claw, walking legs, or even oral appendages (i.e. maxillipeds) to manipulate prey after the autotomy of the major claw (e.g. De Oliveira, Christofoletti, & Barreto, 2015; Flynn et al., 2015; Smith & Hines, 1991; Wasson et al., 2002). However, as discussed above, stinger loss drastically reduces the subduing success of large prey by males, which are constrained to feed mostly on small prey for the rest of their lives. Thus, we expect males to show other compensatory mechanisms, such as more surface

activity to increase the likelihood of finding small prey. In many arthropods, including arachnids, crustaceans and insects, an increase in activity is associated with higher exposure to predators (e.g. Gwynne, 1987; Koga, Backwell, Christy, Murai, & Kasuya, 2001; Polis, Barnes, Seely, Henschel, & Enders, 1998; Requena & Machado, 2015). Consequently, autotomized males would face a behavioural conflict: either increase exposure to predators or decrease foraging activities. This trade-off has been rarely studied in terrestrial animals (reviewed in Verdolin, 2006), and

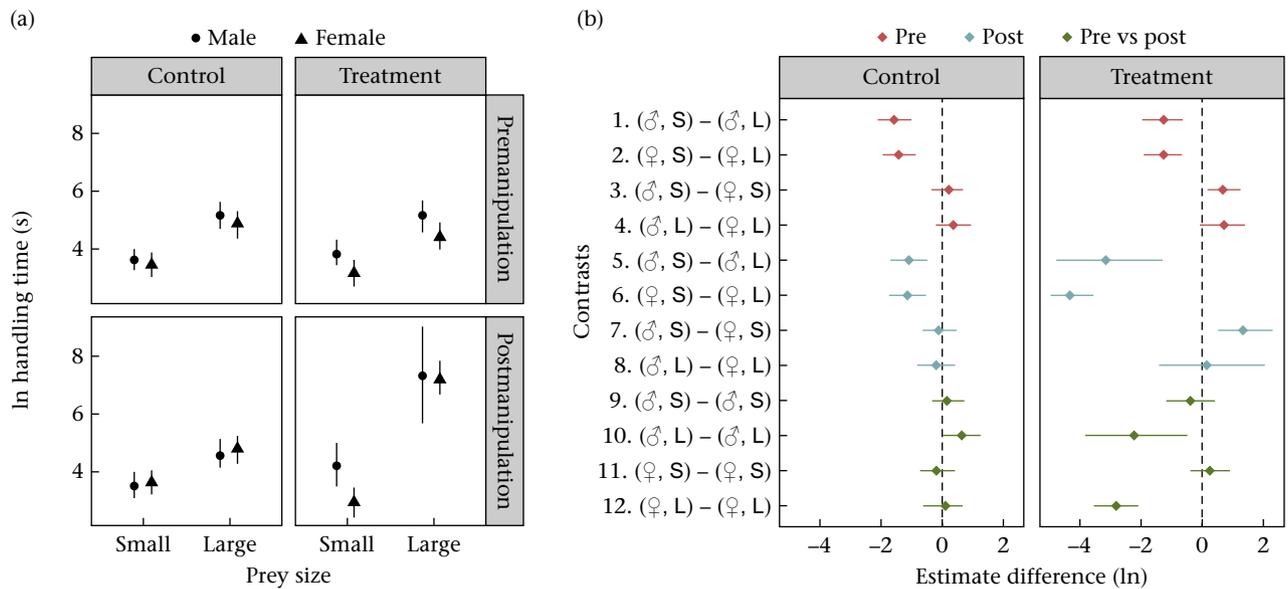


Figure 6. Effects of ‘tail’ autotomy on handling time of the scorpion *Ananteris balzani*. Estimated (a) handling time (ln scale) of males and females depending on prey size (small or large), experimental group (control or treatment) and phase of the experiment (pre- or postmanipulation of the tail). Contrasts of the (b) handling time (ln scale) for combinations of sex (♂: male; ♀: female) and prey size (S: small; L: large) within and between phases of the experiment. Each contrast value is calculated as ((sex, prey size) minus (sex, prey size)), so that a positive value indicates that the estimated parameter for the first term was higher than the second term, whereas a negative value indicates the opposite. Symbols indicate mean values (a) or mean differences (b). In both panels, bars indicate 95% confidence intervals. Values of all estimated contrasts in (b) are presented in Tables A3 and A6.

