SOLIMARY GARCÍA HERNÁNDEZ

Como os escorpiões lidam com a perda permanente de sua "cauda"?

How do scorpions cope with the permanent loss of their "tail"?

SÃO PAULO

2020

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo como parte dos requisitos para obtenção do título de Doutor em Ciências, na área de Ecologia de Ecossistemas Terrestres e Aquáticos.

Orientador: Glauco Machado
Departamento de Ecologia, Universidade de São Paulo, Brasil

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Título (em português): Como os escorpiões lidam com a perda permanente de sua "cauda"?

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Aprovada em: ____________________

Banca examinadora:

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Prof. Dr. Glauco Machado
(Orientador)

Agradecimentos

Para mim, o doutorado foi muito mais do que você lerá nos próximos três capítulos que formam esta tese. Como diz Dexler em sua música, o meu doutorado foi um *"Ir y venir, seguir y guiar, dar y tener, entrar y salir de fase… amar la trama más que el desenlace"* ("Ir e vir, seguir e guiar, dar e obter, entrar e sair de fase... amar a jornada mais que o destino").

Glauco, foi um prazer embarcar com você nesta aventura. Sinto-me muito privilegiada por ter tido você como meu orientador e espero que sigamos colaborando enquanto divulgamos as histórias que fazem nossos olhos brilhar. Sou grata, em especial, pelo entusiasmo que você compartilhou comigo e por sempre me incentivar a explorar aspectos interessantes sobre o comportamento animal — ou, quando necessário, por colocar limites na minha empolgação. *Gracias*, também, por termos trabalhado em equipe inclusive quando, com coragem, decidimos "autotomizar" algumas partes do nosso trabalho. Foi através dessa decisão que conseguimos transmitir a mensagem de forma mais clara. Por fim, muchísimas *gracias* pela maravilhosa idéia de divulgar a nossa pesquisa, não só para o público leigo, mas também para um público infantil. Os quadrinhos que produzimos abriram a minha mente de uma forma que você não imagina.

John, é um prazer enorme tê-lo como companheiro para descobrir as belezas da vida, e da natureza que tanto nos encanta, seja com a câmera fotográfica na mão ou caminhando atentos ao nosso redor. Não tem preço ter você ao meu lado, como um grande incentivador das minhas atividades acadêmicas. Com certeza as apresentações que fiz ao longo do meu doutorado não seriam tão atraentes (e até elogiadas!) se não tivessem sido ilustradas pelas lindas fotos que você tira. Graças à qualidade do seu trabalho, assim como a sua paciência e presença, conseguimos *a foto perfeita* para ilustrar várias das legendas deste texto. *Gracias* enormes pelo carinho e pela mescla perfeita de estabilidade e liberdade que construímos. Foi tudo isso que criou um ambiente ótimo para o desenvolvimento desta tese.

Obrigada aos estudantes, docentes e funcionários que participam ativamente da construção de um programa de pós-graduação mais justo e colaborativo. Um agradecimento especial aos times que fazem do nosso programa um ótimo exemplo de colaboração: EcoEncontros, EcoEscola, Comissão Proex e Café Existencial. Aprendi muito sobre organização, ensino, transparência e amor quando participei dessas atividades com vocês. De coração, *muchas gracias* pelo seu tempo e dedicação. Vera, Paulo, Glauco e representantes discentes (Renato, Diego, Luanne, Eduarda, Mila), muito obrigada pela disposição de vocês em nos escutar. Vocês estão sendo fundamentais para a continuidade das iniciativas dos estudantes.

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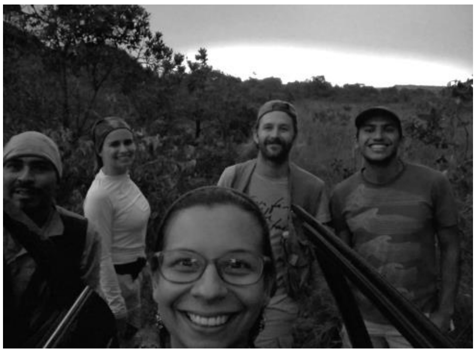
Agradeço ao Danilo e ao Edu pelas orientações analíticas. À Rosita, Andrés, John, Bruna e Diego, *gracias* por me ajudarem em alguma das fases dos experimentos. *Gracias* ao John, Andrés, VP, Danilo, João, Agustin, Bruna e Diego, pela ajuda essencial e pela companhia agradável durante meu trabalho de campo. A perseguição do tamanduá, o churrasco, as muitas risadas, algumas cobras surpresas, a procura do lobo-guará, as comidas chiques, o calmo grito "pode frear, Soly, pode frear", e até a fugida do furação, são memórias que aquecerão sempre meu coração.



Gracias à minha família de origem, meu coração está sempre tranquilo. Apesar da distância, sinto o amor de vocês e lembro dos seus conselhos no meu dia-a-dia. *Gracias* por ficarem tão orgulhosos quando conto sobre as minhas pequenas vitórias na vida. Todas são dedicadas a vocês! *Gracias* à minha família hispanofalante: Irene, Marcelo, Lino, Olivia, Andrés, Dieguito e Jimena. Sua amabilidade, atenção, confiança, sorrisos e momentos de aventuras foram essenciais para que eu e John nos sentíssemos em casa.

Gracias a todos os amigos que exploraram junto comigo as exuberantes paisagens colombianas e brasileiras, cuja diversidade e beleza é inspiradora e acolhedora. Foi muito bom ter passado tanto por desafios quanto por momentos de êxtase juntos :). Falando de desafios e êxtase, aproveito para dar também meus agradecimentos aos inspiradores corredores do Alto do Matão. Vocês são o exemplo sobre como o estímulo e a auto-confiança podem se expandir a outras pessoas e extrapolar para outras atividades além da corrida.

Gracias a Glauco e Eduardo por me convidarem todos estes anos para ser professora na disciplina de comportamento. Inspirada em vocês, tentei melhorar continuamente minha aula e aprendi muito neste processo. *Gracias* também aos professores Marco Mello e Diogo Meyer, pois aprendi muito ao observá-los enquanto ministravam ou preparavam suas aulas. Obrigada a Cris e Janaína que me deram a chance de falar de comportamento animal para crianças. Obrigada ao pessoal da EcoEscola e da Semana Temática por permitirem me arriscar a ministrar aulas mais didáticas.

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A introdução geral e conclusão geral desta tese não seriam tão “palatáveis” se não tivéssemos incluído mudanças sugeridas pelos olhares cuidadosos de Augusto, Marcelo, Fernando, Sonaly, Rosita & Duda. Também agradeço à Pamela pelas sugestões em uma versão preliminar dos agradecimentos. O seu papel editorial foi ótimo!

Obrigada ao meu comitê acadêmico, Murilo, Zé Eduardo e Edu. Sempre saí muito satisfeita das nossas reuniões, pois as sugestões de vocês enriqueceram o trabalho. Também agradeço ao Camilo, pelo importante papel na origem do tema desta tese e por estar sempre disposto a tirar minhas dúvidas escorpionológicas.

Por fim, minha tese foi realizada com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, código de financiamento 001) e da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, processo 2017/05283-1), às quais sou muito grata.

Por fim, agradeço a você, caro leitor, por se interessar em ler meu trabalho. Espero que seja uma experiência proveitosa e divertida.

.

Gracias!

Resumo

GARCÍA-HERNÁNDEZ, S. **Como os escorpiões lidam com a perda permanente de sua “cauda”?** 2018. Tese (Doutorado) – Instituto de Biociências, Universidade de São Paulo, SP.

A perda voluntária de uma parte do corpo, conhecida como autotomia, pode aumentar a chance de sobreviver a uma tentativa de predação. Entretanto, esse mecanismo defensivo também impõe custos aos indivíduos. Embora a autotomia seja bastante estudada, seus custos para espécies que não regeneram a parte do corpo autotomizada são pouco conhecidos. Escorpiões do gênero *Ananteris* realizam uma forma única de autotomia, na qual os indivíduos perdem permanentemente os últimos segmentos abdominais, conhecidos como “cauda”. Após a autotomia “caudal”, os indivíduos perdem massa corporal, o ferrão e a última porção do trato digestivo, incluindo o ânus, o que evita a defecação e leva à constipação. Nesta tese, investigamos experimentalmente como a autotomia “caudal” afeta o desempenho locomotor, o sucesso da predação e o sucesso reprodutivo de machos e fêmeas do escorpião *A. balzani*. Descobrimos que a autotomia “caudal” tem efeitos mínimos de curto prazo para o desempenho locomotor em ambos os sexos. Em longo prazo, porém, a autotomia reduz o desempenho locomotor, principalmente dos machos. O sucesso da predação em ambos os sexos diminui após a autotomia e consequente perda do ferrão. No entanto, machos e fêmeas autotomizados ainda são capazes de capturar presas, especialmente as pequenas. A perda da “cauda” não afeta o sucesso reprodutivo dos machos, mas prejudica a reprodução nas fêmeas, reduzindo sua sobrevivência e número de filhotes. Apesar de indivíduos autotomizados pagarem custos em termos de locomoção e forrageamento, demonstramos que o longo tempo entre a perda da “cauda” e a morte é suficiente para que eles se reproduzam. Assim, a autotomia permanente da “cauda” em escorpiões parece ser adaptativa quando comparada à estratégia alternativa de não autotomizar e eventualmente morrer durante o ataque de um predador.

Palavras-chave: autotomia, desempenho locomotor, dimorfismo sexual, mecanismos de defesa, sucesso de predação, sucesso reprodutivo.

Abstract

GARCÍA-HERNÁNDEZ, S. **How do scorpions cope with the permanent loss of their "tail"?** 2018. Tese (Doutorado) – Instituto de Biociências, Universidade de São Paulo, SP.

The voluntary loss of a body part, known as autotomy, may increase the chance of surviving surviving a predadory attack. However, this defensive mechanism also imposes costs to the individuals. Although autotomy is extensively studied, its costs to species that do no regenerate the lost body part are poorly known. Scorpions of the genus *Ananteris* show a unique form of autotomy in which individuals lose permanently the last abdominal segments, known as the "tail". After "tail" autotomy, individuals lose body mass, the stinger, and the last portion of the digestive tract, including the anus, which prevents defecation and leads to constipation. In this thesis, we experimentally investigated how "tail" autotomy affects the locomotor performance, the predation success, and the reproductive success of males and females of the scorpion *A. balzani*. We found that "tail" autotomy has minimal short-term effects on the locomotor performance of both sexes. In the long-term, however, autotomy reduces locomotor performance, especially in males. The predation success decreases after autotomy and consequent stinger loss. However, autotomized males and females are still able to capture prey, especially the small ones. The reproductive success of males is not affected after "tail" loss, but it impairs female reproduction by reducing their survival and offspring number. Although autotomized individuals pay costs in terms of locomotion and foraging, we demonstrate that the long time between "tail" loss and death is enough for them to reproduce. Thus, permanent "tail" autotomy in scorpions appears to be adaptive when compared with the alternative strategy of not autotomize and eventually die during a predator attack.

Key-words: autotomy, defense mechanisms, locomotor performance, predation success, reproductive sucsess, sexual dimorphism.

Conteúdo

Introdução Geral

Perder a cauda para não perder a vida

Capítulo 1: Short- and long-term effects of an extreme form of autotomy on locomotor performance of a neotropical scorpion 1

Resumo 2
Introdução 3
Material & Métodos 8
Resultados 14
Discussão 22
Referências 29
Material Suplementar 34

Capítulo 2: "Tail" autotomy and consequent stinger loss impair predation success in scorpions 52

Resumo 53
Introdução 54
Materiais & Métodos 56
Resultados 65
Discussão 72
Referências 77
Material Suplementar 82

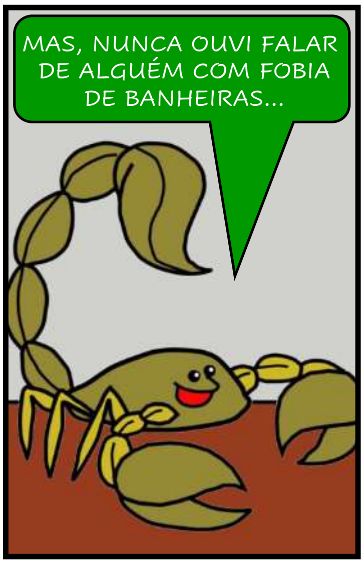
Capítulo 3: Implications of nonlethal injuries on the reproductive success of male and female scorpions 88

Resumo 89
Introdução 90
Materiais & Métodos 92
Resultados 100
Discussão 102
Referências 108
Material suplementar 112

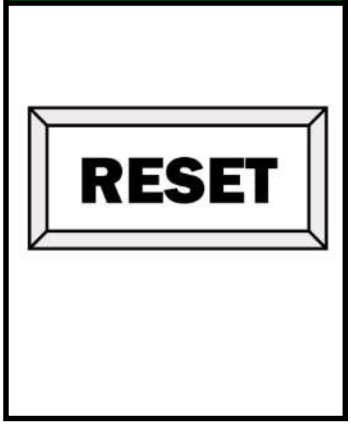
Conclusão Geral

Quatro anos depois

Perder a cauda para não perder a vida

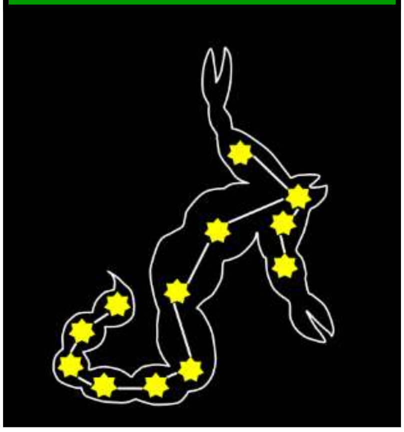


VOCÊ JÁ OUVIU TANTA BESTEIRA SOBRE MIM, QUE ACHO MELHOR COMEÇARMOS DO ZERO.

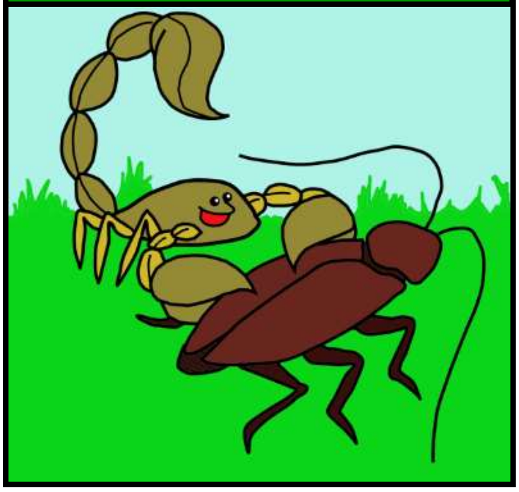


ASSIM COMO OUTROS ARACNÍDEOS, TEMOS OITO PERNAS, DUAS A MAIS DO QUE OS INSETOS. O QUE NOS FAZ ÚNICOS SÃO NOSSAS PINÇAS E NOSSA CAUDA ALONGADA.

É POR CAUSA DESSAS DUAS CARACTERÍSTICAS QUE A CONSTELAÇÃO DE ESCORPIÃO TEM ESTE NOME.

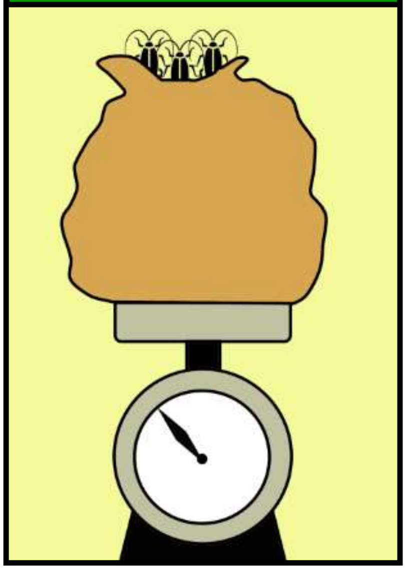


USAMOS AS PINÇAS PARA MUITAS ATIVIDADES, INCLUINDO AGARRAR PRESAS PEQUENAS.

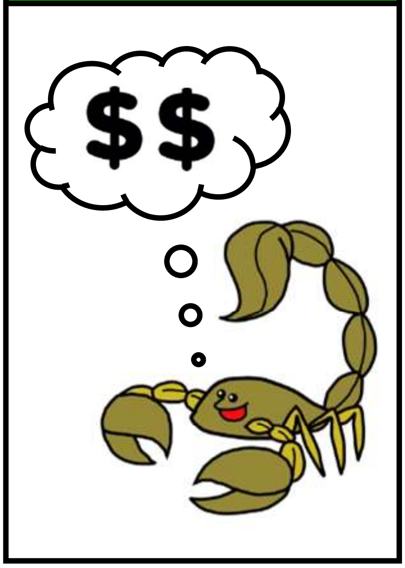


NA CAUDA, ESTÁ O FERRÃO, QUE INJETA O VENENO QUE USAMOS PARA PARALIZAR PRESAS GRANDES.

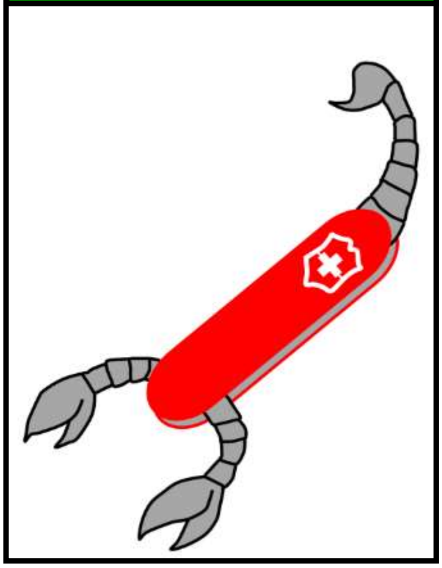
EM UM ANO, ESCORPIÕES CONSOMEM ATÉ 150 QUILOS DE INSETOS POR HECTARE. ISSO EQUIVALE A QUASE 200 MIL BARATAS!



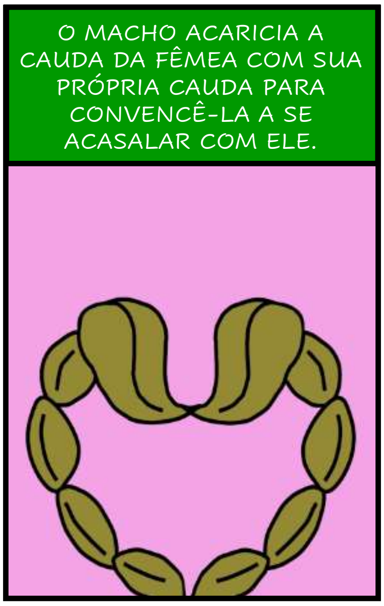
COMO MUITOS DOS INSETOS QUE COMEMOS SÃO NOCIVOS AOS HUMANOS, PRESTAMOS UM SERVIÇO VALIOSO DE GRAÇA PARA VOCÊS.



ALÉM DE SEREM USADAS NA ALIMENTAÇÃO, NOSSAS PINÇAS E NOSSA CAUDA TAMBÉM SÃO ÚTEIS EM MUITOS OUTROS ASPECTOS DA NOSSA VIDA.



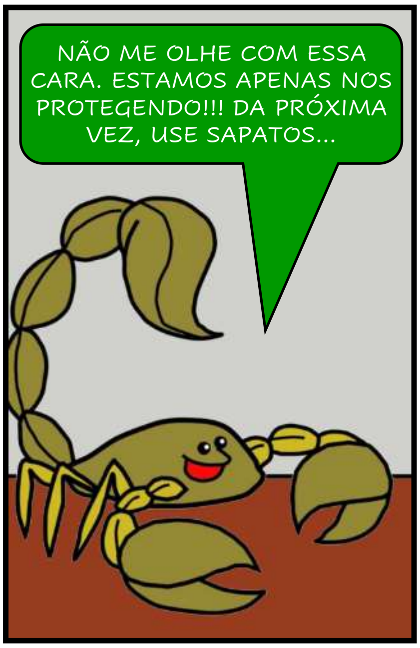

DURANTE O ACASALAMENTO, OS MACHOS, QUE SÃO BEM MENORES QUE AS FÊMEAS, SEGURAM SUAS PARCEIRAS E EXECUTAM UMA DANÇA DE CORTEJO QUE PODE DURAR VÁRIAS HORAS.
NEM ARGENTINOS, NEM URUGUAIOS. QUEM INVENTOU O TANGO FORAM OS ESCORPIÕES!


O MACHO ACARICIA A CAUDA DA FÊMEA COM SUA PRÓPRIA CAUDA PARA CONVENCÊ-LA A SE ACASALAR COM ELE.


SE TIVER SUCESSO, O MACHO DEIXA NO CHÃO UM PACOTE DE ESPERMA QUE É PEGO PELA FÊMEA PARA FECUNDAR SEUS OVOS.

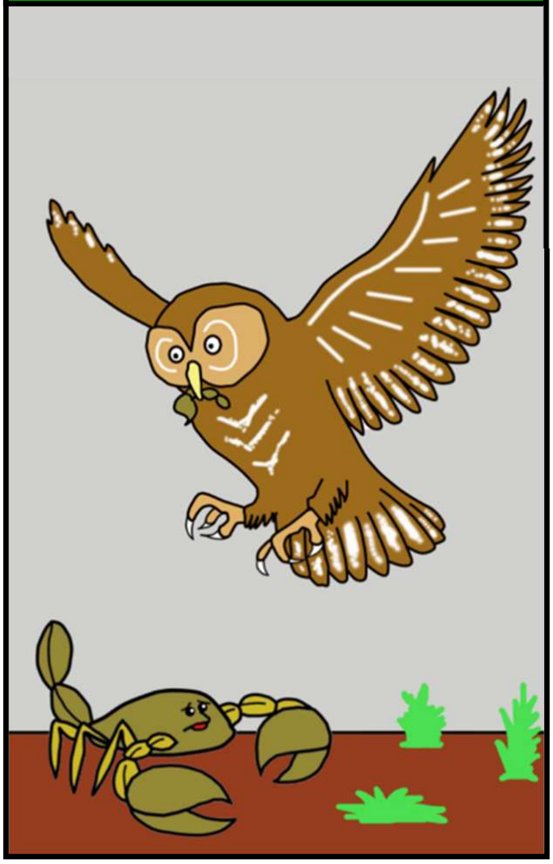

OUTRA UTILIDADE DAS PINÇAS É A DEFESA. ASSIM COMO QUASE TODOS OS ANIMAIS, OS ESCORPIÕES TÊM PREDADORES. ALGUNS DELES PODEM SER AFUGENTADOS COM UMA BOA PINÇADA!


O FERRÃO NA CAUDA TAMBÉM PODE SER USADO PARA DEFESA. É O QUE FAZEMOS QUANDO UM HUMANO PISA EM NÓS.


NÃO ME OLHE COM ESSA CARA. ESTAMOS APENAS NOS PROTEGENDO!!! DA PRÓXIMA VEZ, USE SAPATOS...


AGORA PREPARE-SE, POIS VOU CONTAR UMA NOVIDADE. É UMA DESCOBERTA MUITO RECENTE SOBRE O USO DA CAUDA COMO DEFESA EM ESCORPIÕES.

QUANDO UM PREDADOR AGARRA A CAUDA DE UM *ANANTERIS*, O ESCORPIÃO PODE SOLTAR A CAUDA PARA ESCAPAR DO ATAQUE COM VIDA.



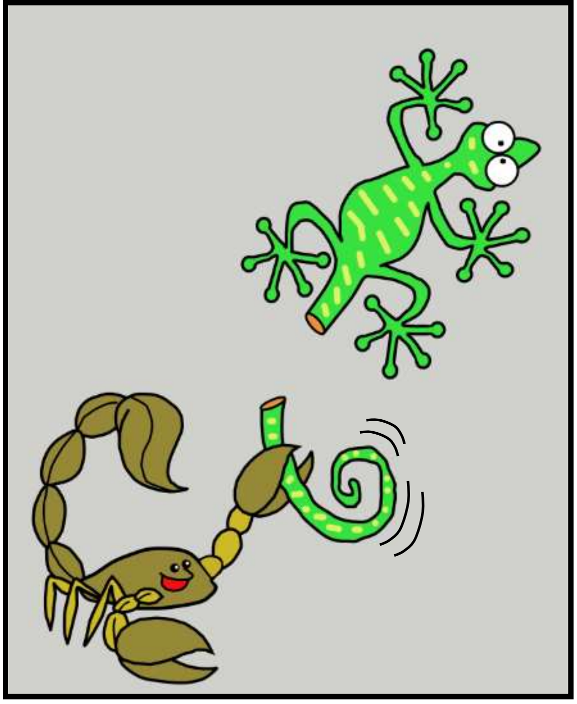
PARÊNTESES

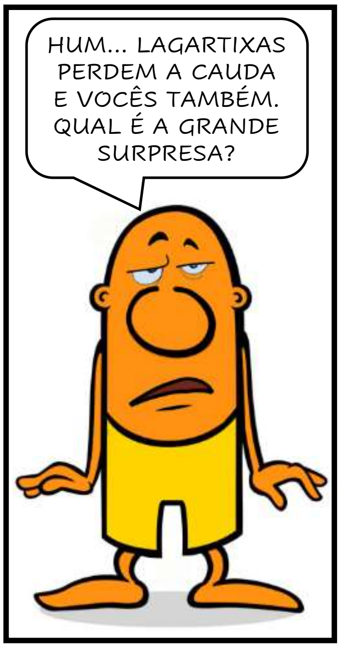
EU, PARTICULARMENTE, NÃO ESTOU SURPRESO, POIS SEMPRE SOUBE QUE NÓS, *ANANTERIS*, PODÍAMOS FAZER ESTA PROEZA. PORÉM, PARA QUE UM HUMANO FICASSE SABENDO, FOI PRECISO QUE UMA EQUIPE DE PESQUISADORES FIZESSE A DESCOBERTA E CONTASSE PARA O RESTO DO MUNDO.

OS PESQUISADORES CHAMAM DE "AUTOTOMIA" A CAPACIDADE DE SE LIVRAR DE UMA PARTE DO CORPO QUANDO AGARRADA POR UM PREDADOR.

A AUTOTOMIA OCORRE EM OUTROS ARACNÍDEOS. NO CASO DAS ARANHAS, POR EXEMPLO, ELAS PODEM SE LIVRAR DE UMA OU MAIS PERNAS PARA ESCAPAR DO ATAQUE DE UM PREDADOR.

TALVEZ O CASO MAIS CONHECIDO DE AUTOTOMIA OCORRA NAS LAGARTIXAS. DEPOIS DE AMPUTAR A CAUDA, A LAGARTIXA SAI CORRENDO, ENQUANTO O PEDAÇO AMPUTADO DA CAUDA FICA SE MEXENDO NAS GARRAS DO PREDADOR.




HUM... LAGARTIXAS PERDEM A CAUDA E VOCÊS TAMBÉM. QUAL É A GRANDE SURPRESA?


A CAUDA DAS LAGARTIXAS POSSUI PRINCIPALMENTE OSSOS E MÚSCULOS. NÃO TEM NENHUM ÓRGÃO IMPORTANTE.


NOS ESCORPIÕES, A CAUDA É MUITO MAIS SOFISTICADA. DENTRO DELA ESTÃO PARTES DOS **SISTEMAS CIRCULATÓRIO** E **DIGESTIVO**, INCLUINDO O ÂNUS. POR FIM, NA PONTA DA CAUDA, ESTÁ A **GLÂNDULA DE VENENO** E O **FERRÃO**.
ÂNUS


DEPOIS DE PERDERMOS O ÂNUS, FICAMOS CONSTIPADOS, O QUE PARECE SER UM PROBLEMA BEM FREQUENTE EM HUMANOS.

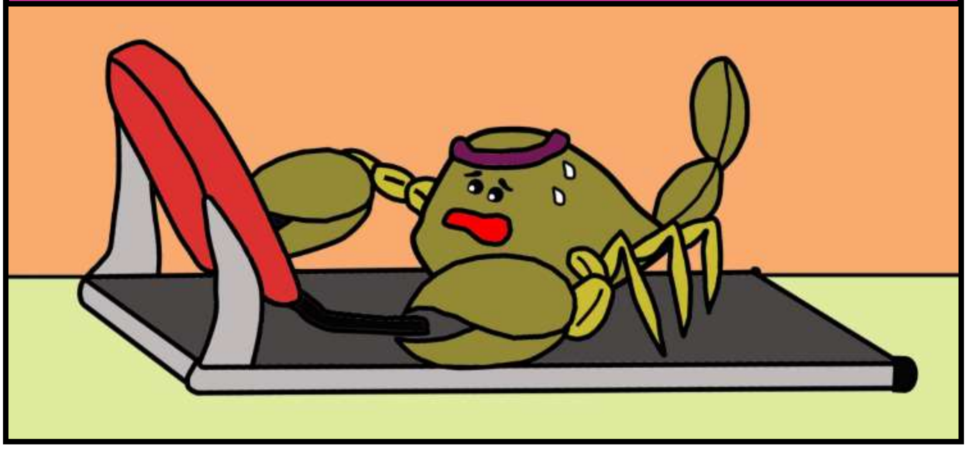

E AO CONTRÁRIO DAS LAGARTIXAS, A NOSSA CAUDA NÃO REGENERA. PORTANTO, O EFEITO DA CONSTIPAÇÃO DURA O RESTO DA NOSSA VIDA.
EU GOSTARIA DE LHE DIZER QUE VAI PASSAR, MEU CARO. MAS NO SEU CASO, NÃO VAI...


COMO A COMIDA QUE ENTRA NÃO SAI, A PERDA DA CAUDA PROMOVE GANHO DE PESO, QUE A LONGO PRAZO QUASE CERTAMENTE NOS LEVA À MORTE.
AMIGA, SOUBE QUE HÁ UMAS SEMANAS VOCÊ ESCAPOU POR POUCO DO ATAQUE DE UM GAMBÁ FAMINTO!
SIM, MAS ESTOU PREOCUPADA, POIS NÃO PAREI DE ENGORDAR DEPOIS DISSO...