A. balzani offers an interesting opportunity to explore it under both laboratory and field conditions.

After metasomal autotomy, the handling time increased for both prey types, and this pattern was especially evident for large prey. We argue that this increase is related to the loss of the stinger and the associated venom glands used to immobilize prey. In many spider species, the use of venom to subjugation depends on prey size, so that a large prey usually requires the injection of more venom (reviewed in Wigger, Kuhn-Nentwig, & Nentwig, 2002). A similar pattern has been reported for a few scorpion species, indicating that individuals adjust venom use based on prey size to minimize the energetic costs associated to subjugation (reviewed in Evans, Northfield, Daly, & Wilson, 2019). When individuals of *A. balzani* lose their metasoma, the entire process of prey subjugation is accomplished using the pedipalps, which are not as effective as the stinger in immobilizing the prey, regardless of the prey's size. Consequently, males and females spend more time handling the prey, which probably implies a greater energy expenditure during predation (e.g. Elner & Hughes, 1978; Rovero, Hughes, & Chelazzi, 2000). The lack of sexual difference in the handling time of large prey is probably a consequence of the low number of autotomized males that were able to subdue this prey type. In fact, once a single autotomized male subdued a large prey, the confidence interval of the handling time estimated by the model was very wide and greatly overlapped the confidence interval estimated for females. Thus, the most evident sex-dependent consequence of metasomal autotomy for the predation success of scorpions seems to rely on subduing success, and not on handling time.

Theoretical models propose that the ratio between energy intake and energy expenditure in handling time has a hump-shaped dependence on prey size (e.g. Persson, Leonardsson, de Roos, Gyllenberg, & Christensen, 1998). On one side of this curve, there are very small prey that still require a minimum of handling time, and thus are of low energetic value. On the other side, there are very large prey that require extremely long handling times, and thus are also of low energetic value. In our experiment, although prey size was the same pre- and postmanipulation of the metasoma, the handling time of the prey showed a marked increase after autotomy, indicating that the functionality of the morphological traits used for hunting also influences prey profitability. For instance, the handling time of large prey was 17 times longer when the females were autotomized. This increase in energy expenditure for subduing prey makes the prey (both small and large) less profitable in terms of energy yield per unit handling time (Charvov, 1976). Among predatory arthropods, low energy intake results in lower female fecundity (e.g. Briceño, 1987; Kessler, 1971; Richardson & Baker, 1997; Sota, 1985; Wise, 1979). Given that scorpions are viviparous, females need to invest great amount of energy in oogenesis and embryogenesis (Warburg, 2011). If autotomy occurs before or at the beginning of pregnancy, then the lower energy intake resulting from low subduing success and long handling time may reduce offspring size (Wheeler, 1996). From the males' perspective, the low energy intake after autotomy may require more investment in foraging activities (as discussed above), which may conflict with mate search, a key component of males' reproductive success in scorpions (Polis & Sissom, 1990). Therefore, the negative effects of metasomal autotomy on the efficiency of predation may have important negative effects on the fitness of both males and females.