DADA A IMPORTÂNCIA DA CAUDA NA VIDA DE UM ESCORPIÃO, SUA PERDA DEVE GERAR UMA SÉRIE DE PROBLEMAS GRAVES.

QUEM INVESTIGOU OS PROBLEMAS RELACIONADOS À PERDA DA CAUDA EM ESCORPIÕES FOI A SOLY. ELA É ALUNA DE DOUTORADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DA USP. ALIÁS, A SOLY FEZ PARTE DA EQUIPE QUE DESCOBRIU A AUTOTOMIA DE CAUDA EM ESCORPIÕES.

1) MINHA EXPECTATIVA ERA QUE LOGO DEPOIS DE PERDER A CAUDA, OS ESCORPIÕES DEVERIAM FICAR MAIS LEVES E CORRER MAIS RÁPIDO, POIS ESTÃO CARREGANDO MENOS PESO. DEPOIS DE ALGUMAS SEMANAS, PORÉM, ELES DEVERIAM FICAR CADA VEZ MAIS PESADOS, POIS NÃO CONSEGUEM DEFECAR. PORTANTO, COM O PASSAR DO TEMPO, OS ESCORPIÕES DEVERIAM FICAR MAIS LENTOS.

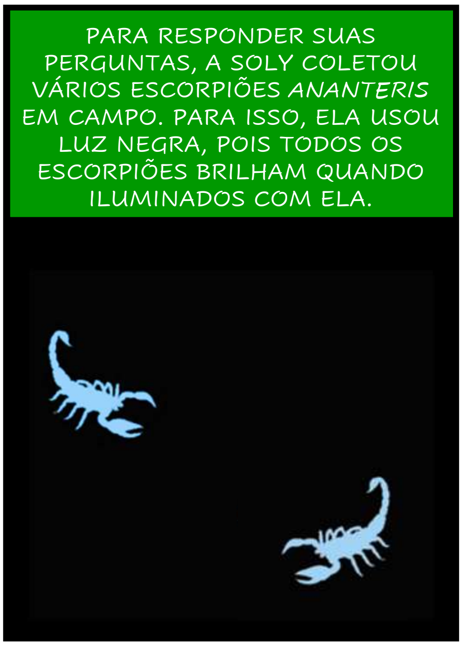


2) AO PERDER A CAUDA, OS ESCORPIÕES PERDEM O FERRÃO, USADO PARA CAPTURAR PRESAS GRANDES. PORTANTO, MINHA EXPECTATIVA ERA QUE APÓS A AUTOTOMIA A DIETA DOS ESCORPIÕES DEVERIA INCLUIR APENAS PRESAS PEQUENAS, QUE PODEM SER CAPTURADAS COM AS PINÇAS. COMO OS MACHOS SÃO MENORES QUE AS FÊMEAS, ELES DEVERIAM COMER PRESAS AINDA MENORES.

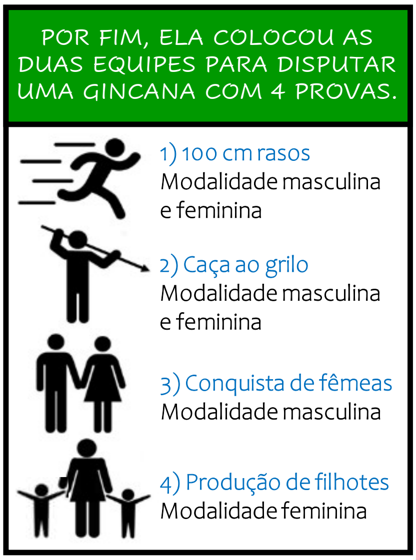

POR ÚLTIMO, QUERIA SABER SE PERDER A CAUDA ATRAPALHA A REPRODUÇÃO DE MACHOS E FÊMEAS.


3) MINHA EXPECTATIVA ERA QUE, COMO OS MACHOS USAM A CAUDA PARA ACARICIAR AS FÊMEAS DURANTE A DANÇA DE ACASALAMENTO, MACHOS SEM CAUDA PODERIAM SE TORNAR MENOS ATRAENTES. COMO CONSEQUÊNCIA, ELES TERIAM UMA CHANCE MENOR DE CONQUISTAR PARCEIRAS PARA A REPRODUÇÃO.
CARA, SINTO QUE PERDI TODA A MINHA MASCULINIDADE...
PODIA SER PIOR... IMAGINE SE SUAS PARTES ÍNTIMAS ESTIVESSEM NA PONTA DA CAUDA.


4) PARA AS FÊMEAS, MINHA EXPECTATIVA ERA QUE A PERDA DA CAUDA DEVERIA TER UM EFEITO NEGATIVO NO NÚMERO DE FILHOTES GERADOS. POR CAUSA DA CONSTIPAÇÃO, A BARRIGA FICA REPLETA DE FEZES E SOBRA POUCO ESPAÇO PARA O DESENVOLVIMENTO DOS FILHOTES.
ESTOU VENDO EM SUA FICHA MÉDICA QUE A SENHORA TEVE MENOS DE 10 FILHOTES...
POIS É, DOUTORA, QUANDO DECIDI SER MÃE, JÁ ESTAVA TÃO CHEIA, QUE NÃO HAVIA MAIS ESPAÇO PARA MUITOS FILHOTES EM MINHA VIDA...


PARA RESPONDER SUAS PERGUNTAS, A SOLY COLETOU VÁRIOS ESCORPIÕES *ANANTERIS* EM CAMPO. PARA ISSO, ELA USOU LUZ NEGRA, POIS TODOS OS ESCORPIÕES BRILHAM QUANDO ILUMINADOS COM ELA.


NO LABORATÓRIO, A SOLY SEPAROU OS ESCORPIÕES EM DUAS EQUIPES: OS COM CAUDA E OS SEM CAUDA.


POR FIM, ELA COLOCOU AS DUAS EQUIPES PARA DISPUTAR UMA GINCANA COM 4 PROVAS.
1) 100 cm rasos
Modalidade masculina e feminina
2) Caça ao grilo
Modalidade masculina e feminina
3) Conquista de fêmeas
Modalidade masculina
4) Produção de filhotes
Modalidade feminina


PARA SABER O QUE A SOLY DESCOBRIU, VOCÊ PODE LER OS ARTIGOS CIENTÍFICOS QUE ELA ESCREVEU OU IR DIRETO PARA AS CONCLUSÕES DA TESE.

Capítulo 1

Short- and long-term effects of an extreme form of autotomy on locomotor performance of a neotropical scorpion*

Solimary García Hernández[1] & Glauco Machado[2]

[1]Programa de Pós-graduação em Ecologia, Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav. 14, no. 321, São Paulo, SP, 05508-900, Brazil

[2]LAGE do Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav. 14, no. 321, São Paulo, SP, 05508-900, Brazil

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Summary

1. Autotomy is one of the most extreme forms of defence against predation. Although autotomy usually increases the chances of escaping a predatory attack, the loss of a body part may also have fitness consequences for the individuals. A widespread consequence of autotomy is a change in locomotor performance, which can be either increased or decreased depending on the function of the autotomized body part in locomotion.

2. Scorpions of the genus *Ananteris* autotomize the last segments of the abdomen, known as the "tail". After "tail" autotomy, individuals lose almost 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 implications of "tail" loss on the locomotor performance of *A. balzani*.

3. In the short-term experiment, the locomotor performances of both males and females did not change after "tail" loss. Moreover, there was no evidence that the relative mass of the lost "tail" affected the locomotor performance in both sexes. In the long-term experiment, "tail" loss had a negative effect on locomotor performance, especially in males. However, contrary to expectations, autotomized over-fed individuals, which suffered from severe constipation, were not slower than autotomized normally fed individuals.

4. In conclusion, autotomy has minimal short-term effects on locomotor performance in both sexes. In the long term, however, autotomy reduces locomotor performance, which may negatively affect food acquisition, the ability to escape future predatory attacks, and the search for mates. The long lag time between "tail" loss and death by constipation is probably a key factor in understanding the evolution of one of the most extreme cases of autotomy in nature.

KEYWORDS

Balance loss, constipation, defensive mechanisms, sex-differences, running speed, weight loss

1 INTRODUCTION

One of the most extreme forms of defence against predation is to voluntarily separate a body part. This defence, known as autotomy, has been reported for numerous animal groups, including vertebrates and invertebrates (Fleming, Muller, & Bateman, 2007; Bateman & Fleming, 2009). 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 limb autotomy in arthropods increases the chances of escaping a predatory attack (e.g., Congdon, Vitt, & King, 1974; Wasson, Lyon, & Knope, 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 increase exposure to infection (Fleming, Muller, & Bateman, 2007).

A widespread consequence of losing a body part is a change in locomotor performance (Fleming, Muller, & Bateman, 2007; Bateman & Fleming, 2009). The specific consequence depends on the function of the autotomized appendage in locomotion. Individuals who 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 terrestrial arthropods decreases after leg loss (Fleming, Muller, & Bateman, 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 locomotor performance (McElroy & Bergmann, 2013). If the tail assists locomotion by balancing the body during movement, autotomy may decrease locomotor performance (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 groups after the loss of large body parts, such as a pedipalp in male spiders (Ramos, Irschick, & Christenson, 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* (Mattoni *et al.*, 2015). Unlike other arthropods, which autotomize 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. 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; Figure 1a). Because there is no regeneration of the "tail", the scar does not allow defecation, so that autotomized individuals accumulate faeces over time and eventually die of constipation (Mattoni *et al.*, 2015; Figure 1b). To understand how this bizarre form of autotomy has evolved, we need to understand the costs and benefits associated with "tail" loss. Here, we investigated the short- and long-term implications of "tail" loss on the locomotor performance of the scorpion *A. balzani* (Buthidae). More specifically, we tested two non-exclusive hypotheses, the *balance loss* and the *weight loss* hypotheses.

The balance loss hypothesis assumes that scorpion "tail" has a balancing role during locomotion, as occurs with some lizards (Jagnandan & Higham, 2017). Although no locomotor function has been reported for the scorpion "tail" (Carlson, McGinley, & Rowe, 2014), our observations show that individuals of *A. balzani* move their "tail" sideways while running (see video in Supplementary Material S1). The balance loss hypothesis predicts that autotomy will have a negative short-term effect on locomotor performance due to balance loss (Table 1). The hypothesis also predicts that individuals with larger "tails" will show greater changes in locomotor performance after autotomy, as already reported in a meta-analysis on tail loss in lizards (McElroy & Bergmann, 2013). Considering that the relative “tail” mass of males is nearly 5% larger than that of females (see STUDY SPECIES), autotomy should have a more pronounced negative effect on the locomotor performance of males (Table 1). If we find that balance indeed has a short-term effect on locomotor performance, we predict that the negative effect of “tail” loss will be later reverted by balance compensation (Table 1). This long-term balance compensation has already been reported for lizards some months after tail autotomy (e.g., Cox, 1969). Because the “tail” of females is relatively smaller than that of males in *A. balzani* (see STUDY SPECIES), we predict that females will recover their original locomotor performance faster than males (Table 1). Finally, we predict that long-term balance compensation will be independent of constipation (Table 1).

The weight loss hypothesis predicts that autotomy will have a short-term positive effect on locomotor performance due to the loss of body mass (Table 1). In the scorpion *Centruroides vittatus* (Buthidae), for instance, the speed of females decreases when they are carrying offspring on their dorsum but increases after the nymphs disperse (Shaffer & Formanowicz, 1996). Similarly, the total body mass in *Ananteris* is considerably reduced after autotomy, which could increase locomotor performance. Because males lose a relatively large percentage of their total body mass after autotomy (see STUDY SPECIES), we predict that the positive effect of “tail” loss on locomotor performance will be higher in males than in females (Table 1). Moreover, given that autotomized individuals are unable to eliminate faeces, constipation will increase the body mass over time. Thus, the long-term effect of “tail” loss will be a reduction in locomotor performance when compared with the period immediately after autotomy (Table 1). This effect will be more pronounced in over-fed than in normally fed individuals because the former will accumulate more faeces over time (Table 1). Finally, we predict that locomotor performance will differ between sexes over time after autotomy. Because the mesosoma of females is relatively large, they will accumulate more faeces, and the negative effect of constipation will be more pronounced in females than in males (Table 1).

TABLE 1 Predictions of the balance loss and weight loss hypotheses for the short- and long-term effects of "tail" autotomy on the locomotor performance of scorpions.

	Hypotheses	
	Balance loss	**Weight loss**
Short-term effects of autotomy		
General tendency	Locomotor performance will *decrease* after autotomy	Locomotor performance will *increase* after autotomy
Effect of relative mass of the lost "tail"	The greater the relative mass of the lost "tail", the *poorer* the locomotor performance will be	The greater the relative mass of the lost "tail", the *greater* the locomotor performance will be
Sexual difference	Males will have a *poorer* locomotor performance than females after autotomy	Males will have a *better* locomotor performance than females after autotomy
Long-term effects of autotomy		
General tendency	Individuals will gradually *increase* locomotor performance after autotomy	Individuals will gradually *decrease* locomotor performance after autotomy
Effect of diet*	Normally fed and over-fed individuals will have a *similar* locomotor performance	Normally fed individuals will have a *better* locomotor performance than over-fed individuals
Sexual difference	Females will *increase* locomotor performance more markedly than males after autotomy	Females will *decrease* locomotor performance more markedly than males after autotomy

* For both the balance loss and weight loss hypotheses, we assume that normally and over-fed individuals have enough energy for the locomotion performance tests.

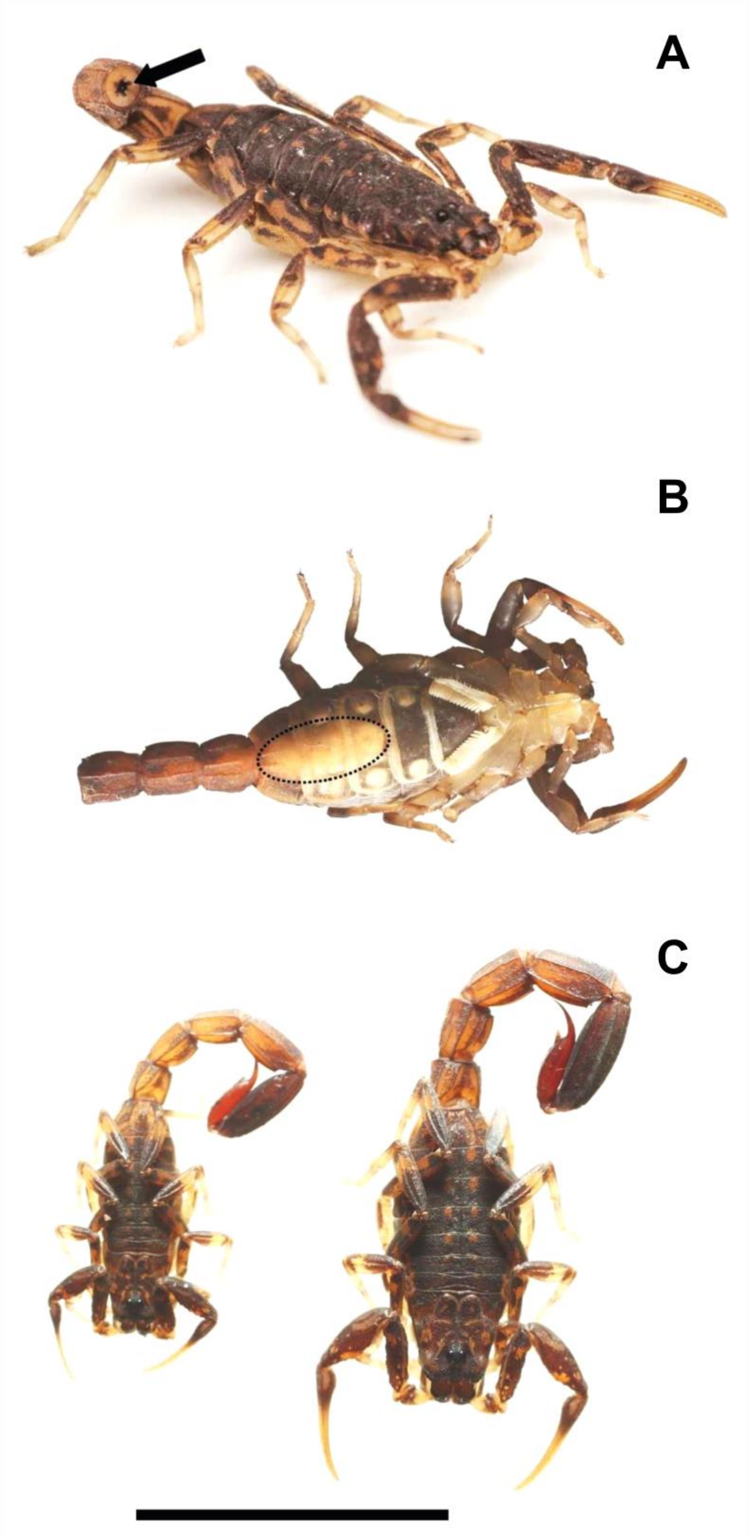

FIGURE 1. Individuals of *Ananteris balzani*. (A) After "tail" autotomy, dark scar tissue (arrow) blocks the posterior end of the digestive tract, preventing defecation and causing constipation. (B) Ventral view of a male 50 days after autotomy. The white patch inside the dashed ellipsis is caused by 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.

2 MATERIALS AND METHODS

2.1 Study species

Ananteris balzani shows marked sexual size dimorphism (Figure 1, Figure S1). Although males are smaller than females, their "tails" are relatively longer (metasoma/carapace length ratio: males=5.17, *N*=16; females=5.03, *N*=17). Moreover, the mean (±SD) mass of the autotomized "tail" in males corresponds to 23.8±2.8% of their total body mass (*N*=28), whereas in females, it corresponds to 19.9±3.6% (*N*=21). After autotomy, both males and females may live as long as seven months (Mattoni *et al*., 2015).

2.2 Collection and maintenance

We collected 154 adult individuals (102 males, 52 females) from October 2016-April 2017 at Santa Bárbara Ecological Station (24º48´S, 49º13´W), São Paulo, Brazil. The specimens were maintained under quarantine conditions for two 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. During the quarantine period, we fed the individuals every 10 days with cricket nymphs (*Acheta* sp.). Because the body mass of females is larger than that of males (Figure S1), females received twice as much food as males. We kept the individuals under a photoperiod of 12:12 h and conducted the experiments at room temperature, which ranged from 24.5 to 26.5 °C.

We induced "tail" autotomy by grasping and pushing 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 (Figure 1a). In individuals assigned to the control group (see below), we used the same protocol to induce “tail” autotomy, but we grasped the first metasomal segment, where “tail” autotomy is impossible. We excluded females bearing embryos from the experiment to avoid the possible confounding effects that the extra load caused by the offspring could have on their locomotor performance.

2.3 Locomotor performance trials

At the beginning of each trial, we placed an individual inside a vial at one end of a 1 m-long racetrack (3 cm wide, 5 cm high) with sand as the substrate; the racetrack was covered by a transparent plastic lid. After 5 min, the plastic vial was removed, and the individual was stimulated to run by repeated taps on its dorsum with forceps (see video in Supplementary Material 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 two trials with the same individual to remove possible chemical cues left on the substrate. We repeated this procedure 24 h later to complete four trials for each individual. All trials were conducted at night (18:00-23:00 h) under dim red illumination. We filmed the trials and, based on the footage, recorded the mean running speed (estimated at each 10 cm-interval of the racetrack in each trial) and maximum running speed (estimated as the highest speed over a 10 cm-interval in each trial).

2.4 Short-term effects of "tail" autotomy

First, we estimated the *original* locomotor performance of 113 intact individuals, including males (*N*=75) and females (*N*=38). To control for possible individual variation in locomotor performance, each individual was tested four times over two consecutive days (two trials on the first day and two trials on the second day). After the last trial, we randomly allocated each individual to one of two experimental groups. The *autotomized* group included 46 males and 25 females for which we induced "tail" autotomy. The *intact* group included 29 males and 13 females that were left intact. We allocated more individuals to the autotomized group because individual mortality was higher than in the intact group during the long-term experiment. The original performance did not differ between individuals in the two groups (Figure S2).

We estimated the locomotor performance of all individuals belonging to both experimental groups three days after the measurement of the original locomotor performance when the healing process of the autotomized individuals was completed. To evaluate the effect of "tail" weight on locomotor performance, we submitted half of the individuals in each experimental group to two burden 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. To load intact individuals, we glued a cylinder (males: length=7 mm, diameter=1.2 mm; females: length=9 mm, diameter=1.8 mm) made of raw spaghetti, weighing 24% and 20% of the total body mass of males and females, respectively, onto their dorsum. These values corresponded to the mean relative mass of the lost "tail" in individuals of each sex. By gluing the "tail" or the cylinder along the central axis of the mesosoma, we tried to maintain the centre of mass of the individuals. For unloaded individuals in both the intact and autotomized groups, we applied only a glue drop onto their dorsum. Finally, three days after the second round of trials, we inverted the burden treatment of each individual (loaded and unloaded) in each experimental group (intact and autotomized) and estimated their locomotor performance again (see scheme of the experimental design in Figure S3).

At the end of the short-term experiment, we had repeated estimates of locomotor performance for each individual of both experimental groups: four measures of the original locomotor performance (before “tail” manipulation), four measures of locomotor performance for autotomized and intact loaded individuals, and four measures for autotomized and intact unloaded individuals. The short-term experiment lasted 12 days, during which individuals were not fed to prevent great mass changes that could influence their locomotor performance.

2.5 Long-term effects of “tail” autotomy

After the short-term experiment, we used a subset of intact (29 males, 13 females) and autotomized individuals (43 males, 24 females) to evaluate the long-term effects of autotomy on locomotor performance (always with unloaded individuals). Half of the males and half of the females in each experimental group (intact and autotomized) were randomly allocated to two diet treatments: *normally fed*, in which individuals received 10% of their body mass in the form of cricket nymphs every 10 days, and *over-fed*, in which individuals received 20% of their body mass in the form of cricket nymphs every 10 days.

The diet treatments started on day 13, one day after the end of the short-term experiment (Figure S3), but the first estimate of locomotor performance for the long-term experiment was obtained only 10 days later. We called the first trials of the long-term experiment *10 days*. The following trials were conducted at 10-day intervals and were referred to *20*, *30*, *40*, and *50 days*. On all these days, the locomotor performance was estimated using the same protocol as in the short-term experiment. At the end of the five rounds of trials (10 to 50 days), we had repeated estimates of locomotor performance for each individual. Due to the long duration of this experiment, some individuals died, and thus we had a complete dataset for 27 intact males, 26 autotomized males, 13 intact females, and 15 autotomized females.

2.6 Data analysis

2.6.1 Short-term effects

We adjusted linear mixed-effect models (LMMs) for the mean and maximum running speed. In both models, we included an interaction between sex (males and females), experimental group (intact and autotomized), and burden treatment (loaded and unloaded) as categorical predictor variables (factors). For the model on mean running speed, we also included the 10 cm intervals along the racetrack (distance) as a continuous predictor variable. Body length and body mass were not included as predictor variables because they are highly correlated with sex (Table S1, Figure S1). We used individual identity as a random factor in both analyses. Based on each model, we estimated the marginal means (EMMs), which are not biased by the imbalance in the sample sizes. For the model on mean running speed, we estimated the slope of linear regressions for each combination between the levels of the factors. We also computed *mvt* contrasts among the estimates using the functions *emmeans* and *emtrends* of the package *emmeans* (Lenth, 2016, 2018), which show where the significant differences are when the estimates of each combination between the levels of the factors are compared with one another.

To test the effect of the relative mass of the lost "tail" on the maximum running speed, we adjusted the linear models using the *lm* function of the package *stats*. The predictor variables were sex and relative weight loss after autotomy, estimated as the weight of the lost “tail” divided by total body mass before autotomy. The response variable was the change in maximum running speed after autotomy, estimated as the difference in maximum speed before and after autotomy (always for unloaded individuals). Negative values indicate a decrease, and positive values indicate an increase in maximum running speed after autotomy.

2.6.2 Data analysis: long-term effects

To test for long-term effects of “tail” autotomy on mean running speed, we adjusted the LMM for each diet treatment (normally and over-fed) to avoid multiple interactions and to simplify the interpretation of the results. In each model, we included an interaction between sex, experimental group, and time (beginning=10 days, end=50 days) as categorical predictor variables, and the 10 cm intervals along the racetrack as a continuous predictor variable. To test for the long-term effects of “tail” autotomy on maximum running speed, we adjusted the LMM with an interaction between sex, experimental group, and diet treatment as categorical predictor variables and time (10 to 50 days) as a continuous predictor variable. Individual identity was used as a random factor. In both analyses, we included the individual identity as a random factor. Based on the model, we obtained the EMMs and slopes and computed the *mvt* contrasts using the *emmeans* and *emtrends* as described for the short-term experiment.

The LMMs were performed in the package *nlme* (Pinheiro *et al*., 2019). In all models in which it was necessary, we incorporated an error structure to account for heterocedasticity and/or autocorrelation of the data. All analyses were performed using the software R version 3.5.2. (R Core Team, 2018). The measures of variance presented in the results always refer to standard error.

2.7 Ethical note

Autotomy is a natural process because 6-10% of the individuals of *Ananteris* spp. collected in the field are found without a "tail" (Mattoni *et al.*, 2015). All trials of induced autotomy were performed carefully, stimulating the voluntary "tail" autotomy and minimizing the stress of the individuals. Both the collection and maintenance of the scorpions were conducted with proper permits from the Brazilian Government (SISBIO/ICMBio, Permit 56081).

3 RESULTS

3.1 Short-term experiment: mean running speed

The interaction between sex, experimental group, burden treatment, and distance was significant (Table S3). For each combination between the levels of the factors, we present the intercept and slope of the linear regression, showing how mean running speed decreases along the racetrack (Table 2). The mean running speed of loaded individuals (males and females) was slower than that of unloaded individuals along the racetrack (Figure 2a, b). The mean running speed of autotomized and intact individuals (males and females) in the first 10 cm interval of the racetrack was similar (Figure 2a, b). Intact and unloaded individuals had similar mean running speeds when compared with autotomized and unloaded individuals (t-ratio$_{\text{females}}$=-1.63, P=0.734; t-ratio$_{\text{males}}$=-2.08, P=0.432). Moreover, intact and loaded individuals had similar mean running speeds when compared with autotomized and loaded individuals (t-ratio$_{\text{females}}$=-1.39, P=0.999; t-ratio$_{\text{males}}$=-0.67, P=0.998).

The mean running speed decreased along the racetrack for individuals of both sexes (Figure 2a, b). However, at the last 10 cm interval, the mean running speed was similar for both sexes, ranging from 2.98 to 5.15 cm/s in females and 1.20 to 3.47 cm/s in males (Figure 2a, b). In females, the decrease was higher for autotomized and unloaded than for autotomized and load individuals (Table 2). In males, the decrease was higher for intact and unloaded individuals than for intact and loaded individuals and also for autotomized and unloaded individuals (Table 2). Estimates of repeatability showed that the mean running speed was consistent between individuals during the short-term experiment (Table S8).

3.2 Short-term experiment: maximum running speed

The interaction between sex, experimental group, and burden treatment was not significant (Table S4). The maximum running speed of intact and unloaded individuals was similar in males and females (*t*-ratio=-0.74, *P*=0.995; Figure 2c). For intact and autotomized males and for autotomized females, the maximum running speed of loaded individuals was lower than that of unloaded individuals (Figure 2c). The reduction in the maximum running speed of loaded individuals was greater in autotomized females (3.76±0.62 cm/s; *t*-ratio=6.17, *P*<0.001) than in autotomized males (2.08±0.45 cm/s; *t*-ratio=4.62, *P*<0.001; Table 2). Estimates of repeatability showed that maximum running speed was consistent between individuals during the short-term experiment (Table S8).

3.3 Short-term experiment: effect of relative mass of the lost “tail”

There was no significant effect of the relative mass of the lost “tail” on the change in the mean running speed of males (slope=-0.05±0.12; $F_{1,40}$=0.170, *P*=0.683, R^2=0.004) and females (slope=-0.10±0.18; $F_{1,20}$=0.333, *P*=0.571, R^2=0.016). However, there was a marginally significant negative effect of relative mass of the lost “tail” on the change in the maximum running speed 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 the maximum running speed (slope=0.54±0.32; $F_{1,20}$=2813.0, *P*=0.191, R^2=0.123).

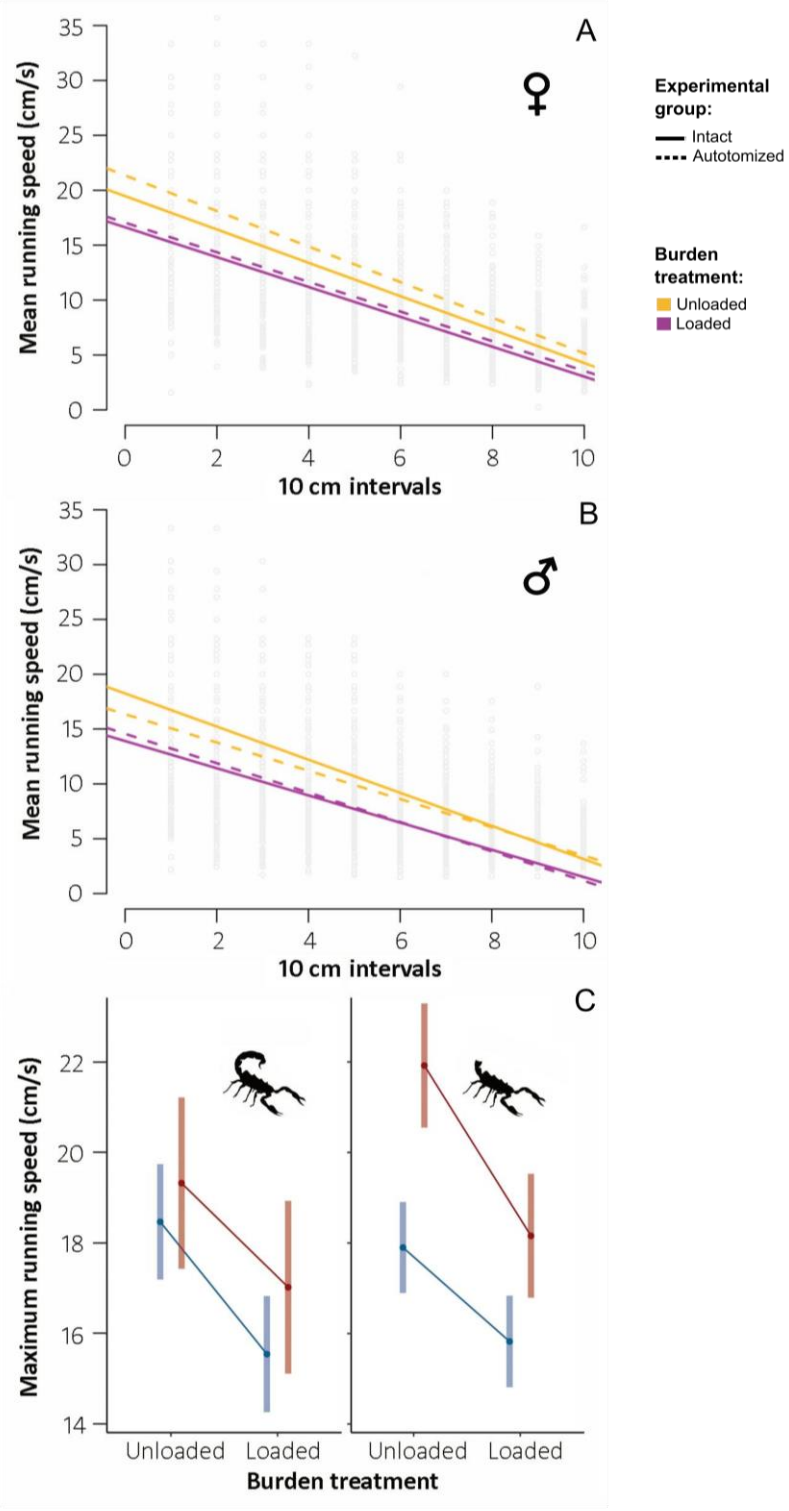

FIGURE 2. Short-term effects of "tail" autotomy on the (A-B) mean and (C) maximum running speeds of a scorpion species. For both sexes, the longer the distance was, the lower the mean running speed estimated in each 10 cm-interval of the racetrack. (C) Marginal means for intact (left) and autotomized individuals (right). Females are in red and males are in blue. Error bars indicate the 95% confidence interval of the estimates. Estimates of all models are presented in Table 2.

TABLE 2. Results of the short-term experiment to evaluate the effect of "tail" autotomy on the locomotor performance of individual scorpions. For the mean running speed, we estimated the intercept and slope of the linear regression for each combination of the levels of the predictor categorical variables (sex, experimental group, and burden treatment), showing how the mean running speed changes along the racetrack. For the maximum running speed, we estimated the marginal means (EMMs) for each combination of the levels of the predictor categorical variables. For all estimates, we provide the standard error and the 95% confidence intervals (in parentheses) following the *mvt* method. Combinations sharing the same letter in the column 'Contrast' are not significantly different (α = 0.05, *mvt*-adjusted). df = degree of freedom.

Factors	**Mean running speed**						**Maximum running speed**		
	Estimate (Intercept)	**df**	**Contrast**	**Estimate (Slope)**	**df**	**Contrast**	**Estimate (EMMs)**	**df**	**Contrast**
Females									
Intact – Unloaded	19.47±0.90 (17.02, 21.92)	109	ef	-1.52±0.06 (-1.68, -1.36)	4914	ab	19.32±0.95 (16.69, 21.95)	109	cd
Intact – Loaded	16.63±0.93 (14.08, 19.18)	109	abcd	-1.36±0.07 (-1.55, -1.18)	4914	abc	17.02±0.96 (14.37, 19.67)	109	abc
Autotomized – Unloaded	21.35±0.71 (19.41, 23.30)	109	f	-1.62±0.05 (-1.76, -1.48)	4914	a	21.92±0.69 (20.02, 23.83)	109	d
Autotomized – Loaded	17.05±0.74 (15.02, 19.09)	109	cde	-1.35±0.06 (-1.51, -1.18)	4914	bc	18.16±0.69 (16.26, 20.06)	109	abc
Males									
Intact – Unloaded	18.25±0.62 (16.55, 19.94)	112	de	-1.51±0.04 (-1.62, -1.39)	4914	ab	18.47±0.64 (16.70, 20.24)	112	c
Intact – Loaded	13.91±0.64 (12.18, 15.65)	112	ab	-1.24±0.05 (-1.38, -1.11)	4914	c	15.55±0.65 (13.77, 17.33)	112	ab
Autotomized – Unloaded	16.36±0.57 (14.80, 17.91)	109	bde	-1.29±0.05 (-1.42, -1.16)	4914	c	17.90±0.51 (16.50, 19.30)	109	bc
Autotomized – Loaded	14.57±0.60 (12.94, 16.20)	109	ac	-1.34±0.06 (-1.50, -1.17)	4914	bc	15.83±0.51 (14.42, 17.23)	109	a

3.4 Long-term experiment: mean running speed

In the analyses performed with each diet treatment separately, the interaction between sex, experimental group, time, and distance was not significant for both normally and over-fed individuals (Table S5). For both diet treatments, however, there was a significant interaction between experimental group, time, and distance (Table S5). The main difference between diet treatments was that the mean running speed of intact and over-fed females decreased more markedly along the racetrack (-1.88±0.17 and -1.67±0.19 cm/s for 10 and 50 days, respectively; Table S6; Figure S4a, b) than that of intact and normally fed females (-1.44±0.12 and -1.07±0.11 cm/s for 10 and 50 days, respectively; Table 3; Figure 3a, b). The other patterns were similar for normally and over-fed individuals, and thus we describe the results only for the former.

At the beginning of the experiment, the mean running speeds of intact and autotomized individuals of both sexes in the first 10 cm interval were similar (t-ratio$_{\text{females}}$=1.42, P=0.846; t-ratio$_{\text{males}}$=0.91, P=0.984; Figure 3a, b). Moreover, there was no sexual difference in the mean running speed of both the intact (0.38±1.55 cm/s; t-ratio=-0.25, P=1.000) and autotomized individuals (2.54±1.58 cm/s; t-ratio=-1.62, P=0.739; Figure 3a, b). At the end of the experiment, the mean running speeds of the intact and autotomized females in the first 10 cm interval were similar (t-ratio=1.49, P=0.810; Figure 3a). In turn, the mean running speed of autotomized males in the first 10 cm interval was 4.67±1.43 cm/s slower than that of intact males (t-ratio=3.62, P=0.038; Figure 3b). Finally, there was no difference in the mean running speed between intact males and females (4.36±1.71 cm/s, t-ratio=-2.55, P=0.200) and between autotomized males and females (3.03±1.50 cm/s; t-ratio=-2.02, P=0.479).

The mean running speed at the end of the experiment decreased when compared with that obtained at the beginning of the experiment (Figure 3a, b). In the first 10 cm interval, the differences in the mean running speeds of intact females at 10 and 50 days (5.85±1.41 cm/s; *t*-ratio=4.14, *P*=0.001) were similar to those of autotomized females at 10 and 50 days (5.30±1.05 cm/s; *t*-ratio=4.94, *P*<0.001; Figure 3a, b). In turn, the differences in the mean running speed between autotomized males at 10 and 50 days (5.30±1.05 cm/s; *t*-ratio=5.05, *P*<0.001) were three times higher than those of intact males at 10 and 50 days (1.87±0.97 cm/s; *t*-ratio=1.91, *P*=0.546; Figure 3a, b). Estimates of repeatability show that the mean running speed was consistent between individuals during the long-term experiment (Table S8).

3.5 Long-term experiment: maximum running speed

The interaction between sex, experimental group, diet treatment, and time was significant (Table S7). The maximum running speed of the individuals was similar at the beginning of the experiment but decreased over time ($F_{1,1709}$=189.28, *P*<0.001; Table 3; Figure 3c, d). The reduction in the maximum running speed over time was more marked when individuals were autotomized ($F_{1,100}$=10.49, *P*<0.002; Table 3; Figure 3c, d). The diet treatment had no significant effect on the maximum running speed of both sexes (Table 3; Figure 3c, d). For normally fed individuals, the reductions in the maximum running speed over time were 0.06±0.02 cm/s and 0.05±0.03 cm/s for intact males and females, respectively. For autotomized males and females, these reductions were 0.14±0.02 cm/s and 0.16±0.02 cm/s, respectively (Figure 3c, d). For over-fed individuals, the reductions in the maximum running speed over time were 0.04±0.02 cm/s and 0.13±0.02 cm/s for intact males and females, respectively. For autotomized males and females, these reductions were 0.11±0.02 cm/s and 0.07±0.02 cm/s, respectively (Table 3; Figure 3c, d). Estimates of repeatability show that maximum running speed was consistent between individuals during the long-term experiment (Table S8).

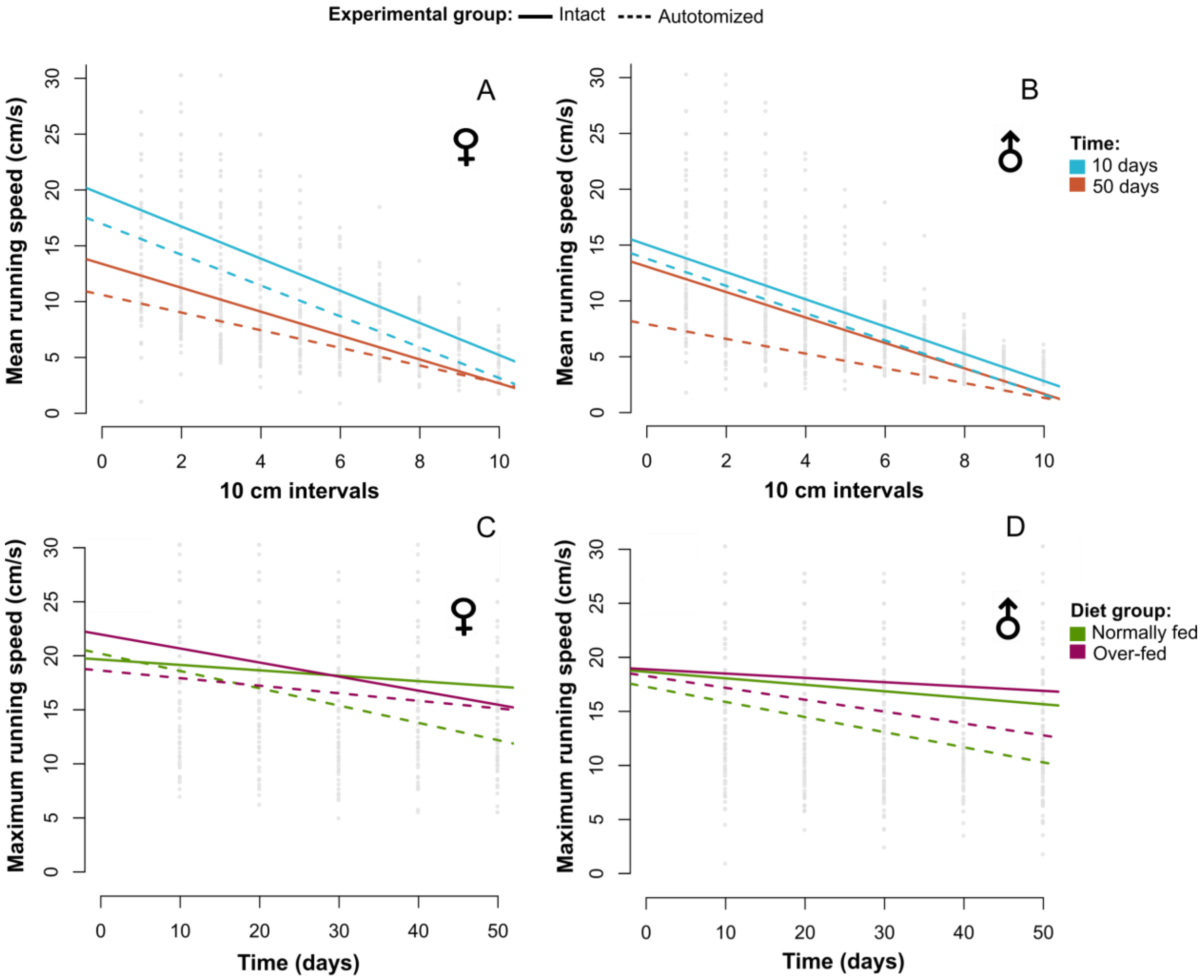

FIGURE 3. Long-term effects of "tail" autotomy on two proxies of locomotor performance in scorpions: (A-B) the mean running speeds of normally fed females and males, respectively, (C-D) the maximum running speeds of females and males, respectively. Estimates of the models are presented in Table 3.

TABLE 3. Results of the long-term experiment to evaluate the effect of “tail” autotomy on the locomotor performance of a scorpion species. For mean running speed, we adjusted a model using only individual of normally fed diet group, and we estimated two parameters: (i) the intercept, which can be interpreted as the initial running speed, and (ii) the slope of the linear regression, which can be interpreted as the decrease in the mean running speed along the racetrack. For the maximum running speed, we estimated two parameters: (i) the intercept, which can be interpreted as the maximum running speed at the beginning of the long-term experiment (i.e., day 10), and (ii) the slope of the linear regression, which can be interpreted as the change in the maximum running speed along the long-term experiment (i.e., from day 10 to day 50). The parameters were estimated for each combination of the levels of the predictor categorical variables of each model. For all estimates, we provide the standard error and the 95% confidence intervals (in parentheses). Combinations sharing the same letter in the column ‘Contrast’ are not significantly different (α = 0.05, *mvt*-adjusted). These contrasts are valid only for combinations within the same proxy of locomotor performance. df = degree of freedom.

Factors	**Intercept**			**Slope**		
	Estimate	**df**	**Contrast**	**Estimate**	**df**	**Contrast**
Mean running speed: Females (normally fed group)						
Intact (10 days)	19.61±1.52 (15.30, 23.91)	52	d	-1.44±0.12 (-1.78, -1.11)	1639	a
Intact (50 days)	13.38±1.33 (9.62, 17.14)	52	abc	-1.07±0.11 (-1.36, -0.77)	1639	abc
Autotomized (10 days)	16.96±1.23 (13.48, 20.45)	52	cd	-1.38±0.11 (-1.69, -1.07)	1639	a
Autotomized (50 days)	10.60±1.23 (7.13, 14.07)	52	ab	-0.79±0.11 (-1.10, -0.49)	1639	bc
Mean running speed: Males (normally fed group)						
Intact (10 days)	15.02±1.03 (12.12, 17.92)	55	bcd	-1.22±0.08 (-1.45, -0.99)	1639	ab
Intact (50 days)	13.07±1.00 (10.26, 15.88)	55	bc	-1.14±0.09 (-1.38, -0.89)	1639	ab
Autotomized (10 days)	13.78±1.05 (10.81, 16.75)	52	bc	-1.22±0.10 (-1.49, -0.95)	1639	ab
Autotomized (50 days)	7.92±1.20 (4.54, 11.30)	52	a	-0.66±0.13 (-1.01, -0.31)	1639	c
Maximum running speed: Females						
Intact – Normally fed	19.65±1.69 (14.95, 24.34)	100	a	-0.05±0.03 (-0.12, 0.02)	1709	bcd
Intact – Over-fed	21.97±1.56 (17.62, 26.31)	100	a	-0.13±0.02 (-0.20, -0.07)	1709	abc
Autotomized – Normally fed	20.19±1.24 (16.74, 23.64)	100	a	-0.16±0.02 (-0.22, -0.10)	1709	a
Autotomized – Over-fed	18.63±1.27 (15.08, 22.18)	100	a	-0.07±0. 02 (-0.13, -0.01)	1709	abcd
Maximum running speed: Males						
Intact – Normally fed	18.66±1.08 (15.68, 21.65)	107	a	-0.06±0.02 (-0.11, -0.01)	1709	cd
Intact – Over-fed	18.90±1.11 (15.82, 21.99)	100	a	-0.04±0.02 (-0.08, 0.01)	1709	d
Autotomized – Normally fed	17.28±0.89 (14.81, 19.76)	100	a	-0.14±0.02 (-0.18, -0.09)	1709	ab
Autotomized – Over-fed	18.28±0.94 (15.66, 20.91)	100	a	-0.11±0.02 (-0.16, 0.07)	1709	abc

Discussion

Here, we investigated the short- and long-term effects of an extreme form of autotomy, including abdomen loss and subsequent constipation, in a scorpion species. We proposed and tested predictions of two non-mutually exclusive hypotheses: balance loss and weight loss. In the short term, “tail” loss alone had a minor effect on the locomotor performance of males and females. However, loaded individuals showed a poorer locomotor performance than unloaded individuals, and this effect was more pronounced in autotomized females. Moreover, we found no evidence that the relative mass of the lost “tail” affects locomotor performance. Taken together, these results do not unequivocally support either of the two hypotheses we tested (Table 1). In the long term, “tail” loss had a negative effect on locomotor performance, as expected by the weight loss hypothesis. However, contrary to what would be expected with this hypothesis, autotomized over-fed individuals were not slower than autotomized normally fed individuals. In the following sections, we interpret these results, explore their ecological consequences and discuss how “tail” autotomy may have evolved in scorpions.

4.1 Short-term effects on locomotor performance

Regardless of the sex, the mean running speed decreased along the racetrack, and the maximum running speed was always recorded in the first or second 10 cm intervals. These findings suggest that the escape behaviour of *A. balzani* is characterized by an initial sprint, but the running speed is reduced by 72-79% (depending on the sex) after a distance of 1 m. Considering that the metabolic rates of scorpions are very low and that running imposes high metabolic demands (Lighton *et al.*, 2001), individuals are likely unable to sustain high speed for long distances. The low metabolic rate may also explain how scorpions can cope with long-term constipation after “tail” autotomy. If individuals are food-limited or feed at low rates under natural conditions (McCormick & Polis, 1990), the negative effects of constipation are reduced. Consequently, individuals can increase their lifespan after autotomy and have enough time to mate and give birth before constipation causes their death.

The results of the short-term experiment were not fully consistent with the predictions of either the balance loss or the weight loss hypotheses. First, "tail" autotomy alone did not impose marked changes in the mean and maximum running speeds of both sexes, a pattern that has already been reported for some lizard species (Bateman & Fleming, 2009). Second, contrary to meta-analytical results reported for lizards (McElroy & Bergmann, 2013), the relative mass of the lost "tail" in *A. balzani* had no effect on the locomotor performance of males and females. Finally, the mean and maximum running speeds of females followed the predictions of the weight loss hypothesis: intact and loaded individuals (the heaviest ones) showed the lowest values, whereas autotomized and unloaded individuals (the lightest ones) showed the highest values. However, the patterns found for the mean and maximum running speeds of males were different: intact and loaded individuals also showed the lowest values, but the highest values were recorded for intact and unloaded individuals. Autotomized and unloaded individuals showed intermediate values, suggesting that weight loss does not increase the locomotor performance of males, although the relative length of their "tail" is greater than that in females. Thus, if we consider only unloaded individuals, "tail" autotomy does not have a marked short-term effect on the locomotor performance of both sexes.

Under natural conditions, there are at least two situations in which scorpions can carry an extra load: when individuals are transporting dead prey on their dorsum (Ojanguren-Affilastro *et al.*, 2016) and when females are carrying nymphs on their dorsum (Polis & Sissom, 1990). The extra load of carrying nymphs decreases the locomotor performance of intact females (Shaffer & Formanowicz, 1996), which may reduce their foraging efficiency and increase their chances of being singled out by predators. In lizards, slow locomotion during pregnancy indeed makes females more vulnerable to predation (Downes & Shine, 2001), and this reduction is regarded as the main cost of reproduction in this group (Shine, 2003). The results of our short-term experiment indicate that the negative effect of carrying a load is more severe when females of *A. balzani* have lost their "tail". This finding provides a putative explanation for the fact that *Ananteris* females are much more reluctant than males to autotomize the "tail" and that the frequency of autotomized females in natural populations is much lower than that of males (Mattoni *et al.*, 2015). Given that nymphs may remain on the mother's dorsum for almost one month after she leaves the brooding chamber, the long period of vulnerability may impose higher mortality costs for autotomized females than for autotomized males.

One aspect of "tail" loss that we did not explore is how the precise fracture point affects locomotor performance. Individuals of *A. balzani* frequently detach their "tail" between metasomal segments III-IV, which corresponds to the most distal fracture point recorded so far (Mattoni *et al.*, 2015). There are two other fracture points (between segments I-II and II-III) where natural or induced autotomy is rarely recorded (Mattoni *et al.*, 2015). For lizards, the *economy of autotomy* hypothesis proposes that individuals should not detach the entire tail to minimize the costs associated with autotomy (Daniels, 1985). Experimental studies with the lizards *Trachylepis maculilabris* (Mabuyidae) and *Takydromus septentrionalis* (Lacertidae), for instance, showed that locomotor performance was almost unaffected until at least 66% and 71% of tail was lost, respectively (Lin & Ji, 2005; Cooper & Smith, 2009). If the economy of autotomy hypothesis also applies to scorpions, it may be the case that the average mass of the lost "tail" in males (24%) and females (20%) is not enough to impose detectable short-term effects on their locomotor performance. 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 segments III-IV may later induce autotomy between segments II-III to eliminate faeces accumulated in 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.

4.2 Long-term effects on locomotor performance

In the long term, both the mean and maximum running speeds decreased over time for intact and autotomized individuals, which clearly refutes the balance loss hypothesis (Table 1). This pattern can be partially explained by the long duration of the experiment. In the laboratory, the metabolic rate of the scorpion *Uroplectes carinatus* (Buthidae) decreased by 38% over a period of 22 days, although the body mass of the individuals remained constant (Terblanche, Janion, & Chown, 2007). Thus, laboratory acclimation decreases metabolic rates in scorpions, which may have negative effects on their locomotor performance. However, the results obtained for the normally fed individuals showed that the negative effect on the mean and maximum running speeds was more pronounced when individuals (especially males) were autotomized. This pattern supports a prediction of the weight loss hypothesis because autotomized individuals are unable to defecate, thus accumulating faeces after “tail” loss; therefore, their locomotor performance should indeed be more negatively affected than that of intact individuals (Table 1).

At the end of the long-term experiment, the mean running speed of intact males was higher than that of autotomized males. For females, however, there was no difference in running speed between intact and autotomized individuals. Regarding the maximum running speed, the reduction over time was higher for autotomized individuals than for intact individuals of both sexes. The first general implication of these findings is that different proxies of locomotor performance lead to different results of the effects of autotomy. Previous work with lizards and arthropods reached a similar conclusion (e.g., Chapple & Swain, 2002; Apontes & Brown, 2005), but most experimental studies on the effects of autotomy use one single proxy of locomotor performance. Our findings on "tail" autotomy in scorpions also show that the effects of autotomy are sex-dependent, a pattern already reported for tail loss in lizards and leg loss in spiders (e.g., Chapple & Swain, 2002; Brown & Formanowicz, 2012). Although the weight loss hypothesis predicts that the negative effect of autotomy and that subsequent constipation should be more marked in females (Table 1), we found that their mean running speed was not affected by autotomy, and the reduction in the maximum running speed was similar for autotomized individuals of both sexes. In the long term, therefore, "tail" autotomy seems to be more detrimental to the locomotor performance of males. Given that females of *A. balzani* live considerably longer than males after autotomy (S. García-Hernández, pers. obs.), we suggest that the locomotor performance of autotomized males is affected by a combination of constipation and ageing. Males of the spider *Pirata sedentarius* have a shorter lifespan than females and are also more affected by autotomy (Apontes & Brown, 2005). As suggested here, the authors propose that reductions in the locomotor performance of males result from a combined effect of autotomy and ageing.

Regarding the effect of diet, the mean running speed of intact and over-fed females along the racetrack decreased more than that of intact and normally fed females. This is an unexpected finding because intact individuals are not constipated, and their locomotor performance should be less affected by diet than that of autotomized individuals. Regardless of the mechanisms that may have caused this pattern, the predictions of the weight loss hypothesis are refuted because autotomized over-fed females (the most constipated) did not show the greatest reduction in mean running speed along the racetrack. For both intact and autotomized males, diet had no effect on the mean running speed, which also refutes the predictions of the weight loss hypothesis. Because constipation is a particular side-effect of autotomy in scorpions, it is difficult to compare our results with those obtained for other animals that automize limbs. However, the locomotor performance of spiders may be negatively affected by an increase in body mass due to over-feeding. For sit-and-wait foragers, body mass increases by 22-34% after a large meal, promoting a decrease in sprint speed (Pruitt, 2010). For active foragers, body mass increases by only 5-6%, and there is no change in sprint speed (Pruitt, 2010). In the scorpion *A. balzani*, the body mass of normally and over-fed autotomized individuals increased by 8-11% and 27-30%, respectively, during the long-term experiment (Supplementary Material S9). Although the increase in body mass of over-fed autotomized individuals was higher than that of normally fed autotomized individuals, the percentage mass increase is comparable to that of sit-and-wait spiders, and the mean running speed of autotomized individuals was similar between diet treatments. Considering that diet also had no effect on maximum running speed, we argue that locomotor performance is not affected by over-feeding in scorpions, even when their "tail" is autotomized.

5 Conclusions

The main conclusion of the short-term experiment is that recently autotomized and unloaded individuals are not slower than intact and unloaded individuals; thus, their chances of escaping subsequent predatory attacks are probably not reduced. Among arachnids, leg loss usually imposes costs in terms of locomotor performance, so autotomized individuals are more susceptible to subsequent predatory attacks (Fleming, Muller, & Bateman, 2007). A similar pattern has also been reported for tail loss in lizards, in which autotomized individuals show a decline in their antipredator defences and are more exposed to predation (Bateman & Fleming, 2009). This study is the first to investigate the costs of "tail" loss in scorpions, and based on our results, we predict that predation pressure on autotomized and unloaded individuals under field conditions should be similar to that of intact and unloaded individuals in the short term. However, for females carrying a load (such as nymphs), autotomy has a short-term negative effect on locomotor performance, making them more vulnerable to predation. This vulnerability may induce behavioural changes to avoid encounters with predators, including a reduction in the movement rate, a decreased home range, and more time spent foraging close to shelter, as occurs with lizards (Cooper *et al.*, 1990).

In the long term, there is a reduction in the locomotor performance of both sexes, but the negative effects of autotomy are more pronounced in males than in females. The locomotor performance affects several fitness components, including the ability to escape from predators (Foster *et al.*, 2016), foraging activities (Higham, 2007), and the intensity of mate searching (Kasumovic & Seebacher, 2018). Thus, if our results apply to field conditions, we expect that autotomized males would be more vulnerable to predation over time. Moreover, reduced locomotor performance may also decrease the mating success of male scorpions, which target receptive females during the breeding season through typical scramble competition polygyny (Polis & Sissom, 1990). However, given that death caused by constipation may take several months, autotomized males may have enough time to find a mating partner and females may have enough time to give birth to their offspring. Thus, the long lag time between "tail" loss and death by constipation is probably a key factor in understanding the evolution of one of the most extreme cases of autotomy in nature.

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AUTHORS´ CONTRIBUTIONS

SGH and GM conceived the study and designed the experiments. SGH collected and analysed the data. SGH and GM wrote the manuscript. Both authors are accountable for the content and approved the final version of the manuscript.

DATA ACCESSIBILITY

Data will be available from the Dryad Digital Repository.

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Supplementary Material

A. The locomotor performance of intact individuals

Video S1. Intact males of scorpion *Ananteris balzani* running in the field and during the experiment. Link: https://youtu.be/zRTCw3oRxzQ

B. Original locomotor performance

Statistical analyses

To test the assumption that the original locomotor performance did not differ between individuals that would be later allocated to the two experimental groups (intact and autotomized) in the short-term experiment, we adjusted linear mixed-effect models (LMMs) for the mean and maximum running speed of the individuals (response variables). In both models, we adjusted the additive effect of sex (males and females) and experimental group, both of them included as categorical predictor variables (factors). For the model of mean running speed, we also included the 10 cm intervals along the racetrack (i.e., distance) as a continuous predictor variable. Body length and body mass were not included as predictor variables because they are both highly correlated with sex (Table S1; Fig. S2). Moreover, we used the following random factors (as crossed effects): date of the trial, trial order (first to fourth), and individual identity. However, the explained residual variance of the date of the trial and the trial order was near zero, and 10 times lower than the residual variance explained by individual identity. Thus, to simplify the model, we used only individual identity as random effect. This same random factor was also used in the model on the maximum running speed.