In conclusion, we found that metasomal autotomy and the consequent stinger loss decrease predation success in scorpions. Autotomized individuals had lower subduing success and longer handling time than intact individuals, but we stress that both males and females were still able to capture prey (especially the small

ones) after stinger loss. Thus, despite the importance of the stinger in the immobilization of large prey, autotomized individuals may use their pedipalps to subdue small prey, mitigating the negative effects of stinger loss. This finding sheds light on the maintenance of metasomal autotomy in scorpions, a defensive behaviour that involves not only the permanent loss of an important organ used in prey subjugation (i.e. the stinger), but also the posterior part of the nervous, circulatory and digestive system, including the anus (Mattoni et al., 2015). Future research should explore how decreased predation success influences the survival and reproduction of the individuals. Given that metasoma loss affects several aspects of the behaviour, ecology and physiology of scorpions, the ultimate fitness costs of autotomy would be better understood using an integrative approach under field conditions, where individuals face problems such as food unpredictability and predation risk.

Data Accessibility

The analyses reported in this manuscript can be reproduced using the data and scripts available in the Mendeley Data Repository (<https://doi.org/10.17632/cgpcggnvw8.1>).

Author Contributions

Conceptualization: S.G.H. and G.M.; methodology: S.G.H. and G.M.; data collection: S.G.H.; formal analysis: S.G.H.; data curation: S.G.H.; writing (original draft): S.G.H. and G.M.; writing (review and editing): S.G.H. and G.M.; visualization: S.G.H. and G.M.; supervision: G.M.; project administration: S.G.H.; funding acquisition: S.G.H. and G.M.

Funding

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant number 2017/05283-1 to S.G.H.) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, grant number 306550/2014-8 to G.M.).

Competing Interests

We declare no competing or financial interests.

Acknowledgments

We thank J. A. Uribe, A. Rojas, D. G. Muniz, A. V. Palaoro and J. Menezes for their assistance in the fieldwork, J. A. Uribe for taking the photos used in Figs 1 and 2, the staff of Santa Bárbara Ecological Station and M. R. Martins for logistical support, and A. V. Palaoro, E. S. A. Santos, J. E. Carvalho and two anonymous referees for comments on the manuscript.

References

- ASAB/ABS. (2012). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*, 83, 301–309.
- Botero-Trujillo, R., & Florez, D. E. (2011). A revisionary approach of Colombian *Ananteris* (Scorpiones, Buthidae): Two new species, a new synonymy, and notes on the value of trichobothria and hemispermatophore for the taxonomy of the group. *Zootaxa*, 2904, 1–44.
- Briceño, R. D. (1987). How spiders determine clutch size. *Revista de Biología Tropical*, 35, 25–29.
- Brueseke, M., Rypstra, A., Walker, S., & Persons, M. (2001). Leg autotomy in the wolf spider *Pardosa milvina*: A common phenomenon with few apparent costs. *The American Midland Naturalist*, 146, 153–160.
- Charnov, E. L. (1976). Optimal foraging: Attack strategy of a mantid. *The American Naturalist*, 110, 141–151.

Complementary Results of Subduing Success and Handling Time

Table A2

Summary of the probability of subduing success and handling time of small and large prey by males and females of the scorpion *Ananteris balzani* in the pre- and post-manipulation phase of the experiment, estimated by the Bayesian generalized mixed models