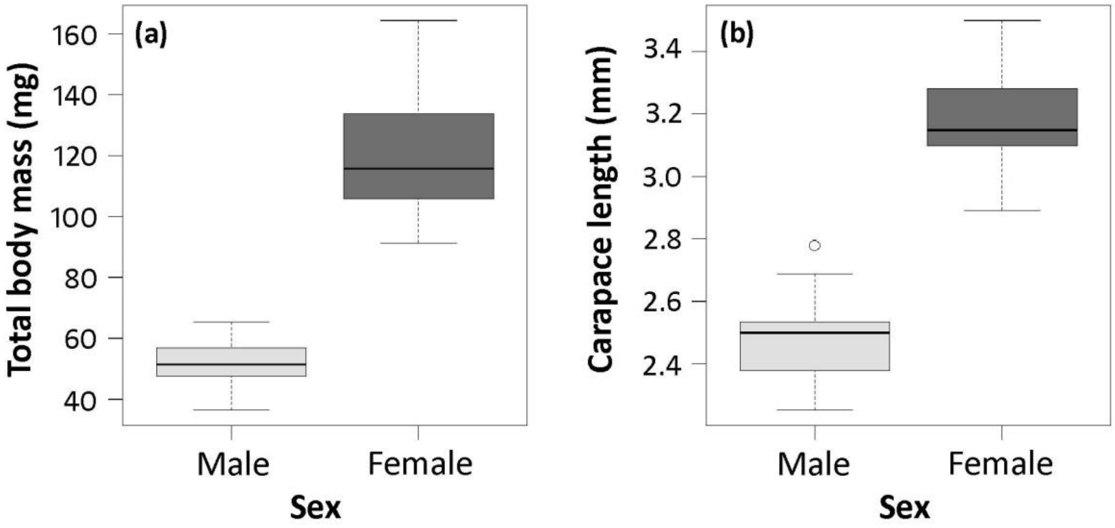


Figure S1. Sexual differences between body mass and body size of the scorpion *Ananteris balzani*. (a) Total body mass of intact males (*N* = 27) and females (*N* = 21). (b) Carapace length of intact males (*N* = 27) and females (*N* = 21). Horizontal lines represent the median, the upper and lower limits of the box represent the third and first quartile (75th and 25th percentile), respectively, vertical lines represent the range, and the circle represents an outlier (i.e., any data beyond 1.5 times the interquartile range).

After a visual inspection of the residuals, we corroborated a normal distribution of the data. We also checked and controlled for temporal autocorrelation and heterocedasticity of the data using the function *weights* of the *nlme* package (Pinheiro *et al*. 2019). Based on each model, we estimated the marginal means (EMMs), which are not biased by imbalances of sample sizes. For the mean running speed model, we estimated the slope of the linear regression for each combination between the levels of the factors. We also computed *mvt* contrasts among the estimates using the *emmeans* and *emtrends* functions of the package *emmeans* (Lenth 2016, 2018). These functions show where the statistical differences are when the estimate of each combination of the levels of the factors are compared with one another.

A significant effect of sex indicates that the original locomotor performance of males and females was different. In turn, a significant effect of experimental group indicates that the original locomotor performance of the individuals that would be later allocated to the intact and autotomized groups had unexpected and undesired differences.

Table S1. Results of two linear regressions between sex and total body mass, and sex and carapace length of individuals of *Ananteris balzani*. SE = Standard Error.

Trait	Estimate ± SE		Correlation
	Male	**Female**	
Total body mass (mg)	51.73 ± 2.78	119.61 ± 3.15	$F_{1, 46} = 261.2$, $P < 0.001$, $R = 0.85$
Carapace length (mm)	2.47 ± 0.03	3.18 ± 0.03	$F_{1, 46} = 341.5$, $P < 0.001$, $R = 0.88$

Results

The mean running speed of males and females decreased -1.55 ± 0.03 cm/s along the racetrack (df = 2908, $t = -61.41$, P <0.000; Fig. S3 and Table S2). The mean running speed of the females was 1.62 ± 0.52 cm/s faster than males (df = 110, $t = 3.08$, $P = 0.003$). Thus, there was a difference in the original mean running speed of males and females, indicating that the categorical variable “sex” should be included in all analyses presented in the main text. However, there was no difference in the original mean running speed between individuals that would be later allocated to the intact and autotomized groups (df = 110, $t = -0.08$, $P = 0.936$; Table S2). Thus, there was initial difference between experimental groups, indicating that the original mean running speed should not be included in the analyses.

In 93.7% of the trials the maximum running speed coincided with the first or second 10 cm intervals of the racetrack. Females had a higher maximum running speed than males (df = 110, $t = 2.57$, $P = 0.012$; Table S2). Thus, there was a difference in the original maximum running speed of males and females, indicating that the categorical variable “sex” should be included in all analyses presented in the main text. However, there was no difference in the original maximum running speed between individuals that would be later allocated to the intact and autotomized groups (df= 110, $t = 0.12$, $P = 0.902$; Table S2). Thus, there was no unexpected and undesired initial difference between experimental groups, indicating that the original maximum running speed should not be included in the analyses presented in the main text.

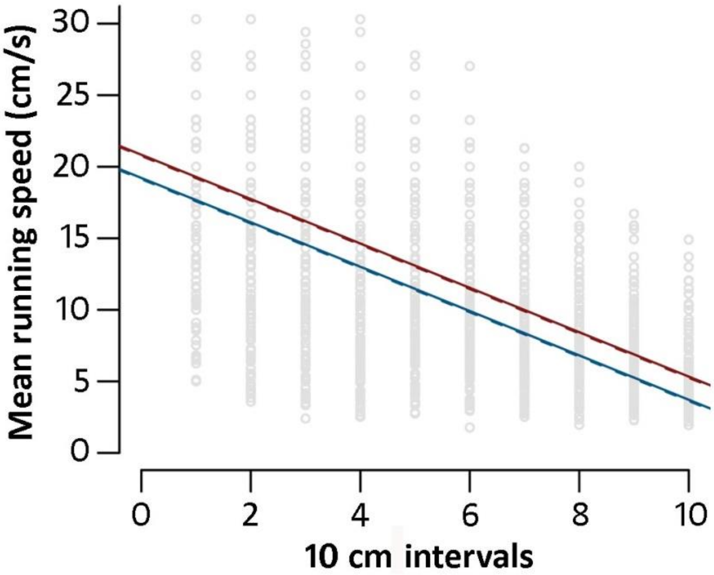


Figure S2. Mean running speed of intact individuals of the scorpion *Ananteris balzani* along the 1 m long racetrack. The longer the distance, the lower the mean running speed estimated in each 10 cm intervals. Lines indicate the adjustments estimated by the model for males (blue) and females (red) that would be later allocated to one of two experimental groups: intact (continuous line) and autotomized (dashed line). The lines of the two groups are overlapped for both males and females because there is no significant difference between them before experimental manipulation (see estimates in Table S2).

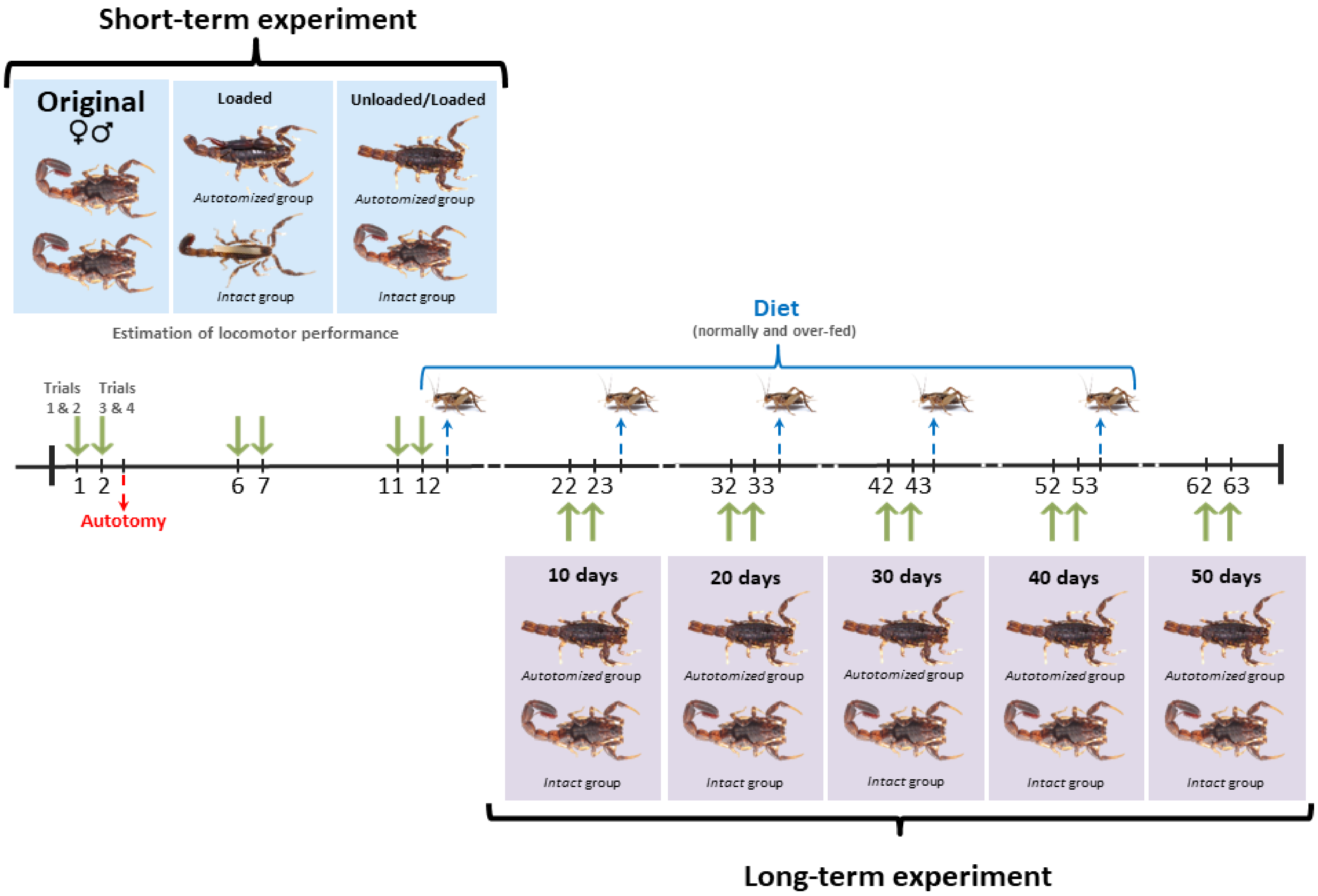
Table S2. Results of the models adjusted to test the assumption that the original locomotor performance did not differ between individual scorpions that would be later allocated to the two experimental groups (intact and autotomized) in the short-term experiment. For the mean running speed, we estimated the intercept and slope of the linear regression for each combination of the levels of the predictor categorical variables (sex and experimental group), showing how the mean running speed changes along the racetrack. For the maximum running speed, we estimated the marginal means (EMMs) for each combination of the levels of the predictor categorical variables. For all estimates, we provide the standard error and the 95% confidence intervals (in parentheses) following the *mvt* method; combinations of factors sharing the same letter in the column 'Contrast' are not significantly different (α = 0.05, *mvt*-adjusted). df = degree of freedom.

Factors	Mean running speed						Maximum running speed		
	Estimate (Intercept)	df	Constrast	Estimate (Slope)	df	Constrast	Estimate (EMMs)	df	Constrast
Females									
Intact	20.82 ± 0.60 (19.34, 22.31)	112	bd	-1.55 ± 0.03 (-1.60, -1.50)	2908	a	20.54 ± 0.75 (18.66, 22.42)	110	cd
Autotomized	20.78 ± 0.52 (19.48, 22.09)	110	cd	-1.55 ± 0.03 (-1.60, -1.50)	2908	a	20.62 ± 0.64 (19.03, 22.21)	110	bd
Males									
Intact	19.21 ± 0.50 (17.96, 20.45)	110	ac	-1.55 ± 0.03 (-1.60, -1.50)	2908	a	18.67 ± 0.60 (17.17, 20.18)	112	ab
Autotomized	19.17 ± 0.46 (18.03, 20.30)	110	ab	-1.55 ± 0.03 (-1.60, -1.50)	2908	a	18.76 ± 0.51 (17.50, 20.02)	110	ac

C. Scheme of the experimental setup

During the short-term experiment, we conducted three estimations of locomotor performance (days 1-2, 6-7, and 11-12). Each estimation was conducted in two consecutive days (two trials/day to complete the four trials), which are indicated with green arrows in Fig. S3. First, we estimated the original locomotor performance of all individuals, i.e. the mean and maximum running speed before the experimental manipulation (days 1-2). Then we split the individuals into two experimental groups: intact and autotomized (red dashed arrow in Fig. S3). It was given three days for the autotomized individuals to complete the healing process. After that, we performed other two estimations of locomotor performance. In one of them, half of the individuals of both experimental groups (intact and autotomized) were carrying a load and the other half was unloaded (days 6-7). In the other, the individuals that were previous carrying a load were tested unloaded, whereas the individuals that were previous unloaded were tested carrying a load (days 11-12). During the 12 days of the short-term experiment, the individuals were not fed to avoid marked changes in their body mass. On day 13, we begun the long-term experiment, which was performed with all individuals unloaded. For the long-term experiment, we split the individuals of both experimental groups (intact and autotomized) into two diet treatments: normally fed, which received 10% of their body mass in the form of small cricket nymphs every 10 days since day 14, and over-fed, which received 20% of their body mass in the form of small cricket nymphs every 10 days since day 14 (blue dashed lines in Fig. S3, corresponding to days 14, 24, 34, and 54). During the long-term experiment, we performed five estimations of locomotor performance: 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 estimation was conducted in two consecutive days (two trials/day to complete the four trials), which are indicated with green arrows in Fig. S3. The experiment ended on day 64 (Fig. S3).

Figure S3. Scheme of the experimental setup.



D. Complementary results of the short- and long-term experiments

Table S3. Results of the model adjusted for the short-term effect on the mean running speed. The predictor variables are: Group = experimental group (intact and autotomized); Sex = male and female; Distance = 10 cm intervals along the racetrack; and Burden = burden treatment (load and unload). numDF = the numerator degrees of freedom, denDF = the denominator degrees of freedom. Bold p-values denote statistical significance at the P < 0.05 level.

Predictor variables	**numDF**	**denDF**	**F-value**	**p-value**
Intercept	1	1546	114.77	**<0.001**
Group	1	100	3.51	0.064
Sex	1	100	0.37	0.544
Distance	1	1546	1671.91	**<0.001**
Burden	1	1546	37.07	**<0.001**
Group:Sex	1	100	0.03	0.863
Group:Distance	1	1546	7.89	**0.005**
Sex:Distance	1	1546	5.79	**0.016**
Group:Burden	1	1546	0.50	0.481
Sex:Burden	1	1546	2.70	0.101
Distance:Burden	1	1546	0.14	0.706
Group:Sex:Distance	1	1546	0.25	0.620
Group:Sex:Burden	1	1546	0.09	0.765
Group:Distance:Burden	1	1546	1.16	0.283
Sex:Distance:Burden	1	1546	0.47	0.492
Group:Sex:Distance:Burden	1	1546	4.34	**0.037**

Table S4. Results of the model adjusted for short-term effect on the maximum running speed. The predictor variables are: Group = experimental group (intact and autotomized); Sex = male and female; and Burden = burden treatment (load and unload). numDF = the numerator degrees of freedom, denDF = the denominator degrees of freedom. Bold p-values denote statistical significance at the P < 0.05 level.

Predictor variables	**numDF**	**denDF**	**F-value**	**p-value**
Intercept	1	768	3705.32	**<0.001**
Group	1	109	0.97	0.328
Sex	1	109	15.62	**<0.001**
Burden	1	768	87.27	**<0.001**
Group:Sex	1	109	2.44	0.121
Group:Burden	1	768	0.01	0.923
Sex:Burden	1	768	2.04	0.154
Group:Sex:Burden	1	768	3.28	0.070

Table S5. Results of the models adjusted for long-term effect on the mean running speed for individuals in the both diet treatments (normally and over-fed). The predictor variables are: Group = experimental group (intact and autotomized); Sex = male and female; Time = beginning and end of the experiment (10 and 50 days); Distance = 10 cm intervals along the racetrack. numDF = the numerator degrees of freedom, denDF = the denominator degrees of freedom. Bold p-values denote statistical significance at the P < 0.05 level.

Predictor variables	**Normally-fed diet treatment**				**Over-fed diet treatment**			
	numDF	**denDF**	**F-value**	**P-value**	**numDF**	**denDF**	**F-value**	**P-value**
Intercept	1	1639	96.08	**<0.001**	1	1795	135.37	**<0.001**
Group	1	52	1.54	0.221	1	48	4.36	**0.042**
Sex	1	52	2.01	0.162	1	48	0.20	0.658
Time	1	1639	2.88	0.090	1	1795	5.72	**0.017**
Distance	1	1639	772.26	**<0.001**	1	1795	1148.99	**<0.001**
Group:Sex	1	52	0.14	0.715	1	48	1.35	0.250
Group:Time	1	1639	0.63	0.429	1	1795	1.07	0.301
Sex:Time	1	1639	3.56	0.059	1	1795	0.93	0.335
Group:Distance	1	1639	2.72	0.099	1	1795	0.30	0.584
Sex:Distance	1	1639	0.14	0.711	1	1795	8.23	**0.004**
Time:Distance	1	1639	32.86	**<0.001**	1	1795	10.80	**0.001**
Group:Sex:Time	1	1639	1.96	0.161	1	1795	11.67	**0.001**
Group:Sex:Distance	1	1639	0.01	0.920	1	1795	7.10	**0.008**
Group:Time:Distance	1	1639	9.46	**0.002**	1	1795	5.67	**0.017**
Sex:Time:Distance	1	1639	1.50	0.221	1	1795	0.22	0.643
Group:Sex:Time:Distance	1	1639	1.10	0.293	1	1795	2.63	0.105

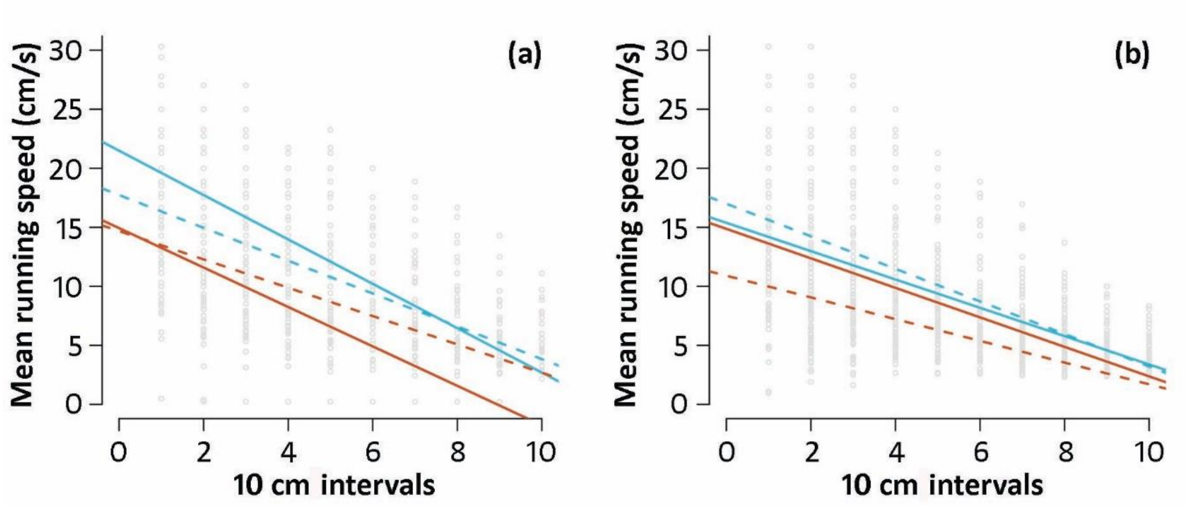


Figure S4. Long-term effects of "tail" autotomy on locomotor performance of in scorpions: (a-b) the mean running speeds of over-fed females and males, respectively. Solid and dashed lines indicate the adjustments estimated by the model for intact and autotomized individuals, respectively. Blue lines indicate data for the beginning of the long-term experiment (10 days), whereas red lines indicate data for the end of the long-term experiment (50 days).

Table S6. Results of the long-term experiment to evaluate the effect of "tail" autotomy on the mean running speed of scorpions belonging to the over-fed diet treatment. For the mean running speed, the factors included in the analyses were sex, experimental group (intact and autotomized), and time (10 and 50 days after experimental manipulation). For each combination of the levels of the factors, we estimated the intercept and slope of the linear regression showing how the mean running speed changes along the racetrack. For all estimates, we provide the standard error and the 95% confidence intervals (in parentheses) following the *mvt* method. Combinations sharing the same letter in the column 'Contrast' are not significantly different (α = 0.05, *mvt*-adjusted). df = degree of freedom.

Factors	**Intercept**			**Slope**		
	Estimate	**df**	**Constrast**	**Estimate**	**df**	**Contrast**
Females						
Intact (10 days)	21.49 ± 1.66 (16.80, 26.19)	48	c	-1.88 ± 0.17 (-2.34, -1.43)	1795	a
Intact (50 days)	14.93 ± 1.71 (10.09, 19.78)	48	ab	-1.67 ± 0.19 (-2.17, -1.16)	1795	ab
Autotomized (10 days)	17.73 ± 1.22 (14.29, 21.18)	48	bc	-1.39 ± 0.10 (-1.66, -1.13)	1795	abc
Autotomized (50 days)	14.68 ± 1.38 (10.76, 18.60)	48	ab	-1.20 ± 0.11 (-1.50, -0.90)	1795	bc
Males						
Intact (10 days)	15.38 ± 0.94 (12.72, 18.05)	51	ab	-1.20 ± 0.07 (-1.39, -1.02)	1795	bc
Intact (50 days)	14.87 ± 1.04 (11.94, 17.81)	51	ab	-1.25 ± 0.08 (-1.48, -1.02)	1795	bc
Autotomized (10 days)	17.00 ± 1.01 (14.14, 19.87)	48	bc	-1.38 ± 0.09 (-1.63, -1.13)	1795	ab
Autotomized (50 days)	10.90 ± 1.20 (7.50, 14.30)	48	a	-0.92 ± 0.13 (-1.27, -0.56)	1795	c

Table S7. Results of the model adjusted for long-term effect on the maximum running speed. Predictor variables are: Group = experimental group (intact and autotomized); Time = from 10 to 50 days; Diet = diet treatment (normally and over-fed); Sex = male and female; Time = beginning and end of the experiment (10 and 50 days); Distance = 10 cm intervals along the racetrack. numDF = the numerator degrees of freedom, denDF = the denominator degrees of freedom. Bold p-values denote statistical significance at the p < 0.05 level.

Predictor variables	**numDF**	**denDF**	**F-value**	**p-value**
Intercept	1	1709	2009.01	**<0.001**
Group	1	100	10.49	**0.002**
Time	1	1709	189.28	**<0.001**
Diet	1	100	2.05	0.156
Sex	1	100	3.33	**0.071**
Group:Time	1	1709	17.85	**<0.001**
Group:Diet	1	100	0.24	0.624
Time:Diet	1	1709	1.93	0.165
Group:Sex	1	100	0.73	0.395
Time:Sex	1	1709	1.49	0.223
Diet:Sex	1	100	0.35	0.557
Group:Time:Diet	1	1709	4.62	**0.032**
Group:Time:Sex	1	1709	4.66	**0.031**
Group:Diet:Sex	1	100	0.00	0.985
Time:Diet:Sex	1	1709	0.18	0.669
Group:Time:Diet:Sex	1	1709	8.43	**0.004**

E. Repeatability index

Statistical analyses

Considering that the locomotor performance of each individual was measured several times, we estimated adjusted repeatability (after controlling for fixed effects) of individual identity, which was our grouping factor. The repeatability index was estimated for both the mean and maximum speed measurements using the *rptR* package (Stoffel, Nakagawa & Schielzeth 2017) in RStudio (RStudio Team 2016). The estimation of the repeatability index was performed using the *lmm* method, according to which the statistical significance of the repeatability is tested by likelihood ratio tests (LRT) comparing the fit of a model including the grouping factor of interest and one excluding it. Because the LRT are conducted at the boundary of possible parameter space (against zero group-level variance), the difference in log-likelihoods is assumed to follow a mixture distribution of a χ^2-distribution with 1 degree-of-freedom. We performed the repeatability tests for the original data (i.e., before the experimental manipulation), for the short-term experiment, and for the long-term experiment.

Results

The measurements of the mean and maximum running speeds were highly consistent between individuals in all phases of the experiment (Table S8). This result indicates that there is little within-individual variation in the mean and maximum running speeds between the trials we conducted in the laboratory.

Table S8. Estimates of repeatability index for the two proxies of locomotor performance (mean and maximum running speed) in three phases of the experiment: before the experimental manipulation (“Original data”), during the short-term experiment, and during the long-term experiment. In the later phase, data are split into two groups: well-fed and over-fed individuals. The repeatability index ranges from 0 (low repeatability, high within-individual variance) to 1 (high repeatability, low within-individual variance).

Phase	**Repeatability index**	
	Estimate	***P*-value**
ORIGINAL DATA		
Mean running speed	0.49 ± 0.04 (0.41, 0.56)	D = 1410, *P* < 0.001
Maximum running speed	0.35 ± 0.05 (0.25, 0.45)	D = 57.6, *P* < 0.001
SHORT-TERM EXPERIMENT		
Mean running speed	0.36 ± 0.03 (0.29, 0.42)	D = 1520, *P* < 0.001
Maximum running speed	0.29 ± 0.04 (0.21, 0.36)	D = 126, *P* < 0.001
LONG-TERM EXPERIMENT		
Mean running speed (Well-fed group)	0.46 ± 0.05 (0.35, 0.56)	D = 561, *P* < 0.001
Mean running speed (Over-fed group)	0.37 ± 0.05 (0.27, 0.46)	D = 418, *P* < 0.001
Maximum running speed	0.40 ± 0.04 (0.32, 0.48)	D = 504, *P* < 0.001

F. Body mass analyses in the long term experiment

Body mass measurements

We measured the body mass of each individual before the trials 1 and 3 conducted to estimate the locomotor performance at the beginning (10 days) and at the end (50 days) of the long-term experiment. Based on these two measurements, we estimated the mean body mass of each individual that was used as our proxy in the following analyses.

Statistical analyses

To estimate the percentage of body mass change of the individuals between the beginning (10 days) and the end (50 days) of the long-term experiment, we adjusted linear mixed-effect models (LMMs) to the mean body mass (response variable) of each individual. In the model, we included sex (male and female), experimental group (intact and autotomized), diet treatment (normally fed and over-fed) and time (10 and 50 days) as categorical predictor variables. Moreover, we used individual identity as a random factor. Based on the model, we estimated marginal means (EMMs), which are not biased by imbalances of sample sizes, using the *emmeans* function of the package *emmeans* (Lenth 2016, 2018). Finally, we estimated the mean body mass and also the difference in body mass between 10 days and 50 days. Moreover, we calculated the corresponding percentage of change in body mass for each combination between the levels of the predictor categorical variables.

Results

In comparison to the beginning (10 days), the mean body mass of intact and normally-fed individuals at the end of the long-term experiment (50 days) changed in -4 and 5% for males and females, respectively. Additionally, for intact and over-fed males and females, the body mass changed in -1 and 10%, respectively. For autotomized and normally fed males and females, the body mass increased in 9 and 11%, respectively. However, for autotomized and over-fed individuals, the body mass increased in 27 and 30 % for males and females, respectively.

Table S9. Result of the model adjusted to estimate de mean body mass change duration the long term-experiment. For each combination of the levels of the factors (experimental group, diet treatment and sex), we estimated the mean body mass at the beginning (10 days) and at the end of the long-term experiment (50 days). Additionally, we estimated the absolute difference in mean body mass between 10 and 50 days, and also calculate the percentage (%) of body mass change at the end of the experiment for each combination of the levels of the factors. For each estimate, we provide the standard error and the 95% confidence intervals (in parentheses) following the *mvt* method. In the column 'Body mass change (%)', negative values indicate a decrease and positive values indicate an increase in the body mass after 50 days of experiment.

Factors	**Estimated body mass (g)**		**Absolute difference**	**Body mass change (%)**
	10 days	**50 days**		
INTACT				
Normally fed males	0.054±0.004 (0.046, 0.063)	0.057 ± 0.005 (0.048, 0.067)	0.003 ± 0.005	5.56
Normally fed females	0.125 ± 0.007 (0.111, 0.138)	0.120 ± 0.007 (0.106, 0.134)	0.005 ± 0.007	-4.00
Over-fed males	0.058 ± 0.004 (0.049, 0.067)	0.057 ± 0.004 (0.048, 0.066)	0.001 ± 0.005	-1.72
Over-fed females	0.121 ± 0.006 (0.108, 0.133)	0.133 ± 0.006 (0.120, 0.145)	0.012 ± 0.007	9.92
AUTOTOMIZED				
Normally fed males	0.046 ± 0.004 (0.039, 0.053)	0.050 ± 0.004 (0.041, 0.059)	0.004 ± 0.005	8.70
Normally fed females	0.107 ± 0.005 (0.097, 0.116)	0.119 ± 0.006 (0.108, 0.131)	0.013 ± 0.006	11.21
Over-fed males	0.048 ± 0.004 (0.040, 0.055)	0.061 ± 0.005 (0.052, 0.070)	0.014 ± 0.005	27.08
Over-fed females	0.110 ± 0.005 (0.100, 0.121)	0.143 ± 0.006 (0.131, 0.155)	0.032 ± 0.006	30.00

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Capítulo 2

"Tail" autotomy and consequent stinger loss decrease predation success in scorpions*

Solimary García Hernández[1] & Glauco Machado[2]

[1]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; [2]LAGE do Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav. 14, no. 321, São Paulo, SP, 05508-090, Brazil.

Author for correspondence (solimarygarcia@gmail.com)

Running header: Tail autotomy impairs predation in scorpions

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ABSTRACT

Predators need to be in good physical condition to subdue their prey. Thus, injuries that compromise the functionality of morphological traits used for hunting may reduce predation success. Autotomy can compromise morphological traits used for predation because the body part detached in response to a predatory attack may be used for hunting. Scorpions of the genus *Ananteris* show a unique form of autotomy in which individuals detach the last abdominal segments, known as "tail". We investigated how "tail" autotomy and the consequent loss of the stinger used for venom inoculation affect predation success in *A. balzani*. Using a paired experimental design, we found that for both small and large prey, the subduing success was higher when individuals were intact than when they were autotomized. The negative effect of "tail" autotomy was more pronounced when the prey was large and the scorpion was a male, which is the smaller sex. After autotomy, large prey demanded longer time to be subdued, but no sexual difference in handling time was found for both prey types. Although "tail" autotomy clearly decreases predation success, males and females were still able to capture prey (especially the small ones) after stinger loss. 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 one of the most extreme cases of autotomy in the animal kingdom.

KEYWORDS: Compensatory mechanism, Defense, Foraging success, Handling time, Prey capture, Prey profitability, Prey size, Stinger use, Subduing success.

INTRODUCTION

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 and 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 prey-predator interactions may compromise the functionality of the morphological traits used for hunting, thus reducing the predation success (reviewed in Mukherjee and 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 and 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 carnivore 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 Maginnis 2006 and Fleming et al. 2007). 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., Riechert 1988; Guffey 1999; Brueseke et al. 2001). In some crabs, individuals missing a claw tend to feed mostly on smaller items because they show a reduction in the maximum crushing force when compared with intact individuals (e.g., Smith and Hines 1991; Juanes and Smith 1995; Wasson et al. 2002; Flynn et al. 2015). 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 autotomized individuals to feed mostly on smaller prey items (e.g., Lawrence et al. 1986; Ramsay et al. 2001). Thus, limb autotomy may have many negative consequences to different phases of the foraging process, especially prey capture and manipulation.

An interesting animal group to investigate the costs of autotomy on foraging behavior are scorpions of the genus *Ananteris*, in which individuals autotomize the metasoma (Lira et al. 2014; Mattoni et al. 2015). Unlike other arachnids, such as spiders, whip-spiders, and harvestmen, in which individuals autotomize legs (reviewed in Roth and Roth 1984), scorpions autotomize 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 (Mattoni et al. 2015). In a previous study, we showed that "tail" loss in *A. balzani* has minimal short-term effects, but well-marked long-term negative effects on the locomotor performance of the individuals, especially males (Chapter 1). Here, we experimentally test how "tail" autotomy may affect the predation success of males and females in this same scorpion species to expand our understanding on the costs of this extreme form of defense.

In scorpions, the probability of using the stinger to subdue a prey seems to be related to prey size. When the prey is small, scorpions can subdue it 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 in small-bodied scorpion species with slender pedipalps (Rein 1993; Edmunds and Sibly 2010), as it is the case of all species in the genus *Ananteris* (Botero-Trujillo and Florez 2011; Figure 1). In fact, we know that autotomized individuals of *A.*

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 "tail" loss on the capture of large prey is still unknown. 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 and Florez 2011; Figure 1), the negative effects of "tail" loss on the predation success should be sex dependent. For small prey, we expect that "tail" autotomy should have similarly low negative effects on both males and females, which are likely to subdue the prey using only their slender pedipalps. For large prey, however, "tail" 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.

MATERIALS AND 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 (24º48´S, 49º13´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 two months in individual plastic containers with a piece of wet cotton to provide water and moisture, and a dry leaf that could be used as a diurnal shelter. Because many individuals collected in the field are parasitized by a nematomorph that makes them lethargic and may kill them 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 stinger in our experiment.

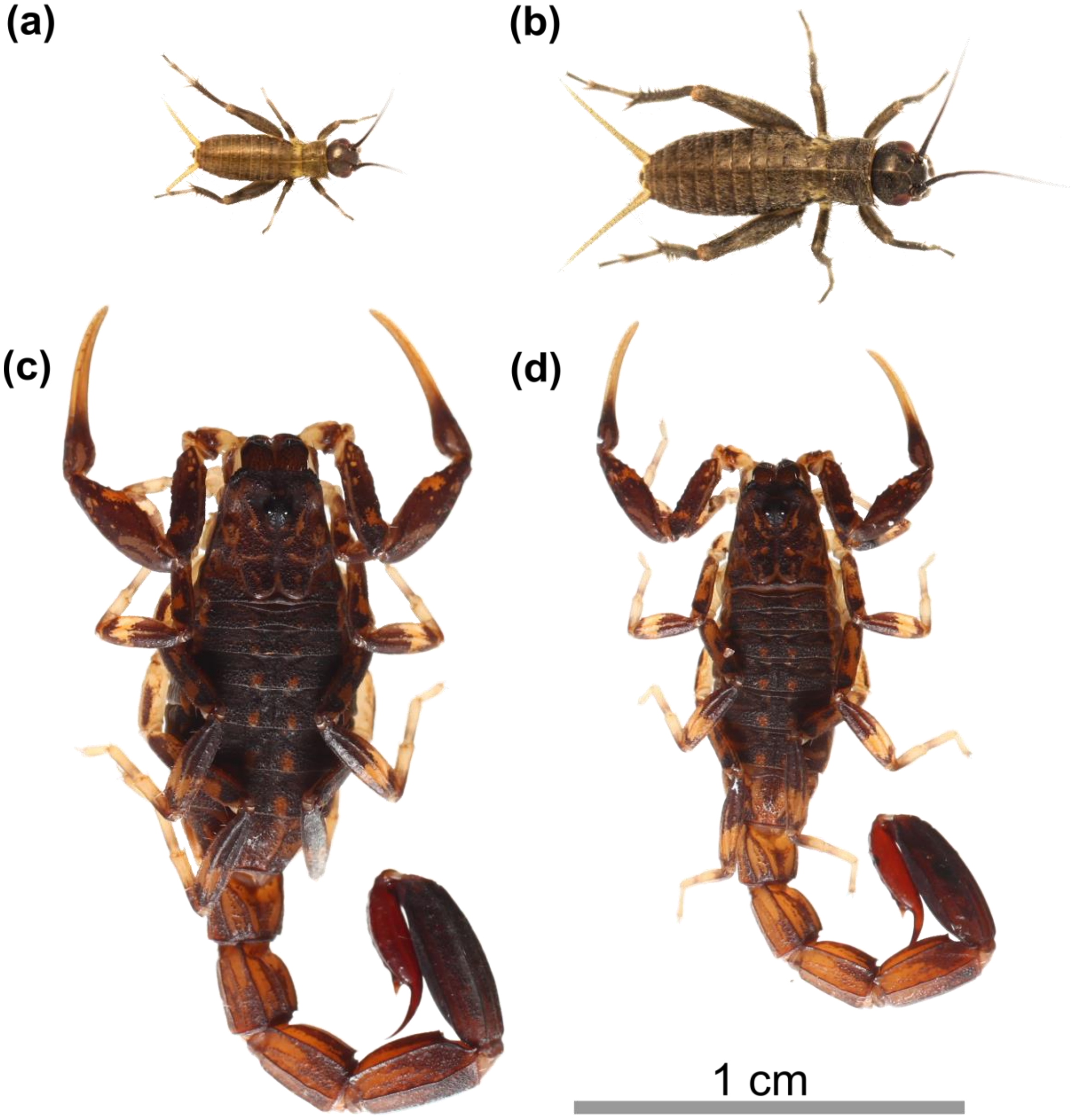

Figure 1. Nymphs of the cricket *Acheta* sp. offered as prey for individuals of the scorpion *Ananteris balzani* (photos by John Uribe). (a) Small prey, with 3-4 mm of total body length. (b) Large prey, with 7-8 mm of total body length. Dorsal view of a (c) female and a (d) male showing the well-marked sexual size dimorphism. Note that the relative size of both small and large prey is lager for males than for females.

During the quarantine, we fed the individuals every 10 days with cricket nymphs (*Acheta* sp.). Given that female body mass is two times higher than male body mass (see Figure S1 in the Chapter 1), at each feeding event males received two cricket nymphs with 3 mm of total length, whereas females received four cricket nymphs of same size. During the entire period of maintenance in the laboratory, we kept the individuals in a photoperiod of 12:12 h, 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., non-autotomized). In the post-manipulation phase of the experiment, we induced "tail" 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 pushing its last metasomal segment with the same forceps. If after 2 min the individual did not release its "tail", we repeated the protocol after 2 hours of resting. In individuals assigned to the control group, we used the same protocol to induce "tail" autotomy, but we grasped the first metasomal segment, where "tail" autotomy is impossible.

To increase the hunger level of the scorpions and their motivation to hunt, two weeks before the beginning of the experiment (i.e., the pre-manipulation phase), we fed both males and females once a week with half of the number of cricket nymphs they received during the quarantine. Thus, at the beginning of the trials, individuals had seven days of fasting. In the day before the beginning of the experiment, we placed each scorpion inside a plastic box (20 x 15 cm of base; 15 cm of height) with sand as substrate, a piece of wet cotton to provide water and moisture, and a dry leaf that could be used as diurnal shelter. The next day, we removed the wet cotton and the dry leaf 1 hour before the beginning of the trial because these objects could provide shelter for the prey we offered to the scorpions, thus interfering in the foraging process. All foraging trials were conducted at night (18:00-23:00 h) 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, and (2) large, which corresponds to crickets with 8-9 mm of total body length, and 45 mg of total body mass (Figure 1). The general morphology and behavior are similar between small and large cricket nymphs, so the main difference between them is size. The prey/predator mass ratio for small prey was 0.086 in the case of intact males and 0.038 in the case of 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 (Figure 1). The prey/predator mass ratio for large prey was 0.86 in the case of intact males and 0.38 in the case of 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 (Figure 1). For both males and females, the prey/predator mass ratio for large prey was 10 times larger than for small prey and, when compared to 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 pre-manipulation phase: one with a small prey and another with a large prey. The two trials were spaced out by a 3 hour-interval, and the order each scorpion received the small and the large prey was randomized among the individuals. We followed each interaction between the scorpion and the cricket for up to 10 min after the first physical contact between them. If the prey was not subdued within this 10 min-interval, we considered that the scorpion was unsuccessful. To avoid that the consumption of the prey affected the subsequent trial, 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 hours inside the experimental box. In the first two hours of resting, we put the wet cotton and the dry leaf back into the box to provide a water source and a shelter to 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 a prey with size different from 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 pre-manipulation phase, we fed each individual with the same amount of cricket nymphs they were receiving in the two weeks before the beginning of the experiment. The next day, we started the post-manipulation phase, when 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 enough for the autotomized individuals to complete healing of the fracture point. One week after the healing period, we estimated the predation success of the individuals using the same procedure explained above. Thus, we have repeated measures of the predation success of each individual for small and large prey in two phases (i.e., pre- and post-manipulation of its "tail").

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 the *subduing success*, i.e., whether the scorpion immobilized the prey. There are two behavioral sequences that clearly indicate that the prey has been immobilized: (a) when the scorpion releases the grip of one or both pedipalpal chelae, grasping the prey using only the chelicerae and start walking around with the prey hanging on the mouthparts, or (b) while still grasping the prey with its pedipalpal chelae, the scorpion touches the prey with the chelicerae to start feeding on it. The second proxy was the *handling time*, which was the period between the scorpion first grasped the cricket with one or both pedipalpal chelae and the moment when the prey was considered subdued. In each trial of the pre-manipulation phase in which the scorpion subdued the prey, we also recorded the *stinger use*, i.e., whether the scorpion used the stinger to inject venom and immobilize the prey. The stinger can be easily observed in the footage because the scorpion bends its "tail" forward (which is a very conspicuous behavior) and it is usually possible to see the stinger penetrating the prey's exoskeleton (Figure 2a).

Data analysis

To test the effect of "tail" autotomy on the probability of subduing a prey (i.e., subduing success), we adjusted a Bayesian generalized linear mixed model (GLMM), which is indicated to deal with datasets 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 and Hill 2006). The Bayesian approach gives estimates and credible intervals for all parameters (Gelman and Hill 2006). The response variable of our model was the 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 post-manipulation). Because we have repeated measures of the same scorpions, we used individual identity as a random effect. Moreover, due to the impossibility to perform the experiment with all individuals in the same day, we separated the trials in six temporal blocks. To control for any possible temporal difference in the behavior of the individuals, we thus included the temporal blocks as an additional random effect in the model.

To shed light on the importance of the tail on the subduing success of small and large prey by females and males, we tested if the probability of using the stinger depends on the sex of the scorpion and prey size. As in the previous analysis, we adjusted a Bayesian GLMM. The response variable was the 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 stinger) that captured prey in the pre-manipulation trials (N = 44 males and 39 females). Again, we used individual identity and temporal blocks as random effects.

To test the effect of "tail" autotomy on the handling time of captured prey, we adjusted a Bayesian GLMM. 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 blocks as random effects. The dataset used here includes only individuals that captured the prey during the entire experiment (small prey: 70 males and 75 females; large prey: 48 males and 68 females).

To each fitted model, we calculated the relevant contrasts between the levels of the fixed effects using the *emmeans* package (Lenth 2019). We expected to find lower subduing success and longer handling time for individuals of the treatment group when they were in the post-manipulation phase (i.e., autotomized) than when they were in the pre-manipulation phase (i.e., intact). The contrasts in this case were calculated as the pre-manipulation estimates minus the post-manipulation estimates and, according to our prediction, the values should be positive for the subduing success analysis and negative for the handling time analysis. Moreover, the negative effect of autotomy should be more pronounced when the prey was large than when the prey was small. These contrasts in this case were calculated as the estimate for small prey minus the estimate for large prey and, according to our prediction, the values should be positive for the subduing success analysis and negative for the handling time analysis. Moreover, we expected that the negative effect of autotomy should be more pronounced for males than for 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, the values should 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 version 3.5.2 (R Core Team 2018). The parameters of all priors used in each model are presented in Supplementary Table S1.

Ethical note

The scorpion *A. balzani* is abundant at the Santa Bárbara Ecological Station, but our experimental design allowed us to use the minimum number of individuals necessary to obtain trustful results. The experiments reported here meet the Animal Behavior Society guidelines for the ethical treatment of animals (Buchanan et al. 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 “tail” autotomy with minimum stress of the individuals. Both the collection and maintenance of the individuals in captivity were conducted with proper permits of the Brazilian Government (SISBIO/ICMBio, permit 56081).

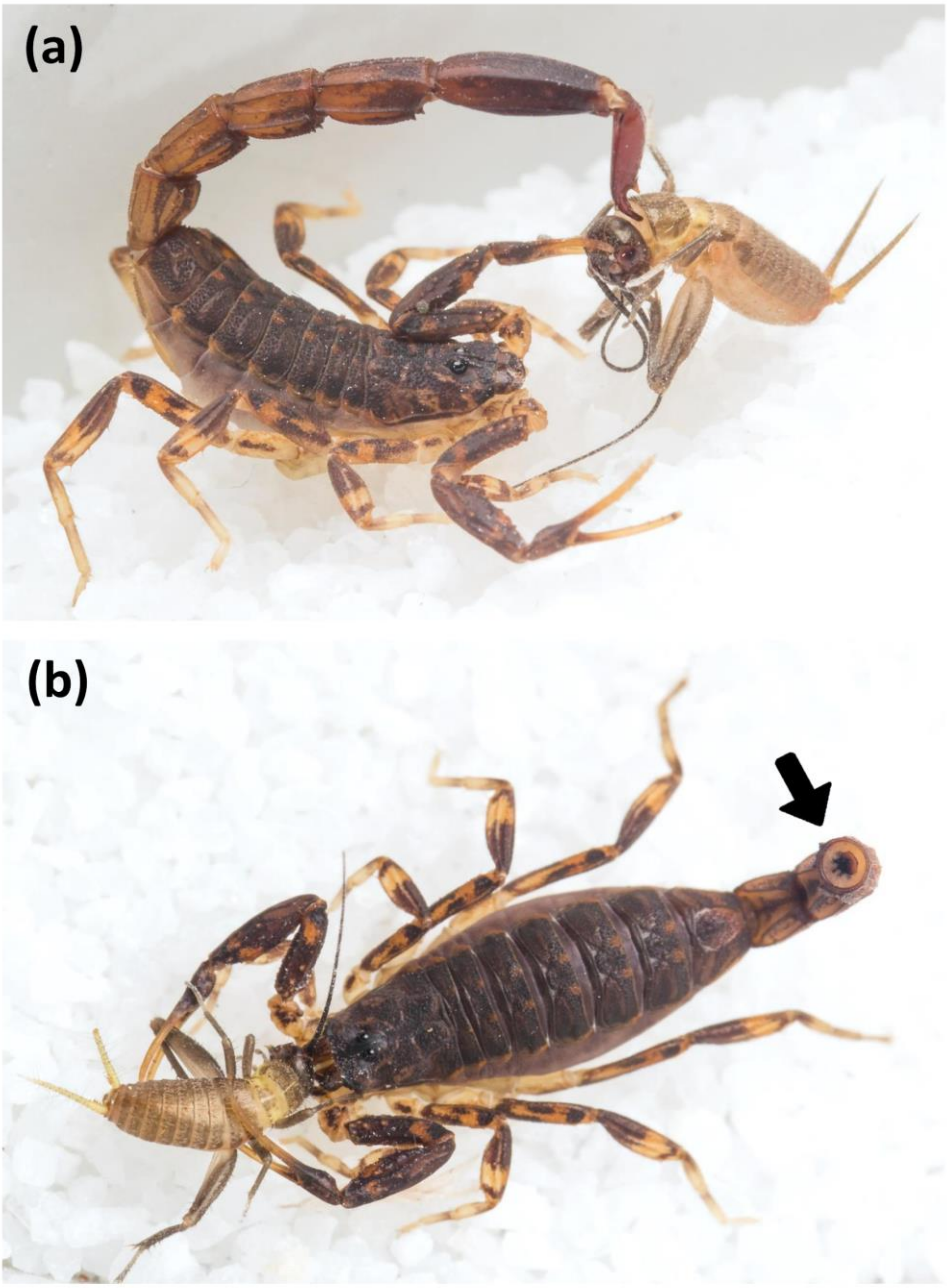

Figure 2. Adults of the scorpion *Ananteris balzani* feeding on cricket nymphs (photos by John Uribe). (a) Adult male with intact "tail" stinging a large cricket. Note that the stinger is inserted in the exoskeleton of the cricket. (b) Adult male with autotomized "tail" grasping a small cricket using 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.

RESULTS

General description of the hunting behavior

Typically, after a cricket was placed inside the plastic box, the focal scorpion started moving the pedipalps while reorienting the body and walking toward the potential prey. When the scorpion was near the cricket (ca. 1 cm), the scorpion attempted to grasp the cricket with one or both pedipalpal chelae. Once the cricket was firmly grasped, the scorpion quickly stung it (Figure 2a). In some cases, however, after touching the cricket for the first time, the scorpion was unable to grasp it, and the prey run away. In some of these occasions, the scorpion chased the cricket that escaped the first attempt of capture. The hunting behavior of autotomized scorpions was similar to that of intact individual, except that they were unable to sting the cricket. However, similar to intact scorpions, autotomized individuals bent forward the first segments of the remaining “tail”, as trying to sting the cricket with a “ghost stinger” (Figure 2b).

Subduing success

In the pre-manipulation phase, when all individuals were intact, the subduing success showed a qualitatively similar pattern between the control and treatment groups (Figure 3a and contrasts 1-4 in Figure 3b; see also Supplementary Tables S2-S3). In the post-manipulation phase, the subduing success differed between the control (i.e., intact) and treatment (i.e., autotomized) groups (Figure 3a and contrasts 5-8 in Figure 3b; see also Supplementary Tables S2-S3). As should be expected, the pattern found for intact individuals of the control group in the post-manipulation phase was qualitatively similar to that found in the pre-manipulation phase, regardless of prey size (contrasts 5-8 in Figure 3b). For autotomized individuals of the treatment group, however, the subduing success was higher when the prey was small than when it was large (contrasts 5-6 in Figure 3b). Moreover, the subduing of males was lower than that of females, regardless of prey size (contrasts 7-8 in Figure 3b).

When comparing the pre- and post-manipulation phases, the subduing success in the control group tended to be higher in the pre-manipulation phase (contrasts 9-12 in the Figure 3b). In the treatment group, the subduing success in the pre-manipulation phase (i.e., when individuals were intact) was higher than in the post-manipulation phase (i.e., when individuals were autotomized) for both small and large prey (contrasts 9-12 in Figure 3b). This difference was smaller for females when the prey was small (contrast 12 in Figure 3b). For males belonging to both experimental groups, the subduing success of small prey in the pre-manipulation phase was higher than in the post-manipulation phase (contrast 9 in Figure 3b). However, in the treatment group the magnitude of this difference was 2.6 times greater than in the control group (Supplementary Table S3), reinforcing 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 (Figure 4). When the prey was large, all males and females used their stinger in the subjugation process (Figure 4). A within-sex comparison shows that the probability of stinger use was lower when the prey was small, but this pattern was more evident for females (contrasts 1-2 in Figure 5a; see also Supplementary Tables S4-S5). A between-sex comparison shows that the probability of stinger use was higher for males than for females when the prey was small (contrast 3 in Figure 5b; see also Supplementary Table S4-S5). However, when the prey was large, there was no sexual difference in the probability of stinger use (contrast 4 in Figure 5b; see also Supplementary Table S4-S5).

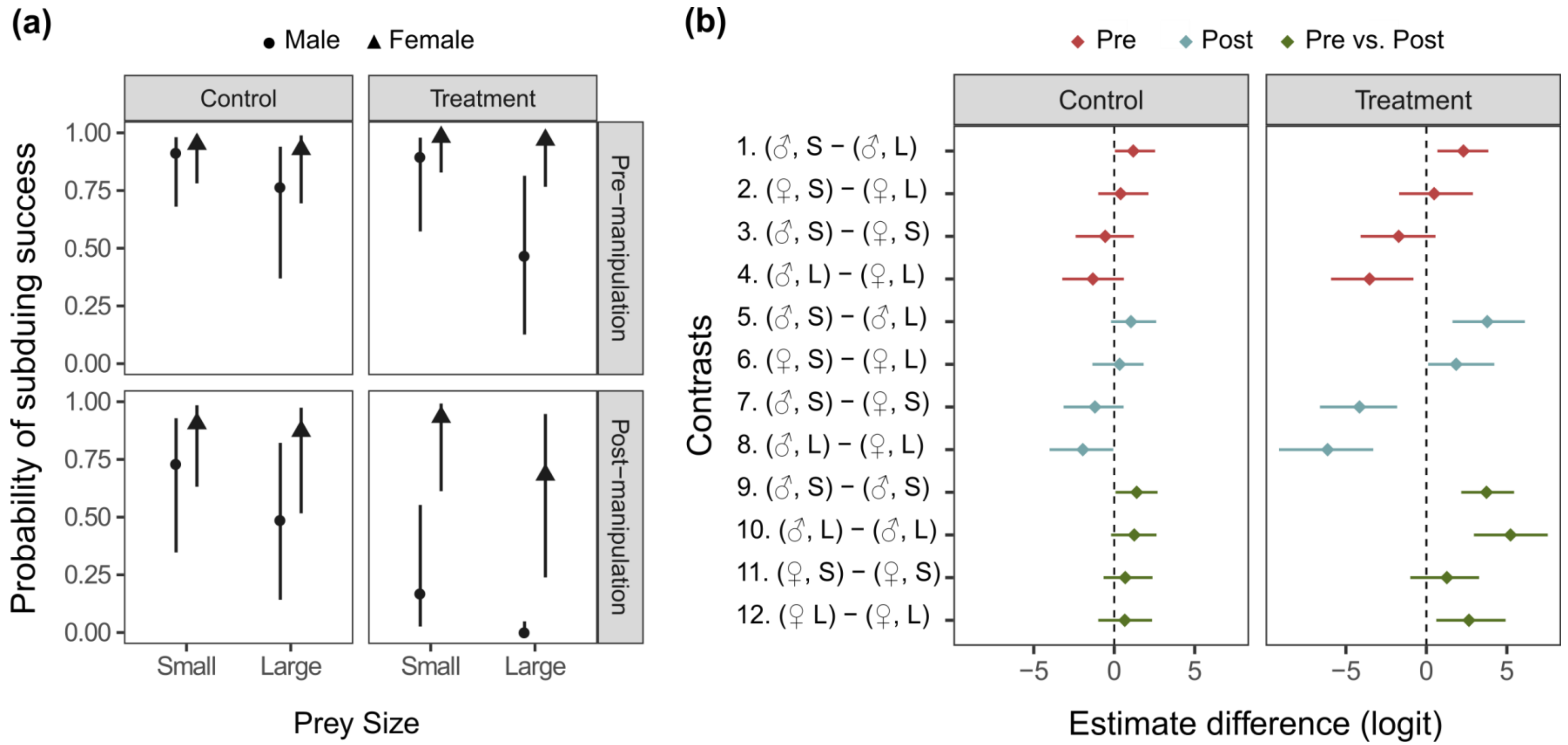

Figure 3. Effects of "tail" autotomy on the subduing success of the scorpion *Ananteris balzani.* (a) Estimated subduing success (back-transformed from logit) of males and females depending on prey size (small or large), experimental group (control or treatment), and phase of the experiment (pre- or post-manipulation of the "tail"). (b) Contrasts of the subduing success (logit scale) for combinations of sex (♂ for male and ♀ for female) and prey size (S for small or L for 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 is higher than the second term, whereas negative values indicate the contrary. 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 S3 and S6.

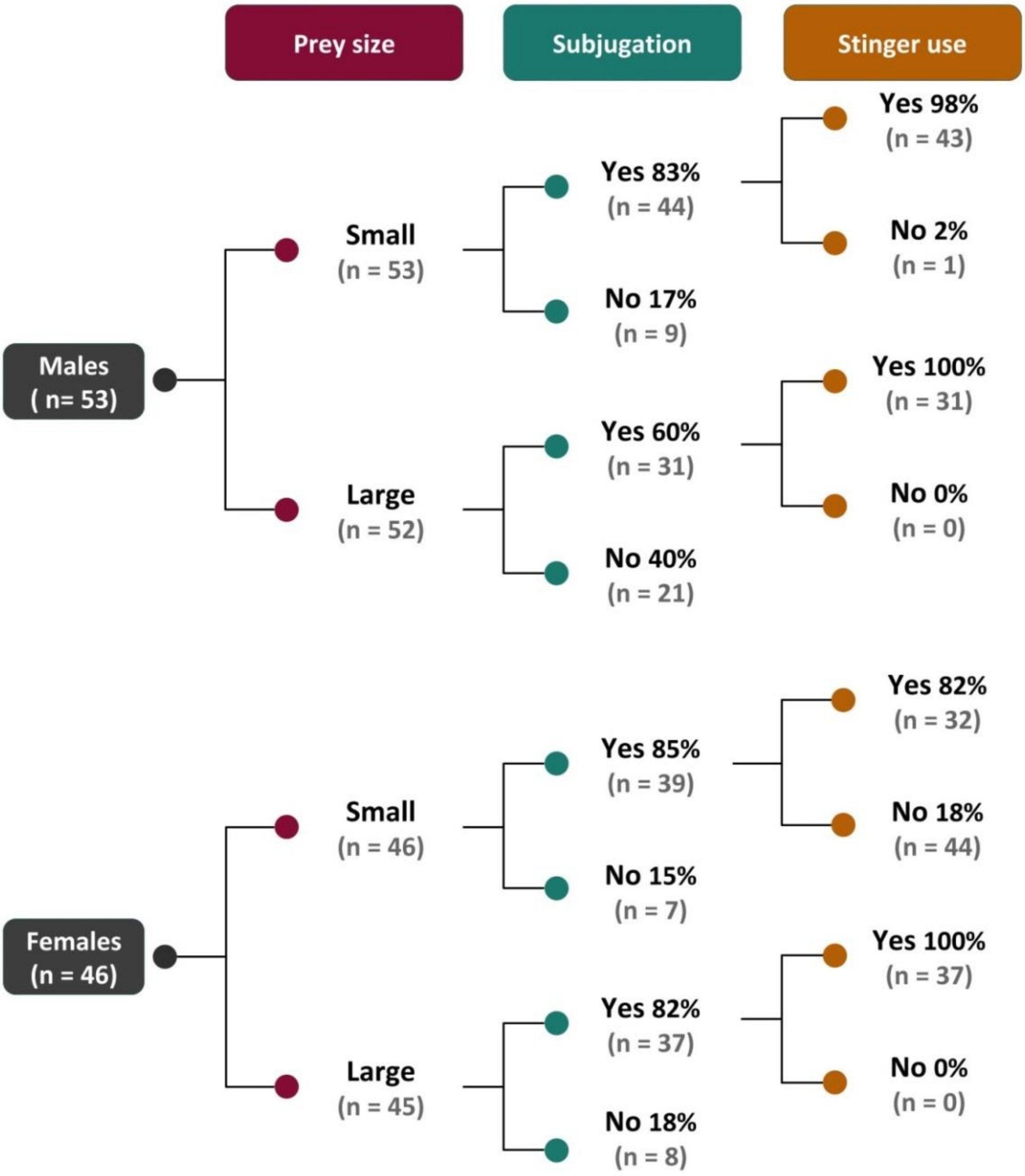

Figure 4. Scheme showing the percentage of trials in the pre-manipulation phase of the experiment in which intact individuals of the scorpion *Ananteris balzani* subdued two types of prey (small or large). The scheme also shows the percentage of trials in which the scorpions used their stinger to inject venom and immobilize the prey. Given that all scorpions were intact in the pre-manipulation phase, data from the control and treatment groups were pooled.