Trials	Probability of subduing success		Handling time (ln)	
	Estimate ± SD	95% CI	Estimate ± SD	95% CI
Control				
<i>Premanipulation</i>				
Male, Small prey	0.917 ± 0.691	0.680 to 0.981	3.635 ± 1.970	3.237 to 4.002
Male, Large prey	0.769 ± 0.699	0.369 to 0.940	5.211 ± 2.281	4.727 to 5.644
Female, Small prey	0.951 ± 0.716	0.781 to 0.992	3.433 ± 2.218	3.001 to 3.858
Female, Large prey	0.929 ± 0.721	0.695 to 0.989	4.849 ± 2.307	4.373 to 5.302
<i>Postmanipulation</i>				
Male, Small prey	0.734 ± 0.695	0.347 to 0.928	3.492 ± 2.307	3.044 to 3.959
Male, Large prey	0.491 ± 0.700	0.142 to 0.822	4.580 ± 2.680	4.081 to 5.112
Female, Small prey	0.906 ± 0.714	0.632 to 0.985	3.608 ± 2.292	3.163 to 4.019
Female, Large prey	0.873 ± 0.714	0.516 to 0.974	4.750 ± 2.512	4.243 to 5.222
Treatment				
<i>Premanipulation</i>				
Male, Small prey	0.899 ± 0.714	0.573 to 0.979	3.837 ± 2.272	3.422 to 4.307
Male, Large prey	0.471 ± 0.710	0.126 to 0.814	5.120 ± 2.881	4.535 to 5.682
Female, Small prey	0.982 ± 0.777	0.828 to 0.998	3.142 ± 2.431	2.659 to 3.613
Female, Large prey	0.969 ± 0.781	0.766 to 0.998	4.414 ± 2.509	3.946 to 4.906
<i>Postmanipulation</i>				
Male, Small prey	0.173 ± 0.726	0.026 to 0.553	4.214 ± 3.958	3.458 to 4.986
Male, Large prey	0.005 ± 0.789	0.000 to 0.048	7.355 ± 8.466	5.679 to 9.035
Female, Small prey	0.933 ± 0.758	0.613 to 0.992	2.891 ± 2.699	2.370 to 3.411
Female, Large prey	0.683 ± 0.747	0.239 to 0.947	7.222 ± 2.930	6.657 to 7.857

Table A3

Summary of relevant contrasts of the probability of subduing success

	Premanipulation	Postmanipulation	Pre- vs postmanipulation
Control	(♂, Small) > (♂, Large) (1.181; 0.040 to 2.540)	(♂, Small) ≈ (♂, Large) (1.038; -0.202 to 2.608)	(♂, Small) > (♂, Small) (1.394; 0.078 to 2.692)
	(♀, Small) ≈ (♀, Large) (0.395; -0.995 to 2.126)	(♀, Small) ≈ (♀, Large) (0.332; -1.358 to 1.825)	(♂, Large) ≈ (♂, Large) (1.243; -0.197 to 2.627)
	(♂, Small) ≈ (♀, Small) (-0.561; -2.401 to 1.219)	(♂, Small) ≈ ♀, (Small) (-1.198, -3.148 to 0.581)	(♀, Small) ≈ (♀, Small) (0.682; -0.662 to 2.377)
	(♂, Large) ≈ (♀, Large) (-1.328; -3.230 to 0.598)	(♂, Large) < (♀, Large) (-1.953; -4.017 to -0.062)	(♀, Large) ≈ (♀, Large) (0.654; -0.993 to 2.351)
	(♂, Small) > (♂, Large) (2.302; 0.683 to 3.851)	(♂, Small) > (♂, Large) (3.783; 1.623 to 6.112)	(♂, Small) > (♂, Small) (3.735; 2.167 to 5.450)
	(♀, Small) ≈ (♀, Large) (0.481; -1.693 to 2.893)	(♀, Small) > (♀, Large) (1.850; 0.113 to 4.221)	(♂, Large) > (♂, Large) (5.227; 2.953 to 7.542)
Treatment	(♂, Small) ≈ (♀, Small) (-1.721; -4.086 to 0.566)	(♂, Small) < (♀, Small) (-4.155; -6.613 to -1.817)	(♀, Small) ≈ (♀, Small) (1.274; -1.001 to 3.274)
	(♂, Large) < (♀, Large) (-3.531; -5.924 to -0.804)	(♂, Large) < (♀, Large) (-6.138; -9.158 to -3.301)	(♀, Large) > (♀, Large) (2.644; 0.615 to 4.921)

Contrast combinations of sex (♂: males; ♀: females) and prey size (small, large) within and between phases of the experiment (pre- and postmanipulation). The direction of difference is indicated as follows: > (i.e. the contrast value was positive and the 95% CI did not overlap zero); < (i.e. the contrast value was negative and the 95% CI did not overlap zero); ≈ (i.e. regardless of the contrast value, the 95% CI overlapped zero). The estimated difference and the 95% CI (both in logit) are given below each contrast.