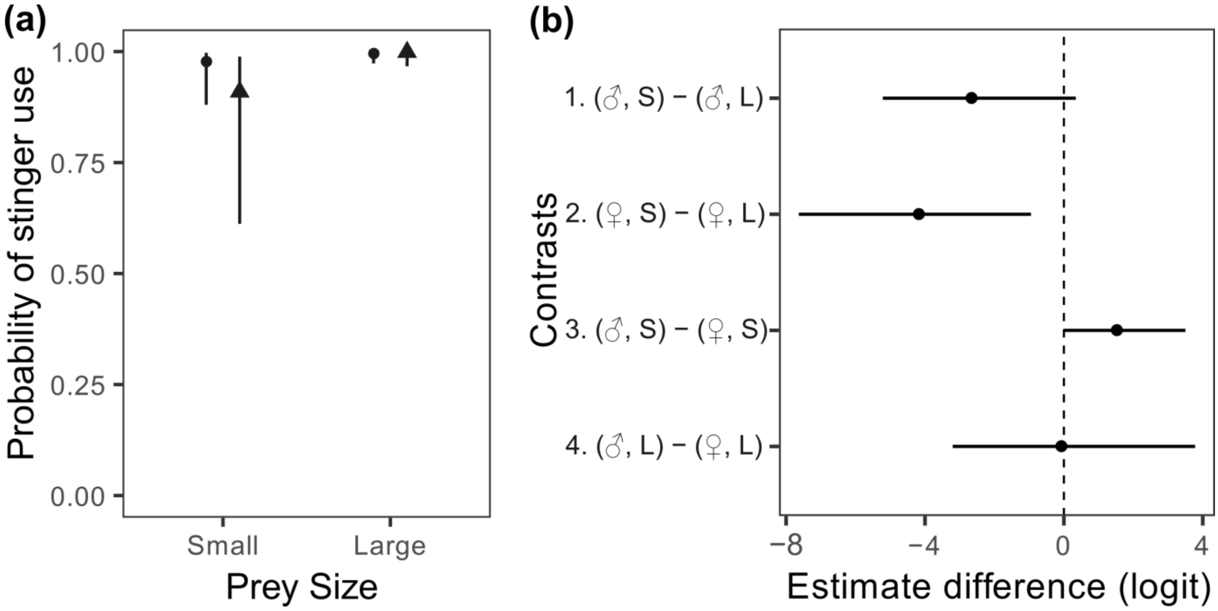

Figure 5. Stinger use for intact (i.e., non-autotomized) individuals of the scorpion of *Ananteris balzani*. (a) Estimated probability (back-transformed from logit) of stinger use for males (circle) and females (triangle) that subdued a prey (small and/or large) during the pre-manipulation phase of the experiment (see also Supplementary Table S4). (b) Contrasts of the estimated probability of stinger use (logit scale) for the relevant combinations of the levels of sex (♂ for male and ♀ for female) and prey size (S for small or L for 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 is higher than the second term, whereas negative values indicate the contrary. In both panels, bars indicate 95% confidence intervals. Values of all estimated contrasts in (b) are presented in Tables S5.

Handling time

In the pre-manipulation phase, when all individuals were intact, the handling time was similar between the control and treatment groups (Figure 6a and contrasts 1-4 in Figure 6b; see also Supplementary Tables S2-S6). In the post-manipulation phase, the handling time differed between the control (i.e., intact) and treatment (i.e., autotomized) groups (Figure 6). As should be expected, intact individuals of the control group showed the same pattern reported for the pre-manipulation phase (contrasts 5-8 in Figure 6b). For autotomized individuals of the treatment group, however, the handling time of small prey was shorter than the handling time of large prey (contrasts 5-6 in Figure 6b). Although this pattern was qualitatively similar to that reported for the pre-manipulation phase, the magnitude of the difference was 2.9 times greater for males and 3.7 times greater for females (Supplementary Table S6), 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 Figure 6b). When the prey was large, there was no sex difference in handling time (contrast 8 in Figure 6b).

When comparing the pre- and post-manipulation phases, the handling time of small prey was similar for both males and females of the control group (contrasts 9 and 11 in Figure 6b). For large prey, females of the control group showed similar handling times in the pre- and post-manipulation phases (contrast 12 in Figure 6b). For males of the control group, however, the handling time of large prey was slightly longer in the pre-manipulation phase (contrast 10 in Figure 6b). In the treatment group, the handling time of small prey in the pre-manipulation phase (i.e., when individuals were intact) was similar to that in the post-manipulation phase (i.e., when individuals were autotomized) for both males and females (contrasts 9 and 11 in Figure 6b). For both males and females, the handling time of large prey was lower when they were intact than when they were autotomized (contrasts 12-13 in Figure 6b).

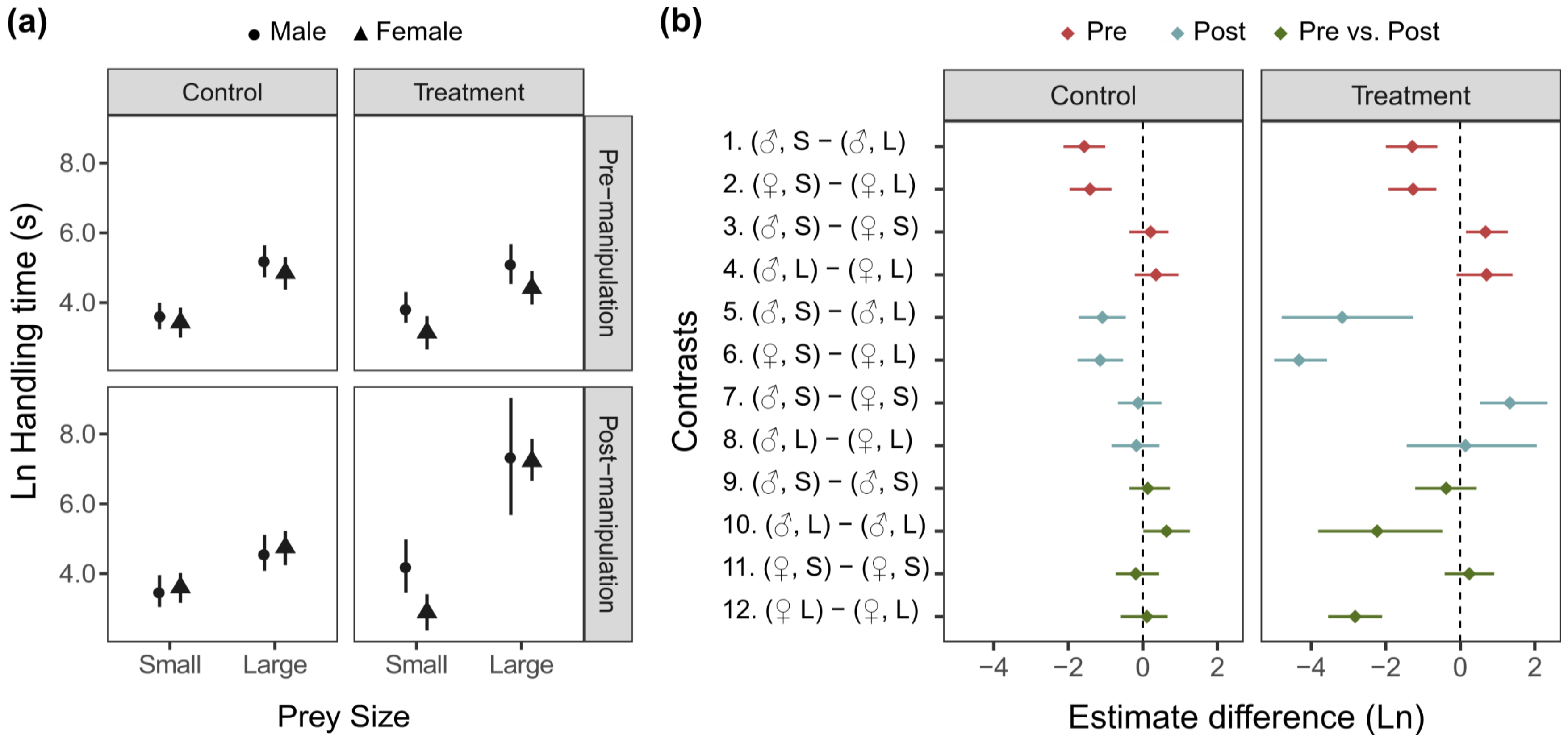

Figure 6. Effects of "tail" autotomy on the handling time of the scorpion *Ananteris balzani*. Estimated (a) handling time (ln scale) of males and females depending on prey size (small or large), experimental group (control or treatment), and phase of the experiment (pre- or post-manipulation of the "tail"). Contrasts of the (b) handling time (ln scale) for combinations of sex (♂ for male and ♀ for female) and prey size (S for small or L for 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 is higher than the second term, whereas negative values indicate the contrary. 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 S3 and S6.

DISCUSSION

We investigated how “tail” 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 by autotomized individuals. For both small and large prey, subduing success was higher when scorpions were intact than when they were autotomized. In accordance with our prediction, “tail” 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 longer 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. In what follows, we compare our results with those reported for other invertebrate species that also lose body parts used in the predation process.

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 (Rein 1993; Edmunds and Sibly 2010). 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 (Figure 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, “tail” autotomy and the consequent loss of the stinger should be more detrimental to males, regardless of prey size. Indeed, after “tail” autotomy, the subduing success of small prey by males decreased from 90% to 17%, whereas the subduing success of large prey decreased from to 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 to 97% to 70%

(Figure 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, generating a positive balance between benefits and costs. A reduction of prey size ranges after autotomy has already been recorded for crabs after the loss of a claw (e.g., Smith and Hines 1991; Juanes and Smith 1995; Wasson et al. 2002; Flynn et al. 2015) 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 on prey size range during only a small 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 "tail", and thus autotomy will entail in decreased niche breadth until the individual perishes.

Although males and females almost always used the stinger to subdue large prey (Figure 4), after autotomy some individuals, especially females, were still able to subdue large prey using only the 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 "tail" autotomy. An analogous compensatory adjustment 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., Smith and Hines 1991; Wasson et al. 2002; Flynn et al. 2015; De Oliveira et al. 2015). 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., Polis 1981; Gwynne 1987; Polis et al. 1998; Koga et al. 2001; Requena and Machado 2015). For *A. balzani* males missing a “tail”, the predation risk may be even higher because the locomotor performance of autotomized individuals decreases over time (Chapter 1). Consequently, autotomized males would face a behavioral conflict: to increase their exposure to predators or to decrease their foraging activities? This trade-off has been rarely studied in terrestrial systems (reviewed in Verdolin 2006), and *A. balzani* offers an interesting opportunity to explore it more detail under natural field conditions.

After “tail” autotomy, the handling time increased for both prey types, and this pattern was especially evident for large prey. We argue 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 large prey usually requires the injection of more venom (reviewed in Wigger et al. 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 et al. 2019). When individuals of *A. balzani* lose their “tail”, the entire process of prey subjugation is accomplished using the pedipalps, which are not so effective as the stinger in immobilizing the prey, regardless of its size. Consequently, males and females spend more time handling the prey, which probably implies a greater energy expenditure during predation (e.g., Elner and Hughes 1978; Rovero et al. 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 “tail” autotomy for the predation success of scorpions seems to rely on the subduing success, and not on the 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 et al. 1998). On one side of this curve, there are very small prey that still require a minimum handling time, and thus are of low energetic value. On the other side, there are very large prey that require extremely long handling time, and thus are also of low energetic value. In our experiment, although prey size was the same pre- and post-manipulation of the "tail", the handling time of these prey showed a marked increase after autotomy. From the females' perspective, for instance, the handling time of large prey was 17 times longer when they were autotomized. This increase of energy expenditure in subduing 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., Kessler 1971; Wise 1979; Sota 1985; Briceño 1987; Richardson and Baker 1997). 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 the pregnancy, the lower energy intake as a result of the 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 and Sissom 1990). Therefore, the negative effects of "tail" autotomy on the efficiency of predation may have important negative effects on the fitness of both males and females.

In conclusion, we found that "tail" autotomy and the consequent stinger loss decrease predation success in scorpions. Autotomized individuals had lower subduing success and higher 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 shed light on the maintenance of “tail” autotomy in scorpions, a defensive behavior that involves not only the permanent loss of an important organ used in prey subjugation, but also the posterior part of the nervous, circulatory, and digestive system, including the anus (Mattoni et al. 2015). Future research should explore how constipation and decreased locomotor performance, two additional consequences of autotomy in scorpions (Chapter 1), interact with decreased predation success to determine the survival of the individuals. Given that “tail” loss affects several aspects of the behavior, 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.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: SGH and GM; Methodology: SGH and GM; Data collection: SGH; Formal analysis: SGH; Data curation: SGH; Writing (original draft): SGH and GM; Writing (review & editing): SGH and GM; Visualization: SGH and GM; Supervision: GM; Project administration: SGH; Funding acquisition: SGH and GM.

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Data accessibility

The analyses reported in this manuscript can be reproduced using the data and the scripts that will be available at Dryad Digital Repository.

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SUPPLEMENTARY MATERIAL

1. Priors used in the Bayesian models

Table S1. Prior specification for the Bayesian generalized mixed models of subduing success, stinger use, and handling time. Each prior includes the following terms: B, which is a list of elements specifying the prior for the mean; R, which is a list of elements specifying the prior for the variance; G, which is a list of elements specifying the prior for random variables. All models ran with the following values: nitt = 10,000,000, burnin = 9,000,000, thin = 1,000.

	MODELS		
Terms	**Subduing success**	**Stinger use**	**Handling time**
B	mu = 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0	mu = 0,0,0,0	mu = 0,0,0,0,0,0,0,0,0,0,0,0.01,0,0,0,0
	V = diag (16) * (1 + $\Pi^{2/3}$)	V = diag (4) * (1 + $\Pi^{2/3}$)	V = diag (16) * (1 + $\Pi^{2/3}$)
R	V = 1	V = 1	V = 1
	fix = 1	fix = 1	fix = 1
G	G1 = list (V = 1, nu = 0.002)	G1 = list (V = 1, nu = 1,	G1 = list (V = 1, nu = 0.002)
	G2 = list (V = 1, nu = 0.002)	alpha.mu = 0, alpha.V = 1000)	G2 = list (V = 1, nu = 0.002)
		G2 = list (V = 1, nu = 1,	
		alpha.mu = 0, alpha.V = 1000)	

2. Complementary results of subduing success and handling time

Table S2. Summary of the probabilities estimated by the Bayesian generalized mixed models. We present data on the subduing success and handling time of small and large prey by individuals of the scorpion *Ananteris balzani*.

	Probability of subduing success		**Handling time (ln)**	
Trials	Estimate ± SD	95% CI	Estimate ± SD	95% CI
CONTROL				
Pre-manipulation				
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
Post-manipulation				
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				
Pre-manipulation				
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
Post-manipulation				
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 S3. Summary of the relevant contrasts of the probability of subduing success. We contrast combinations of sex (♂ for males and ♀ for females) and prey size (Small and Large), within and between the phases of the experiment (pre- and post-manipulation). In parentheses are presented the estimated difference and the 95% CI (both in logit). The direction of the difference is showed using symbols > (the contrast value is positive and the 95% CI does overlap zero), < (the contrast value is negative and the 95% CI does overlap zero), or ≈ (regardless of the contrast value, the 95% CI overlaps zero).

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

Table S4. Summary of the probability estimated by the Bayesian generalized mixed model. We present data on stinger use (back-transformed from logit) for intact individuals (♂ for males and ♀ for females) of the scorpion *Ananteris balzani* that subdued small or large prey during the pre-manipulation phase of the experiment.

Trials	**Estimate ± SD**	**95% IC**
♂, Small	0.981 ± 0.741	0.880 to 0.866
♂, Large	0.999 ± 0.878	0.973 to 1.000
♀, Small	0.910 ± 0.733	0.613 to 0.989
♀, Large	0.999 ± 0.882	0.960 to 1.000

Table S5. Summary of the relevant contrasts estimated by the model of stinger use for intact individuals (♂ for males and ♀ for females) of the scorpion *Ananteris balzani* that subdued small or large prey during the pre-manipulation phase of the experiment. Both the contrast and the 95% CI are in logit.

Contrast	**Estimate**	**Lower 95% IC**
(♂, 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

Table S6. Summary of the relevant contrasts of the probability of handling time. We contrast combinations of sex (♂ for males and ♀ for females) and prey size (small and large), within and between the phases of the experiment (pre- and post-manipulation). In parentheses are presented the estimated difference and the 95% CI (both in logit). The direction of the difference is showed using symbols > (the contrast value is positive and the 95% CI does overlap zero), < (the contrast value is negative and the 95% CI does overlap zero), or ≈ (regardless of the contrast value, the 95% CI overlaps zero).

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

Capítulo 3

Implications of nonlethal injuries on the reproductive success of male and female scorpions*

Solimary García Hernández[1] & Glauco Machado[2]

1. 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

2. LAGE do Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, trav. 14, no. 321, São Paulo, SP, 05508-090, Brazil

Corresponding author: solimarygarcia@gmail.com

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Abstract

Autotomy is a taxonomically widespread form of nonlethal injury in animals. The ability to autotomize a body part and avoid predation enhances fitness, but it may also impose survival and reproduction costs. Although survival costs are well explored, reproductive costs of autotomy are poorly known. Scorpions of the genus *Ananteris* are the only that autotomize the last abdominal segments (i.e. the "tail"). After autotomy, individuals lose part of the digestive tract, including the anus, which prevents defecation and leads to constipation because regeneration does not occur. We experimentally investigated how autotomy affects the reproductive success of males and females, and also the offspring phenotype. Because male "tail" is used during courtship, we expected that autotomy would reduce mating and sperm transfer success. For females, which give birth five months after mating, we expected that autotomy would decrease survival and fecundity due to severe constipation. Finally, we expected that constipation would also reduce offspring mass and size. We found that autotomy had no effect on the reproductive success of males. For females, autotomy imposed a great reduction in fitness (survival x fecundity). Offspring mass and size showed no difference between intact and autotomized females. Autotomy is an adaptive strategy for males because it imposes no immediate reproductive cost and provides the benefit of scaping from a predation attempt. The high cost paid by females may explain why they are more reluctant than males in autotomizing the "tail", and also why the frequency of autotomized females in the field is low.

Keywords: autotomy, *Ananteris*, constipation, courtship, fecundity, fitness, maternal effect, mating success, per-propagule investment, sperm transfer.

Introduction

Nonlethal injuries resulting from unsuccessful predation attempts occur in both plants and animals (Harris 1989). Autotomy of body parts is one of the most intensively studied form of nonlethal injury, being reported for numerous invertebrate and vertebrate taxa, such as cnidarians, flatworms, annelids, mollusks, nematodes, arthropods, echinoderms, amphibians, lizards, snakes, and mammals (Fleming et al. 2007; Emberts et al. 2019). The ability to detach a body part and avoid death imposed by predation clearly enhances fitness, but the nonlethal injury may impose costs to survival and reproduction. The costs of autotomy to survival include, for instance, reduced locomotor and/or foraging performance, reduced growth, loss of energy storage, and increased susceptibility to infections and future predation (Maginnis 2006; Fleming et al. 2007; Bateman and Fleming 2009; Emberts et al. 2019). The costs of autotomy to reproduction are little studied, but in males it may cause reductions in courtship success and resource holding potential, as well as changes in social status. In females, the costs of autotomy to reproduction involve mainly reductions in present and/or future fecundity (Bernardo and Agosta 2005). There are also largely unexplored negative impacts on the offspring of autotomized females, such as reductions in per-propagule investment, brooding behaviors, nest defense, and post-hatching provisioning, as well as changes in oviposition site selection and nest construction, exposing the offspring to predation and/or stressful abiotic conditions (Bernardo and Agosta 2005).

Empirical evidence showing that nonlethal injuries related to autotomy have negative impact on the reproductive success of males and females is scarce (Maginnis 2006; Fleming et al. 2007; Emberts et al. 2019). There are scattered studies among invertebrates, such as polychaetes (e.g., Hill et al. 1982), crustaceans (Juanes and Smith 1995), and sea stars (e.g., Pomory and Lawrence 1999), and, among vertebrates, most studies focus on salamanders and lizards (Bernardo and Agosta 2005). For males, appendage loss per se is likely to be the most relevant cost to reproduction because in many species the autotomized body part is used in female courtship (e.g., Arnold 1977; Uetz et al. 1996) and contests for mates and territories (e.g., Smith 1992; Dodson and Beck 1993). For females, in turn, the reproductive costs seem to be imposed mainly by appendage regeneration, which is an energetically expensive process (Maginnis 2006). In fact, the energy allocated to appendage regeneration may reduce both current and future fecundity, and in the most extreme cases may cause complete inhibition of reproduction (Bernardo and Agosta 2005; Maginnis 2006; Fleming et al. 2007). Finally, nonlethal injuries may also impose reproductive costs via maternal effects, defined as the impact of the parental phenotype (i.e., autotomy) on the offspring phenotype (Bernardo and Agosta 2005). Although this is certainly the most obscure cost of autotomy, there is at least one study showing that the degree of maternal injury in a salamander species has a negative relationship with per-propagule investment, so that the higher the proportion of the lost tail, the smaller the eggs produced (Bernardo and Agosta 2005).

In this study, we explore the implications of a nonlethal injury related to autotomy on the reproductive success of males and females of the scorpion *Ananteris balzani* (Buthidae). The genus *Ananteris* is the only one among scorpions in which individuals have the ability to autotomize the last abdominal segments, known as the "tail" (Lira et al. 2014; Mattoni et al. 2015). This is an extreme form of nonlethal injury because the "tail" bears the telson, which contains a pair of venom glands and the stinger organ, and parts of the nervous, circulatory, and digestive systems, including the anus (Hjelle 1990). Moreover, the "tail" does not regenerate and, given that individuals are unable to defecate for the rest of their lives, they eventually die from constipation some months after autotomy (Mattoni et al. 2015). This study system is interesting because "tail" loss alone may impair courtship behavior and mating success in males, providing a parallel with previous, non-homologous cases of tail loss in salamanders (e.g., Arnold 1977; Houck 1982). For females, we have the opportunity to explore whether the fecundity costs of autotomy also apply to a species in which the lost appendage is not regenerated.

We put forward and tested the hypothesis that "tail" autotomy in scorpions will have reproductive costs to males and females, and also to offspring via maternal effects. Given that males of *A. balzani* use the "tail" to perform a very conspicuous behavior called "tail" wagging during the courtship, and also to support the body during spermatophore transfer (see video in Supplementary Material S1), we predict that autotomized males will have long courtship dance due to high female resistance, low mating success due to failure in spermatophore deposition, and reduced sperm transfer. For females, which give birth five months after mating, we predict that severe constipation may cause high mortality before parturition and low fecundity because the accumulation of feces in the abdomen would reduce the space available to the offspring. Female autotomy and the subsequent constipation may also induce maternal effects, such as reduced per-propagule investment (i.e., smaller nymphs). Finally, pregnant females use the "tail" to construct the gestational chamber (pers. obs.), which provides a shelter for them and for the early instar nymphs. Thus, we predict that autotomized females will have difficulty to construct the gestational chamber and some of them will be simply unable to do so.

Materials and Methods

Collection and maintenance

We collected 53 adult males and 49 adult females of *A. balzani* during the austral spring from 2017-2019 in a neotropical savanna area at Santa Bárbara Ecological Station (24°48´S, 49°13´W), state of São Paulo, Brazil. The individuals were found at night along sand roads, where the substrate had scattered patches of grass, leaf litter, and stones, and also small bushes. We transported the individuals to the laboratory, where they were maintained individually in plastic containers during a quarantine period of two months. This long quarantine was necessary to identify and exclude from the experiments all individuals parasitized by nematomorphs. During the period of maintenance, the individuals were fed every 10 days with cricket nymphs (*Acheta* sp.). Given that female body mass is two times higher than male body mass (Figure S1 in Chapter 1), at each feeding event males received two cricket nymphs (3 mm of total length) whereas females received four cricket nymphs. We kept the individuals under a 12:12 h light:dark cycle and conducted the experiments in a room with minimal temperature variation (mean = 25 °C, range = 24.5 – 26.5 °C).

Experimental design I: Male reproductive success

To assess the possible implications of "tail" autotomy on the mating success of males, we first measured the prosoma length (a standard measure of body size in scorpions) of all individuals and paired males and females according to their sizes. The largest male was paired with the largest female, the second largest male was paired with the second largest females, and so on. This procedure minimized pair differences in body size and their potential confounding effects on the output of mating interactions. As the individuals were collected in different years, the size-assortative arrangement of the mating pairs was done in blocks containing at least 24 individuals each. Then, we split the males into two experimental groups: intact (n = 41) and autotomized (n = 34) individuals. We induced "tail" autotomy in males of the autotomized group by repeatedly touching their body with forceps for 1 min and then grasping and pushing their last metasomal segment with the same forceps. In males assigned to the intact group, we used the same protocol to induce "tail" autotomy, but we grasped the first metasomal segment, where "tail" autotomy is impossible. All males were allowed to rest for 10 days before mating, to complete the healing of the fracture point.

The mating trials were performed inside a glass box (20 x 10 cm of base; 15 cm of height) with sand as substrate, and two pieces of flat wooden boards and two pieces of porous tiles placed in opposite corners of the box. Preliminary trials showed that wooden boards and porous tiles provide a suitable substrate for the males to deposit their spermatophores. We wet the sand in the central part of the box to provide moisture to the individuals during the mating trial. No shelter was provided inside the box to increase the chance that the male would find the female. We placed a male inside the box, and after 30 min of acclimatization, we placed the female previously established for him. We followed and filmed each male-female sexual interaction (Figure 1a,b).

In some mating trials individuals did not engage in courtship within 1 h after the first physical contact between them. This situation usually occurs because the female is pregnant or has already received the maximum number of inseminations, i.e., three or four, depending on the species (Peretti 1993). Thus, whenever a female did not engage in courtship, we followed her during two months after the mating trial to identify signs of pregnancy, i.e., a great increase in abdominal volume or visible embryos through the ventral intersegmentar membranes of the abdomen. Moreover, within 30 days after an unsuccessful mating trial, we tried to re-mate the supposedly unreceptive females with up to two other males. We considered as truly unreceptive females all females that were pregnant or did accept any other male after the first mating trial. In these cases, we discarded the females from the sample and considered all mating trials accomplished with them as invalid. After this process of filtering, we had a dataset of 28 mating trials with intact males and 23 mating trials with autotomized males.

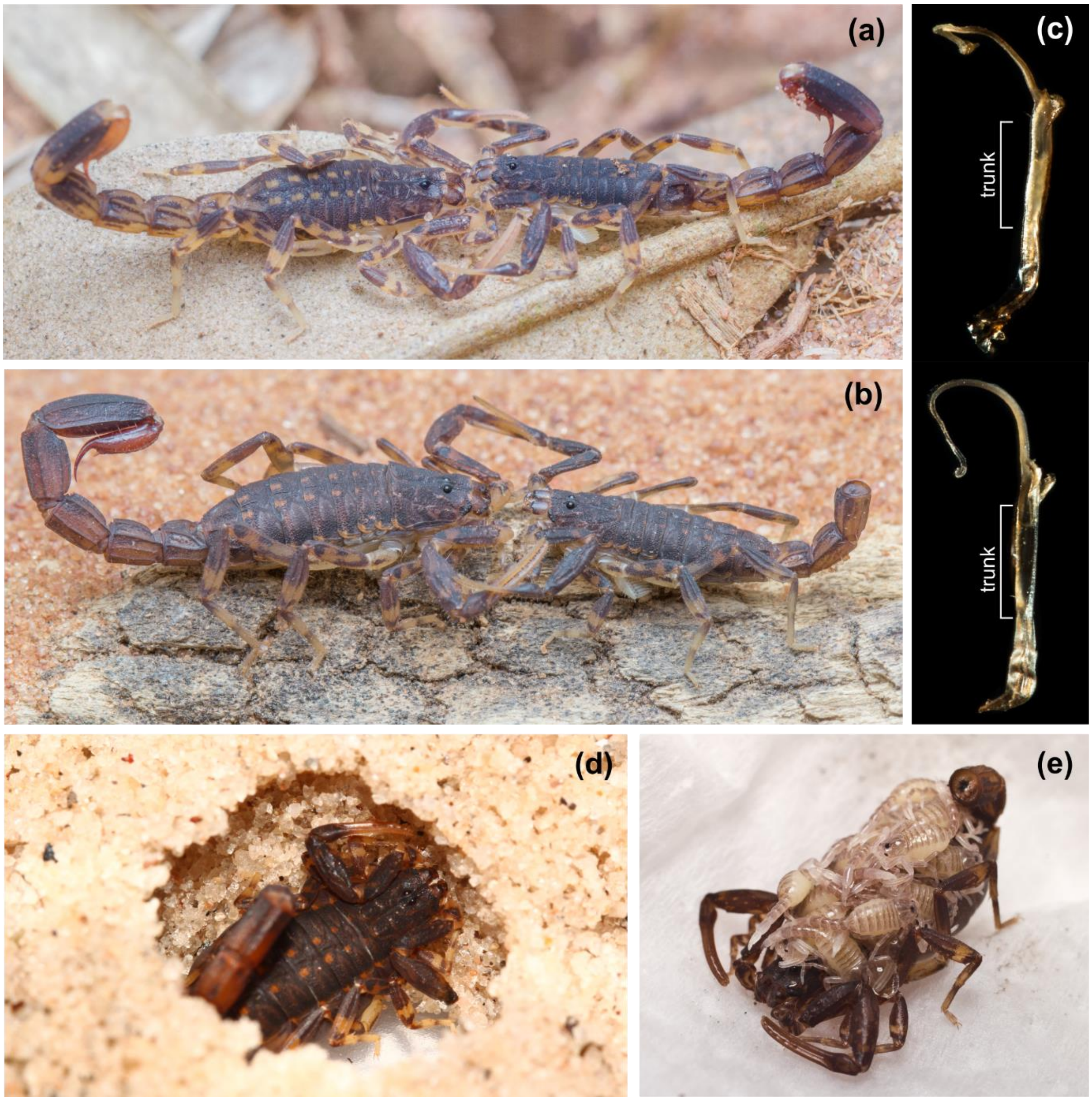

Figure 1. (a-b) Mating pair of the scorpion *Ananteris balzani*. In both photos the female is on the left side and the male is on the right side. During courtship, (a) intact and (b) autotomized males grip the female's pedipalps using their own pedipalps. Note that female coloration is clearly different in (a) and (b). The lighter the tegument, the younger the female is. (c) Two flagelliform spermatophores collected after the mating trials. When sperm transfer is incomplete, it is possible to see through the hyaline walls of the spermatophore's trunk the white mass of sperm (above). An empty spermatophore (bellow) indicates that sperm transfer was complete, and no sperm remained inside the spermatophore. (d) Intact and pregnant female inside her gestational chamber constructed in the sand substrate. The roof of the refuge (i.e., a dry leaf) was carefully removed to show the internal space. (e) An autotomized female with first instar nymphs on her dorsum. Photos: John Uribe.

All mating trials were conducted during the dark phase of the light cycle, under dim red illumination, which does not disturb scorpions (Machan 1968). We estimated male mating success using three proxies. (1) *Spermatophore transfer success*, i.e., whether the male successfully deposited the spermatophore, which is a sclerotized, flagelliform structure that is attached by the male to a solid substrate at the end of the courtship (Figure 1c). Considering only males that successfully transferred their spermatophore, we estimated (2) *sperm transfer success*, i.e., whether the male transferred to the female all sperm contained in his spermatophore. Because the walls of the spermatophore are transparent in *A. balzani*, we used a stereomicroscope to score whether or not some white sperm remained inside the spermatophore (Figure 1c). Spermatophores containing any visible amount of remaining sperm were scored as incomplete transference. Finally, based on the footage, we estimated (3) *courtship duration* of all males that successfully transferred the spermatophore. Courtship duration was the period between pedipalpal grip (i.e., the beginning of the courtship; Figure 1a,b) and the moment when both individuals retracted their pedipalps and separated their bodies after sperm transference (see video in Supplementary Material S1).

Experimental design II: Female reproductive success and maternal effects

To assess the possible implications of "tail" autotomy on the reproductive success of females, we used females from the previous experiment that successfully received a spermatophore during courtship. To increase the sample size, we included 9 additional females that were not used in the previous experiment, but that were paired in the laboratory following the same protocol described above. Because the females used in our experiments were collected in the field, we have no control on their previous mating and pregnancy history. However, we reared some females in the laboratory, from the last nymphal stages until their death. We took periodic photos of these females after they reached adulthood and detected that the tegument coloration of young adult females is clearly lighter than that of older adult females (Figure 1a,b). Therefore, using tegument coloration we divided the experimental females into two age categories: young (*n* = 10), which may have mated in the field but are unlikely to have produced a first litter, and old (*n* = 40), which almost certainly have already mated and produced a first litter in the field. Very old female, i.e., those showing the darkest tegument coloration and damages in the stinger, pedipalps, or pecten, were not included in the experiment.

To avoid any bias related to female mating or pregnancy history, we equally split young and old adult females into two experimental groups: intact (*n* = 25) and autotomized groups (*n* = 25). By using this procedure, we ensured that a similar number of females in each experimental group would give birth to the first or to the second litter. To induce "tail" autotomy we followed the same protocol described in the previous experiment for males. All females were maintained individually in plastic containers with sand as substrate (2 cm depth), a wet piece of cotton to provide moisture, and a dry leave that could be used as shelter. The sand substrate allowed females to build the underground gestation chamber (Figure 1d). During all pregnancy period, females of both experimental groups were feed with four cricket nymphs (3 mm of total length) every 10 days.

We inspected each female at 3 day-intervals during the entire period of experiment. For each female, we considered that the experiment ended when she died or when the second instar nymphs dispersed from her dorsum and she abandoned the gestational chamber. We estimated female reproductive success using three proxies: (1) *survival until offspring birth*, i.e., whether the female remained alive until giving birth; (2) *offspring number*, which is the number of nymphs produced by each female (Figure 1e); and (3) *female fitness*, given by the formula ([Probability of survival until offspring birth] * [Offspring number]).

Finally, we estimated maternal effects on the offspring using three proxies: (1) *construction of the gestational chamber* (Figure 1d); (2) *offspring mass*, which is the total weight of each litter when nymphs were in the second instar; and (3) *offspring size,* which is the mean cephalothorax width of all second instar nymphs of each litter. The size of the nymphs was measured from digital photos using the software *ImageJ* (Schneider et al. 2012).

Data analysis

To test the possible implications of "tail" autotomy on the reproductive success of males, we adjusted a different model to each proxy. For all models the experimental groups (intact or autotomized) were the predictor variable. For the discrete response variables *spermatophore transfer success* (no = 0 and yes = 1) and *sperm transfer success* (incomplete = 0; complete = 1), we adjusted generalized linear models (GLMs) with binomial error distribution and logit link function. For the continuous response variable *courtship duration,* we adjusted a GLM with Gaussian error distribution.

To test the possible implications of "tail" autotomy on the reproductive success of females and also on the offspring, we also adjusted a model to each proxy, and used experimental groups as predictor variable. For the discrete response variables *survival until offspring birth* (no = 0 and yes = 1) and *construction of gestational chamber* (no = 0 and yes = 1), we adjusted GLMs with binomial error distribution and logit link function. Considering that intact and autotomized females begin to construct the gestational camera, on average, 64 days after mating, we excluded from this analysis seven females that survived considerably less than this period after mating. After eliminating these females, the new dataset contained 23 intact and 19 autotomized females. For the counting response variable *offspring number,* we adjusted a GLM with Poisson error distribution to a dataset of 13 intact and 12 autotomized females that survived until giving birth. Then, we adjusted a GLM with

Gaussian error distribution for the continuous response variable *offspring mass.* Because total offspring mass may be influenced by offspring number, we included the total number of nymphs in each litter as a covariable in the model. Finally, for the continuous response variable *offspring size*, we adjusted a generalized linear mixed model (GLMM) with Gaussian error distribution and female identity as random factor because each female has multiple nymphs in her litter. Given that offspring size also may be influenced by offspring number, we included the total number of nymphs in each litter as a covariable in the model.

In the models of offspring number, offspring mass, and offspring size we did not include the experimental group of the father as a predictor variable because we had no prediction about possible paternal effects. However, we performed exploratory analyses including the experimental group of the father in the models and no effect was detected on any offspring trait we measured (Supplementary Material S2). Finally, we also did not include female body size as a covariable in the models of offspring number and offspring size because we found that female body size was similar between the experimental groups, and had no effect on offspring number and offspring size (Supplementary Material S3).

The GLM of mating time, in which we included an error structure to account for heterocedasticity using the *gls()* function, was performed in the package *nlme* (Pinheiro et al. 2019). The other GLMs were performed in the package *stats* (R Core Team, 2019). The GLMM for offspring size was performed in the package *lme4* (Bates et al. 2015). Because the results of the models adjusted with binomial and Poisson error distribution are in logit and log scale, respectively, we used the package *emmeans* (Lenth, 2019) to transform them into probabilities and number of individuals. All analyses were performed using the software R version 3.6.2. (R Core Team, 2019).

Results

Male reproductive success

Two males, one autotomized and one intact, did not engage in courtship behavior. From the males that engaged in courtship, the spermatophore transfer success of intact and autotomized males was similar (Table 1). Among the males that successfully transferred the spermatophore, the sperm transfer success and the courtship duration were also similar between intact and autotomized males (Table 1; Figure 2a). The only difference we found between experimental groups was that the courtship duration varied almost four times more in autotomized males (SD = 92.4 min) than in intact males (SD = 25.6 min) (Levene test: F = 11.503, p = 0.002; Figure 3a).

Female reproductive success and maternal effects

Although the probability of survival until offspring birth for intact females was 42% higher than for autotomized females, the 95% confidence intervals were very large for both experimental groups and there was no significant difference between them (Table 1). The probability of construction of the gestational chamber was similar between intact and for autotomized (Table 1). The offspring number produced by intact females was 29% greater than that produced by autotomized females, and this difference between the experimental groups was significant (Table 1; Figure 2b). After controlling for offspring number, the offspring mass and the offspring size was similar between intact and autotomized females (Table 1). However, the estimated female fitness was almost two times higher for intact females (9.18) than for autotomized females (4.82).

Table 1. Summary of results obtained from the adjusted models to test the implications of "tail" autotomy on the reproductive success of males and females, and also on the offspring phenotype of the scorpion *Ananteris balzani.* For each experimental group (intact or autotomized) we show the estimate value, standard error, sample size (*n*), and the 95% confidence interval (95%CI). All estimate values from models adjusted with binomial and Poisson error distribution were back-transformed to present them in terms of probability or counts, respectively. Offspring mass and offspring size for each experimental group were estimated at the mean of the covariable offspring number (i.e., 12 nymphs).

	Experimental groups		
Proxies	**Intact**	**Autotomized**	**Statistics**
Males			
Spermatophore transfer success (probability)	0.889 ± 0.061 (n = 27), 95%CI: 0.707 to 0.964	0.864 ± 0.073 (n = 22), 95%CI: 0.652 to 0.955	z = 0.268, p = 0.789
Sperm transfer success (probability)	0.708 ± 0.093 (n = 24), 95%CI: 0.502 to 0.854	0.842 ± 0.084 (n = 19), 95%CI: 0.608 to 0.948	z = 1.018, p = 0.308
Courtship duration (min)	42.340 ± 5.025 (n = 22), 95%CI: 32.490 to 52.190)	73.790 ± 21.189 (n = 19), 95%CI: 32.260 to 115.330	z = 1.444, p = 0.157
Females			
Survival until offspring birth (probability)	0.680 ± 0.093 (n = 25), 95%CI: 0.478 to 0.831	0.480 ± 0.099 (n = 25), 95%CI: 0.296 to 0.669	z = 1.421, p = 0.155
Offspring number	13.462 ± 1.018 (n = 13), 95%CI: 11.607 to 15.611	10.500 ± 0.935 (n = 12), 95%CI: 8.818 to 12.503	z = 2.127, p = 0.034
Maternal effects			
Construction of gestational chamber (probability)	0.696 ± 0.096 (n = 23), 95%CI: 0.485 to 0.847	0.650 ± 0.107 (n = 20), 95%CI: 0.426 to 0.823	z = 0.080, p = 0.936
Offspring mass (mg)	50.250 ± 1.991 (n = 13), 95%CI: 46.348 to 54.151	47.515 ± 2.122 (n = 11), 95%CI: 43.355 to 51.674	z = 0.911, p = 0.373
Offspring size (mm)	1.188 ± 0.018 (n = 109), 95%CI: 1.150 to 1.227	1.156 ± 0.018 (n = 106), 95%CI: 1.118 to 1.195	z = 1.225, p = 0.237

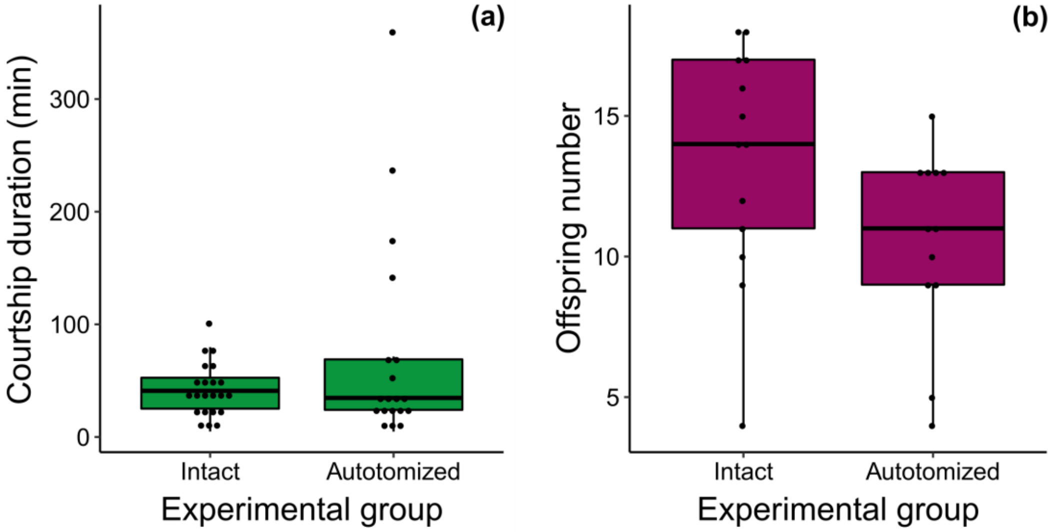

Figure 2. Box-plots showing the effect of "tail" autotomy on the reproduction success of (a) males and (b) females of the scorpion *Ananteris balzani*. In both boxplots, each point represents an individual. (a) The mean courtship duration of intact and automized males was similar, but the variation in latter was much higher. (b) Autotomized females give birth a lower number of nymphs than intact females.

Discussion

We investigated how a nonlethal injury related to "tail" autotomy affects the reproductive success of males and females, and also the offspring phenotype of a scorpion species. We found that the permanent loss of the "tail" has no effect on the reproductive success of males because spermatophore transfer success, sperm transfer success, and courtship duration were similar between intact and autotomized males. "Tail" autotomy, however, impaired the reproduction of females by reducing their survival, offspring number, and ultimately their fitness when compared with intact females. Regarding the maternal effects, we did not detect any effect of female autotomy on the offspring phenotype because the mass and size of the nymphs were similar between the two experimental groups. Finally, the probability of construction of the gestational chamber was also similar between intact and autotomized females, suggesting that their nymphs were equally protected from predators and stressful abiotic conditions, such as dehydration. In what follows we discuss the implications of these findings to the evolution and maintenance of one of the most extreme forms of nonlethal injury among animals.

The “tail” of *A. balzani* males is not crucial for spermatophore transfer. This is surprising because the stinger is used to anchor the body immediately before spermatophore transfer. However, our observations show that autotomized males can use only the basal “tail” segments to perform this anchoring behavior and successfully transfer the spermatophore (see video in Supplementary Material S1). Moreover, although tail wagging may have a stimulatory role during courtship (Gaffin and Brownell 1992), we found no difference in mean courtship duration between intact and autotomized males. In the salamander *Desmognathus ochrophaeus*, males also use their tail to stimulate females, but tail loss does not affect courtship intensity and insemination success (Houck 1982). Therefore, it seems that tail loss *per se* does not necessarily reduce mating or insemination success in species in which males use this appendage during courtship. However, some autotomized males of *A. balzani* needed much more time to successfully transfer the spermatophore than intact males (Figure 2a). We suggest that autotomized males face more female resistance, increasing the variation in courtship duration. A testable prediction of this hypothesis is that autotomized males compensate “tail” loss increasing the frequency of other stimulatory behaviors, such as juddering and cheliceral massage. In fact, studies with other buthid scorpions show that males can adjust the intensity of courtship in response to female receptivity (Peretti and Carrera 2005). If the same happens with *A. balzani*, compensatory adjustments in the intensity of courtship could therefore mitigate the immediate reproductive costs to autotomized males.

Our experiment focuses on the immediate costs of “tail” loss to the reproductive success of males, and no negative effect was found. However, previous studies on the effects of “tail” loss in *A. balzani* show that severe constipation two months after autotomy promotes a reduction in the locomotor performance of males (see Chapter 1). In scorpions, males show intense surface activity during the breeding season, searching for receptive females as in a typical scramble competition polygyny (Polis and Sissom 1990). In the long-term, therefore, reduced locomotor performance could decrease the mating success of *A. balzani* males. Moreover, autotomized males show decreased predation performance because stinger loss impairs subjugation of large prey, constraining male diet to only small items (Chapter 2). In the long-term, autotomized males should be in poorer body condition than intact males due to decreased predation success. Considering that courtship is a condition-dependent behavior in many groups, including scorpions (e.g., Shine et al. 2004; Rypstra and Wilder 2007; Oliveiro et al. 2019), we expect that autotomized males would have a decreased courtship performance after some months. Finally, accumulation of feces in the abdomen of autotomized males could cause deformities in the hemi-spermatophores produced in their reproductive tract. Taking all these effects together, it is reasonable to infer that autotomized males may pay long-term reproductive costs but still can mate several times before constipation causes their death.

Contrary to males, autotomized females of *A. balzani* paid high reproductive costs in the form of 22% reduction in offspring number. This reduction is close to the lower value reported for automized female lizards, in which the reduction in offspring number ranges from 14% to 75% (Bateman and Fleming 2009). The greater reduction among lizards is probably related to a double cost of tail autotomy: (1) the loss of energetic resources stored in the tail that is no longer available to egg production, and (2) the regeneration of the tail, which diverts energy from egg production (Bernardo and Agosta 2005; Maginnis 2006). In contrast, the permanent “tail” loss in scorpions has no direct effect on energetic reserves used for reproduction because there is no storage organ in the metasoma (Hjelle 1990). Moreover, there is no “tail” regeneration in scorpions (Mattoni et al. 2015), and the energetic trade-off between replacement of metasomal tissues and offspring production does not exist. We suggest that the reduction in offspring number reported here for autotomized females is a consequence of severe constipation. At the moment of parturition, the relative litter mass in scorpions ranges from 22% to 56% (e.g., Formanowicz and Shaffer 1993; Brown 2004; Warburg 2011), which implies that embryos occupy a great volume inside pregnant females. In a previous study, we showed that two months after autotomy the mass of non-pregnant females increases 58% due to accumulation of feces in their digestive tract (Chapter 1). Given that the embryos take five months to develop, we argue that some of them die or are resorbed in the beginning of the gestation due to lack of space inside the females. Thus, although lizards and scorpions may suffer a reduction in offspring number after autotomy, the mechanisms underlying this reduction are probably very different.

Less than 50% of the autotomized females survived until offspring birth. The combined effect of increased mortality and reduced fecundity resulted in a great fitness cost to autotomized females, which produced 48% less offspring than intact females. The fact that females pay higher reproductive costs than males may explain sexual differences in the willingness to autotomize the "tail". Under laboratory conditions, females are more reluctant than males to autotomize the "tail" (Mattoni et al. 2015). Moreover, the frequency of autotomized females in natural populations is much lower than that of males (Mattoni et al. 2015). Similar sexual differences related to the frequency of autotomy are found in insects and lizards. In the bushcricket *Scudderia texensis*, for instance, individuals of both sexes autotomize frontal legs, where is located the tympanum, a structure that assists the location of sexual partners. Males are more prone than females to autotomize frontal legs, possibly because mate search is performed mainly by females, which pay higher reproductive costs if they are unable to find sexual partners (Dixon 1989). Males of the gecko *Uta stansburian*a, in turn, autotomize their tail less readily than females. In this species, males and females stablish intrasexual dominance hierarchy, and whereas autotomized females manage to reproduce as subordinates, autotomized males completely lose their social status and are deprived from access to sexual partners (Fox et al. 1998). These examples with scorpions, insects, and lizards clearly show that the frequency of autotomy may differ between males and females in response to the costs paid by each sex (but see Smith 1992).

"Tail" autotomy of females did not impose any detectable change in offspring traits (i.e., body size and body mass) via maternal effects. This finding indicates that, despite severe constipation, autotomized females maintain the same per-propagule investment as intact females. In fact, there is little empirical evidence showing that female autotomy changes offspring traits (lizards: Dial and Fitzpatrick 1981; Wilson and Booth 1998; salamanders: Bernardo and Agosta 2005). Life history theory states that larger offspring have higher fitness and thus are favored by natural selection (Stearns 1976). Among arthropods, several studies show that the effects of initial propagule size on subsequent growth and survival are consistent with the ''bigger is better'' hypothesis (Fox and Czesak 2000). Moreover, a mathematical model predicts that the effects of propagule size on viability should lead to the production of eggs of a constant size by females (Parker and Begon 1986). In the case of scorpions, mortality in the first nymphal instars is strongly size-dependent, so that the larger nymphs have higher rates of survival (Polis 1988). Thus, assuming that pregnant females resorb and transfer nutrients among embryos (Polis and Sissom 1990), autotomized females could maintain the same per-propagule investment as intact females by reducing offspring number. This suggestion provides an alternative explanation to the lower fecundity of autotomized *A. balzani* females reported here.

In conclusion, we found that a permanent nonlethal injury related to "tail" autotomy does not impair the mating process of males. Given that males can live several months after losing the "tail" and can potentially mate many times during this period, autotomy is clearly an adaptive strategy because it imposes no immediate reproductive cost and provides the tremendous benefit of scaping alive from a predation attempt. In the case of females, autotomy greatly reduces the fitness, but imposes no detectable change in offspring traits via maternal effects. The high cost paid by females may explain why they are more reluctant than males in autotomizing the "tail" and also why the frequency of autotomized females is very low in the field. In the future, it would be interesting to conduct a long-term mark-recapture study under natural conditions to understand how the combination of decreased locomotor (Chapter 1) and predation performances of autotomized individuals (Chapter 2) may influence: (i) survival rates and longevity of adults of both sexes, and (ii) frequency of parturitions and mean litter size of females.

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Author contributions: Conceptualization: SGH and GM; Methodology: SGH and GM; Data collection: SGH; Formal analysis: SGH; Data curation: SGH; Writing (original draft): SGH and GM; Writing (review & editing): SGH and GM; Visualization: SGH and GM; Supervision: GM; Project administration: SGH; Funding acquisition: SGH and GM.

Data Archiving

The original data will be available in the Dryad repository upon manuscript acceptance.

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Supplementary material

S1. Courtship behavior of *Ananteris balzani*

Video. Courtship behavior of intact and autotomized males of the scorpion *Ananteris balzani*.

Link: https://youtu.be/CoJMi1m6tFk

S2. Paternal effects on offspring traits

Methods

To explore the possible implications of the experimental group of fathers (intact or autotomized) on offspring traits, we used the experimental group of fathers as predictor variable in the analyses related to *offspring number*, *offspring mass*, and *offspring size*. For the counting response variable offspring number, we adjusted a generalized linear model (GLM) with Poisson error distribution. For the continuous response variable offspring mass, we adjusted a GLM with Gaussian error distribution. Because total offspring mass may be influenced by offspring number, we included the total number of nymphs in each litter as a covariable in the model. Finally, for the continuous response variable offspring size, we adjusted a generalized linear mixed model (GLMM) with Gaussian error distribution and mother identity as random factor because each female has multiple nymphs in her litter. Given that offspring size may also be influenced by offspring number, we included the total number of nymphs in each litter as a covariable in the model.

The GLMs were performed in the package *stats* (R Core Team 2019) and the GLMM was performed in the package *lme4* (Bates et al. 2015). Because the results of the models adjusted with binomial and Poisson error distribution are in logit and log scale, respectively, we used the package *emmeans* (Lenth 2019) to transform them into probabilities and number of individuals. All analyses were performed using the software R version 3.5.2. (R Core Team 2018).

Results

The experimental group of the fathers (intact or autotomized) had no effect on the number of nymphs produced by the females (Table S1). Moreover, after controlling for offspring number, the experimental group of the fathers also had no effect on offspring mass and offspring size (Table S1).

Table S1. Summary of the results obtained with the adjusted models to explore the possible implications of "tail" autotomy of fathers on offspring traits. For each experimental group (intact or autotomized) we show the estimate value, standard error, sample size (*n*), and the 95% confidence interval (95%CI). All estimates values from models adjusted with binomial and Poisson error structure were back-transformed to present them in terms of probability or counts, respectively. Offspring mass and offspring size for each experimental group were estimated at the mean of the covariable offspring number (i.e., 12 nymphs).

	Experimental groups of fathers		
Proxies	**Intact**	**Autotomized**	**Statistics**
Offspring number	12.500 ± 0.884 (*n* = 16), 95%CI: 10.882 to 14.358	11.222 ± 1.117 (*n* = 9), 95%CI: 9.234 to 13.638	*z* = 0.883, *p* = 0.337
Offspring mass (mg)	48.900 ± 1.755 (*n* = 16), 95%CI: 45.460 to 52.339	49.092 ± 2.473 (*n* = 8), 95%CI: 44.244 to 53.940	*z* = 0.063, *p* = 0.950
Offspring size (mm)	1.181 ± 0.016 (*n* = 142), 95%CI: 1.146 to 1.215	1.158 ± 0.022 (*n* = 73), 95%CI: 1.111 to 1.205	*z* = 0.829, *p* = 0.419

S3. Analysis of female body size

Methods

To test whether female body size differed between the two experimental groups (25 intact and 25 autotomized), we adjusted a GLM with Gaussian error distribution using female body size as continuous response variable and experimental group as categorical predictor variable. Then, we explored the possible implications of female body size on offspring number and offspring size. For the 13 intact and 12 autotomized females that gave birth, we adjusted a GLM with Poisson error distribution using offspring number as counting response variable and female body size as predictor variable. Moreover, for the continuous response variable offspring size, we adjusted a generalized linear mixed model (GLMM) with Gaussian error distribution. In this case, female body size was used as predictor variable and mother identity as random factor because each female has multiple nymphs in her litter. The GLMs were performed in the package *stats* (R Core Team 2019) and the GLMM was performed in the package *lme4* (Bates et al. 2015). All analyses were performed using the software R version 3.5.2. (R Core Team 2018).

Results

Female body size was similar between the two experimental groups ($R^2 = 0.026$, $F_{1,21} = 1.580$, $p = 0.223$), and had no effect on offspring number ($z = 0.051$, $p = 0.960$) or on offspring size ($t = -1.051$, $p = 0.308$).

Literature Cited

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1-48.

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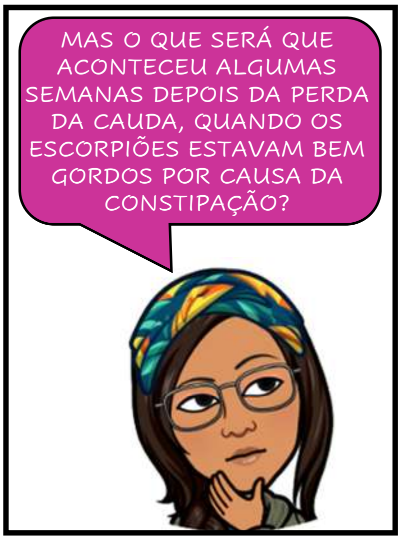
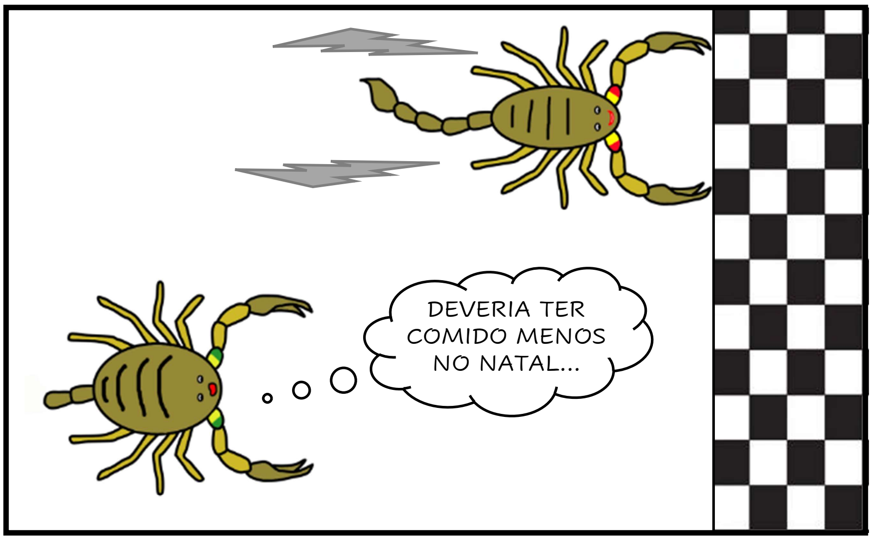
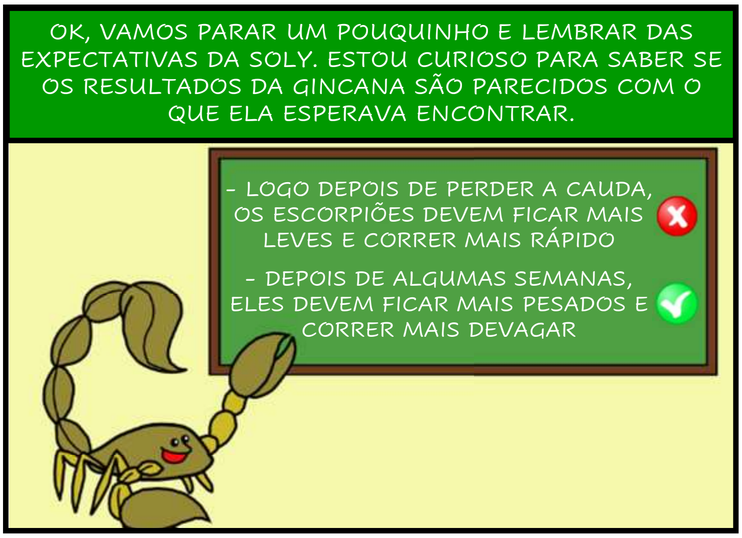
Quatro anos depois...


MAS O QUE SERÁ QUE ACONTECEU ALGUMAS SEMANAS DEPOIS DA PERDA DA CAUDA, QUANDO OS ESCORPIÕES ESTAVAM BEM GORDOS POR CAUSA DA CONSTIPAÇÃO?