Table A4

Summary of the probability of stinger use estimated by the Bayesian generalized mixed model

Trials	Estimate ± SD	95% CI
Male, Small	0.981 ± 0.741	0.880 to 0.866
Male, Large	0.999 ± 0.878	0.973 to 1.000
Female, Small	0.910 ± 0.733	0.613 to 0.989
Female, Large	0.999 ± 0.882	0.960 to 1.000

Data are based on stinger use (backtransformed from logit) for intact individuals (males and females) of the scorpion *Ananteris balzani* that subdued small and/or large prey during the premanipulation phase of the experiment.

Table A5

Summary of relevant contrasts estimated by the model of stinger use for intact males (♂) and females (♀) of the scorpion *Ananteris balzani* that subdued small and/or large prey during the premanipulation phase of the experiment

Contrast	Estimate	Lower 95% CI
(♂, Small) - (♂, Large)	-2.600	-5.216 to 0.343
(♀, Small) - (♀, Large)	-4.117	-7.635 to -0.948
(♂, Small) - (♀, Small)	1.585	0.000 to 3.501
(♂, Large) - (♀, Large)	-0.012	-3.202 to 3.781

Both the contrast and the 95% CI are in logit.

Table A6
Summary of relevant contrasts for handling time

	Premanipulation	Postmanipulation	Pre- vs postmanipulation
Control	(♂, Small) < (♂, Large)	(♂, Small) < (♂, Large)	(♂, Small) ≈ (♂, Small)
	(-1.571; -2.131 to -1.010)	(-1.086; -1.720 to -0.461)	(0.130; -0.362 to 0.728)
	(♀, Small) < (♀, Large)	(♀, Small) < (♀, Large)	(♂, Large) > (♂, Large)
	(-1.416; -1.961 to -0.838)	(-1.145; -1.753 to -0.527)	(0.635; 0.014 to 1.264)
	(♂, Small) ≈ (♀, Small)	(♂, Small) ≈ (♀, Small)	(♀, Small) ≈ (♀, Small)
	(0.209; -0.362 to 0.689)	(-0.125; -0.667 to 0.503)	(-0.188; -0.730 to 0.433)
Treatment	(♂, Large) ≈ (♀, Large)	(♂, Large) < (♀, Large)	(♀, Large) ≈ (♀, Large)
	(0.353; -0.215 to 0.957)	(-0.172; -0.838 to 0.444)	(0.109; -0.602 to 0.665)
	(♂, Small) < (♂, Large)	(♂, Small) < (♂, Large)	(♂, Small) ≈ (♂, Small)
	(-1.288; -1.998 to -0.616)	(-3.169; -4.791 to -1.264)	(-0.381; -1.213 to 0.435)
	(♀, Small) < (♀, Large)	(♀, Small) < (♀, Large)	(♂, Large) < (♂, Large)
	(-1.266; -1.929 to -0.649)	(-4.328; -4.991 to -3.573)	(-2.231; -3.813 to -0.481)
	(♂, Small) > (♀, Small)	(♂, Small) > (♀, Small)	(♀, Small) ≈ (♀, Small)
	(0.676; 0.154 to 1.277)	(1.330; 0.526 to 2.342)	(0.241; -0.421 to 0.911)
	(♂, Large) ≈ (♀, Large)	(♂, Large) ≈ (♀, Large)	(♀, Large) < (♀, Large)
	(0.706; -0.101 to 1.402)	(0.139; -1.441 to 2.051)	(-2.821; -3.547 to -2.095)

Contrast combinations of sex (♂: males; ♀: females) and prey size (small, large) within and between phases of the experiment (pre- and postmanipulation). The direction of difference is indicated as follows: > (i.e. the contrast value was positive and the 95% CI overlapped zero); < (i.e. the contrast value was negative and the 95% CI did not overlap zero); ≈ (i.e. regardless of the contrast value, the 95% CI did not overlap zero). The estimated difference and the 95% CI (both in logit) are given below each contrast.