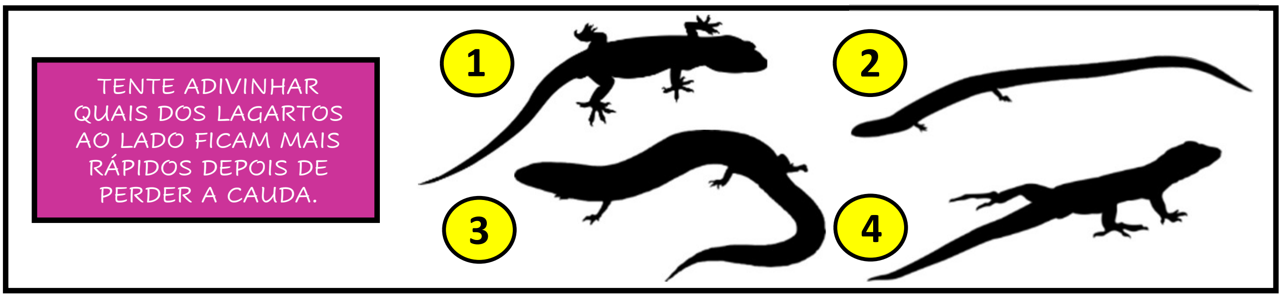

DEVERIA TER COMIDO MENOS NO NATAL...

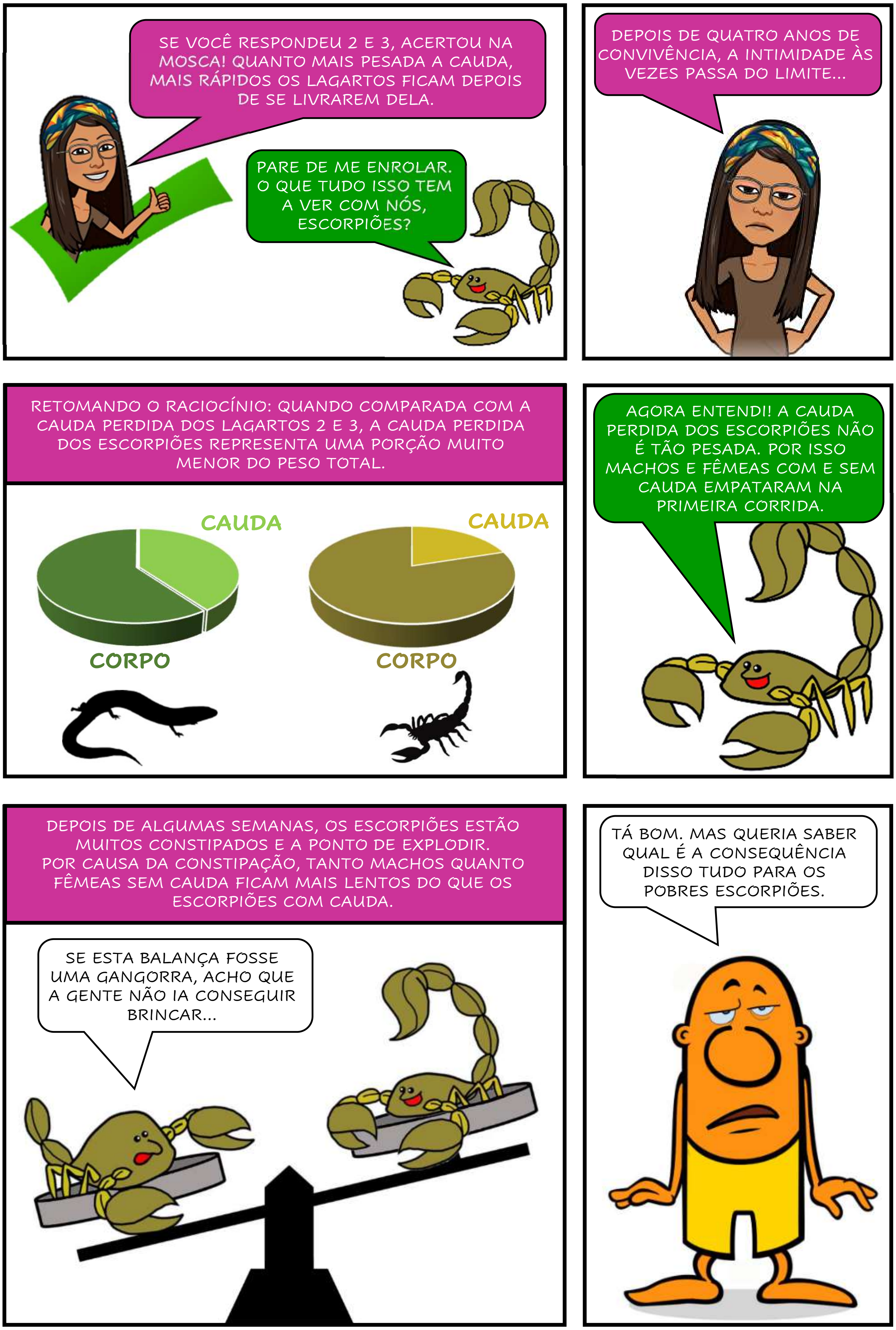

TANTO MACHOS QUANTO FÊMEAS SEM CAUDA PERDERAM A CORRIDA. OS INDIVÍDUOS COM CAUDA FORAM MAIS RÁPIDOS.


OK, VAMOS PARAR UM POUQUINHO E LEMBRAR DAS EXPECTATIVAS DA SOLY. ESTOU CURIOSO PARA SABER SE OS RESULTADOS DA GINCANA SÃO PARECIDOS COM O QUE ELA ESPERAVA ENCONTRAR.
- LOGO DEPOIS DE PERDER A CAUDA, OS ESCORPIÕES DEVEM FICAR MAIS LEVES E CORRER MAIS RÁPIDO ✗
- DEPOIS DE ALGUMAS SEMANAS, ELES DEVEM FICAR MAIS PESADOS E CORRER MAIS DEVAGAR ✓


HUM... PARECE QUE AS COISAS SÃO MAIS COMPLICADAS DO QUE VOCÊ ESPERAVA. SERÁ QUE VOCÊ TEM ALGUMA EXPLICAÇÃO?


PRIMEIRO, VAMOS VER O QUE ACONTECE COM OS LAGARTOS. NEM SEMPRE UM LAGARTO SEM CAUDA É MAIS RÁPIDO DO QUE UM LAGARTO COM CAUDA.


TENTE ADIVINHAR QUAIS DOS LAGARTOS AO LADO FICAM MAIS RÁPIDOS DEPOIS DE PERDER A CAUDA.
1
2
3
4


SE VOCÊ RESPONDEU 2 E 3, ACERTOU NA MOSCA! QUANTO MAIS PESADA A CAUDA, MAIS RÁPIDOS OS LAGARTOS FICAM DEPOIS DE SE LIVRAREM DELA.
PARE DE ME ENROLAR. O QUE TUDO ISSO TEM A VER COM NÓS, ESCORPIÕES?
DEPOIS DE QUATRO ANOS DE CONVIVÊNCIA, A INTIMIDADE ÀS VEZES PASSA DO LIMITE...
RETOMANDO O RACIOCÍNIO: QUANDO COMPARADA COM A CAUDA PERDIDA DOS LAGARTOS 2 E 3, A CAUDA PERDIDA DOS ESCORPIÕES REPRESENTA UMA PORÇÃO MUITO MENOR DO PESO TOTAL.
CAUDA
CORPO
CAUDA
CORPO
AGORA ENTENDI! A CAUDA PERDIDA DOS ESCORPIÕES NÃO É TÃO PESADA. POR ISSO MACHOS E FÊMEAS COM E SEM CAUDA EMPATARAM NA PRIMEIRA CORRIDA.
DEPOIS DE ALGUMAS SEMANAS, OS ESCORPIÕES ESTÃO MUITOS CONSTIPADOS E A PONTO DE EXPLODIR. POR CAUSA DA CONSTIPAÇÃO, TANTO MACHOS QUANTO FÊMEAS SEM CAUDA FICAM MAIS LENTOS DO QUE OS ESCORPIÕES COM CAUDA.
SE ESTA BALANÇA FOSSE UMA GANGORRA, ACHO QUE A GENTE NÃO IA CONSEGUIR BRINCAR...
TÁ BOM. MAS QUERIA SABER QUAL É A CONSEQUÊNCIA DISSO TUDO PARA OS POBRES ESCORPIÕES.

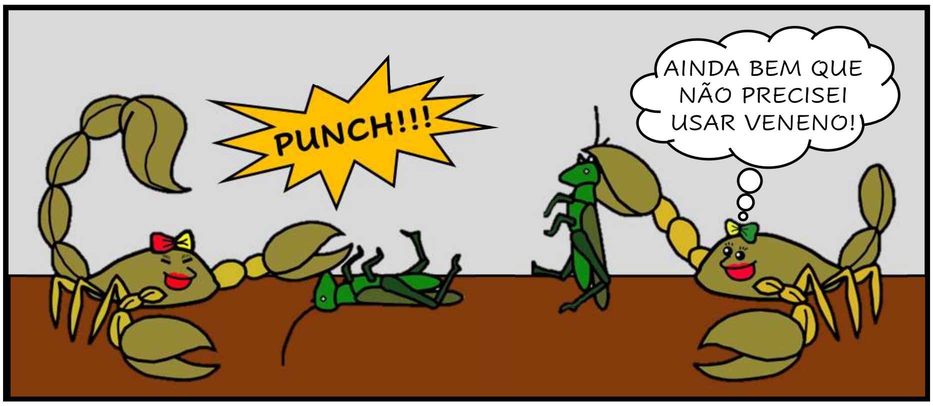

FICAR MAIS LENTO PODE SER MUITO RUIM! IMAGINE SE UM ESCORPIÃO SEM CAUDA E MUITO CONSTIPADO TIVER QUE FUGIR DE UM PREDADOR.
AI, AI! ESTOU MAIS LENTO QUE UMA LESMA. ESPERO QUE ESTE RATO NÃO ME VEJA...


POIS É, PERDER A CAUDA PODE SALVAR NOSSA VIDA DE UM ATAQUE, MAS FICAMOS MAIS VULNERÁVEIS A OUTROS ATAQUES.


Caça ao grilo
VAMOS VOLTAR PARA A GINCANA PORQUE AINDA TEMOS OUTRAS PROVAS.


NESTA PROVA, MACHOS E FÊMEAS TINHAM 10 MINUTOS PARA CONSEGUIR CAÇAR UM GRILO PEQUENO E DEPOIS UM GRILO GRANDE DENTRO DE UMA ARENA.

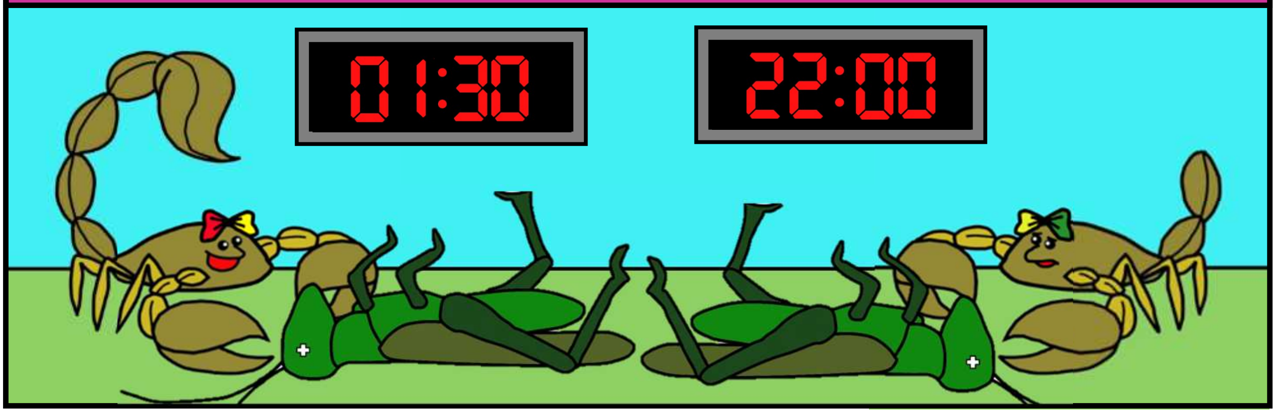

COMECEI COM A PROVA DE GRILOS PEQUENOS E HOUVE EMPATE ENTRE AS FÊMEAS

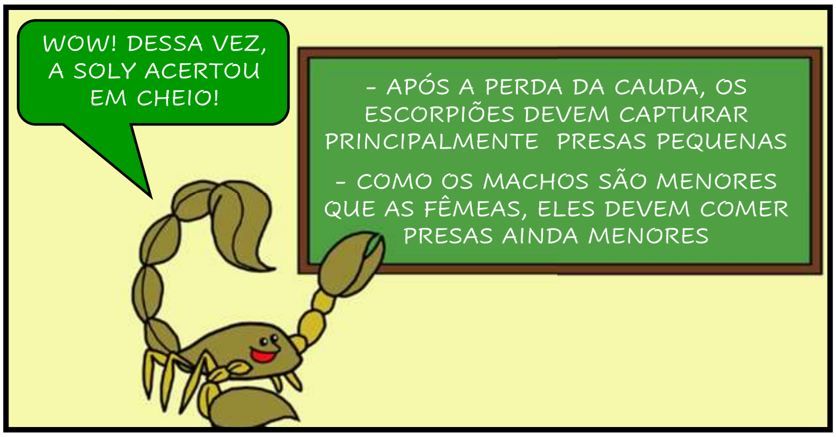

PUNCH!!!
AINDA BEM QUE NÃO PRECISEI USAR VENENO!

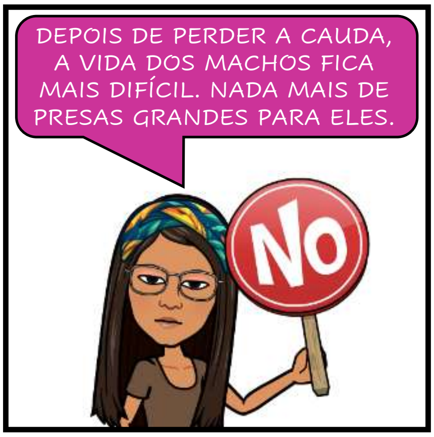

JÁ OS MACHOS NÃO EMPATARAM NESSA PROVA. OS INTACTOS GANHARAM!

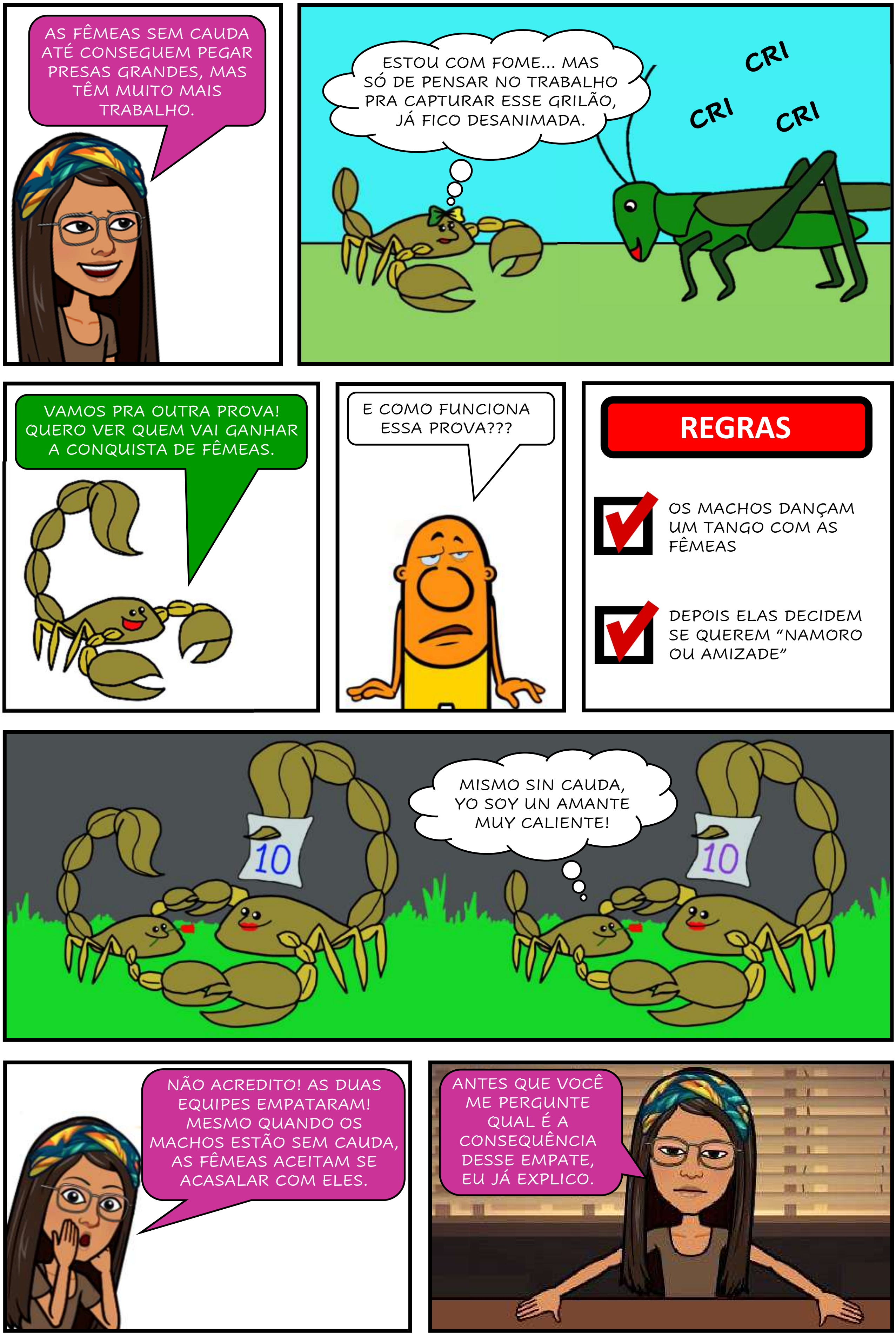
NA PROVA DE CAÇA AO GRILO GRANDE, OS MACHOS COM CAUDA CONSEGUIRAM USAR SEU VENENO PARA MATAR E CAPTURAR A PRESA. JÁ OS MACHOS SEM CAUDA, NÃO CONSEGUIRAM CAÇAR O GRILO GRANDE MESMO DEPOIS DE MUITO ESFORÇO.

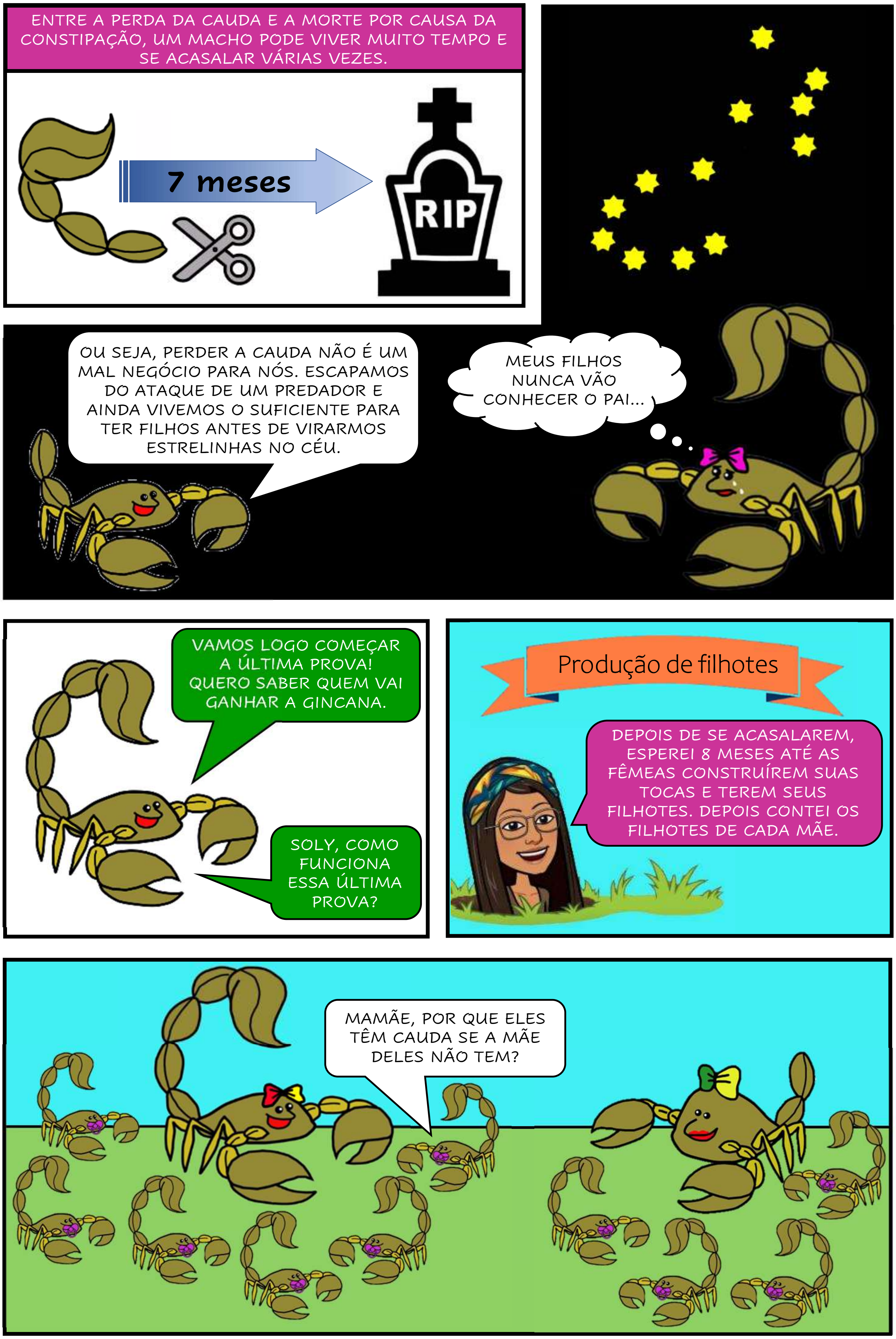
FÊMEAS COM E SEM CAUDA CONSEGUIRAM CAPTURAR O GRILO GRANDE. PORÉM, NO CRITÉRIO DE DESEMPATE DA GINCANA, FÊMEAS COM CAUDA GANHARAM A PROVA PORQUE CONSEGUIRAM CAPTURAR O GRILO MUITO MAIS RÁPIDO.



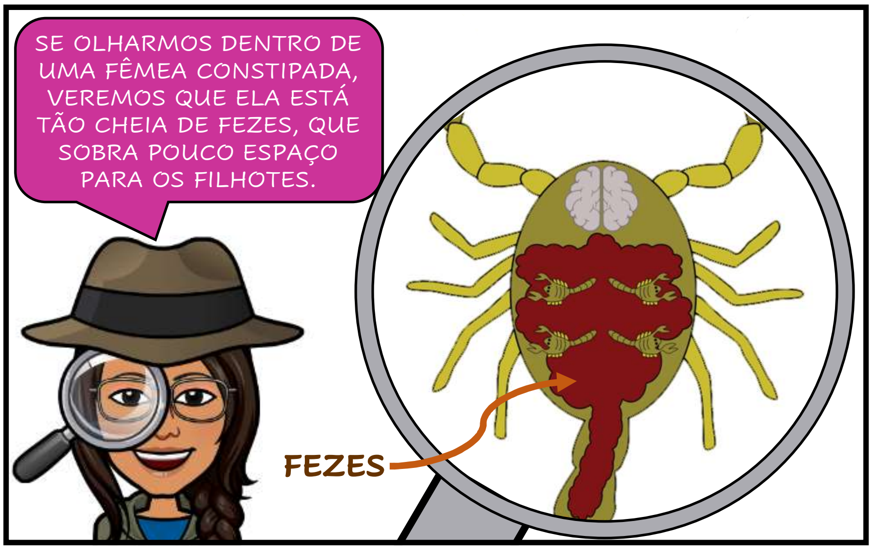


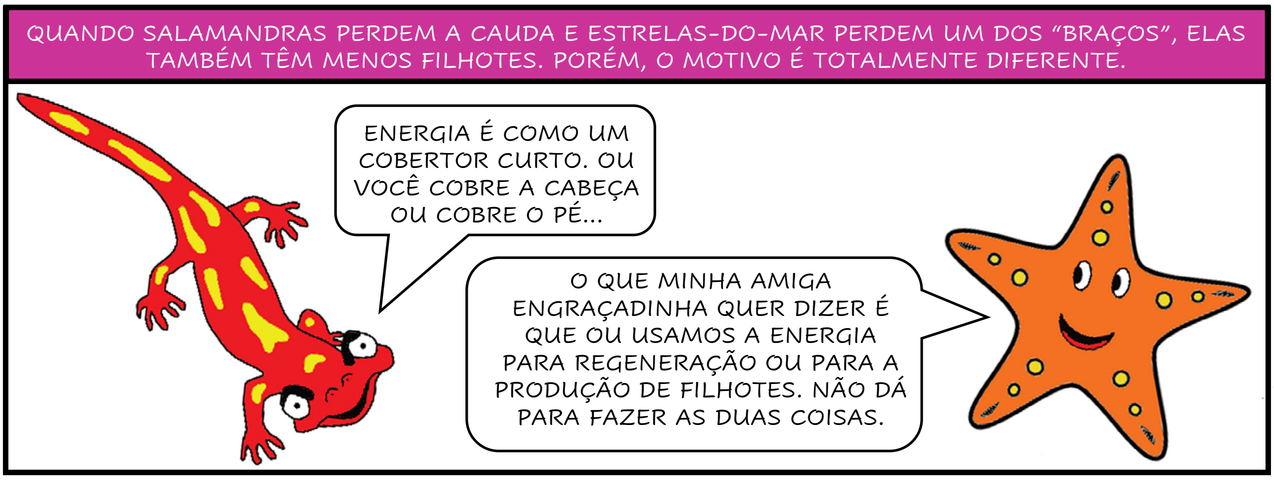


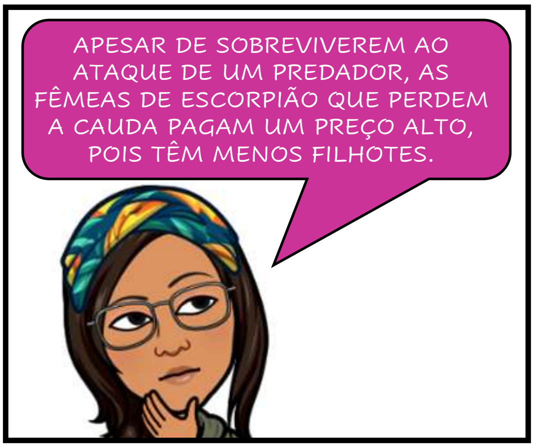

AS FÊMEAS SEM CAUDA ATÉ CONSEGUEM PEGAR PRESAS GRANDES, MAS TÊM MUITO MAIS TRABALHO.
ESTOU COM FOME... MAS SÓ DE PENSAR NO TRABALHO PRA CAPTURAR ESSE GRILÃO, JÁ FICO DESANIMADA.
CRI
CRI
CRI
VAMOS PRA OUTRA PROVA! QUERO VER QUEM VAI GANHAR A CONQUISTA DE FÊMEAS.
E COMO FUNCIONA ESSA PROVA???
REGRAS
OS MACHOS DANÇAM UM TANGO COM AS FÊMEAS
DEPOIS ELAS DECIDEM SE QUEREM "NAMORO OU AMIZADE"
10
MISMO SIN CAUDA, YO SOY UN AMANTE MUY CALIENTE!
10
NÃO ACREDITO! AS DUAS EQUIPES EMPATARAM! MESMO QUANDO OS MACHOS ESTÃO SEM CAUDA, AS FÊMEAS ACEITAM SE ACASALAR COM ELES.
ANTES QUE VOCÊ ME PERGUNTE QUAL É A CONSEQUÊNCIA DESSE EMPATE, EU JÁ EXPLICO.


ENTRE A PERDA DA CAUDA E A MORTE POR CAUSA DA CONSTIPAÇÃO, UM MACHO PODE VIVER MUITO TEMPO E SE ACASALAR VÁRIAS VEZES.
7 meses
RIP
OU SEJA, PERDER A CAUDA NÃO É UM MAL NEGÓCIO PARA NÓS. ESCAPAMOS DO ATAQUE DE UM PREDADOR E AINDA VIVEMOS O SUFICIENTE PARA TER FILHOS ANTES DE VIRARMOS ESTRELINHAS NO CÉU.
MEUS FILHOS NUNCA VÃO CONHECER O PAI...
VAMOS LOGO COMEÇAR A ÚLTIMA PROVA! QUERO SABER QUEM VAI GANHAR A GINCANA.
SOLY, COMO FUNCIONA ESSA ÚLTIMA PROVA?
Produção de filhotes
DEPOIS DE SE ACASALAREM, ESPEREI 8 MESES ATÉ AS FÊMEAS CONSTRUÍREM SUAS TOCAS E TEREM SEUS FILHOTES. DEPOIS CONTEI OS FILHOTES DE CADA MÃE.
MAMÃE, POR QUE ELES TÊM CAUDA SE A MÃE DELES NÃO TEM?

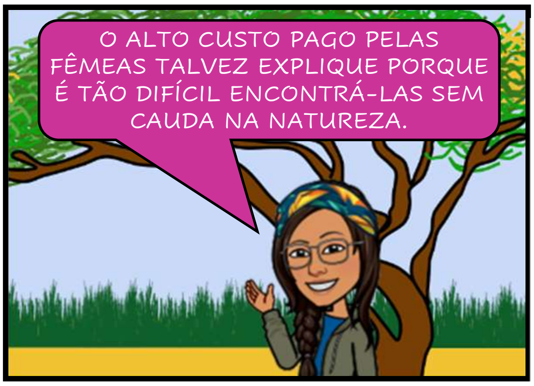

AS FÊMEAS COM CAUDA TIVERAM MAIS FILHOTES, ASSIM COMO VOCÊ ESPERAVA, SOLY!


SE OLHARMOS DENTRO DE UMA FÊMEA CONSTIPADA, VEREMOS QUE ELA ESTÁ TÃO CHEIA DE FEZES, QUE SOBRA POUCO ESPAÇO PARA OS FILHOTES.
FEZES

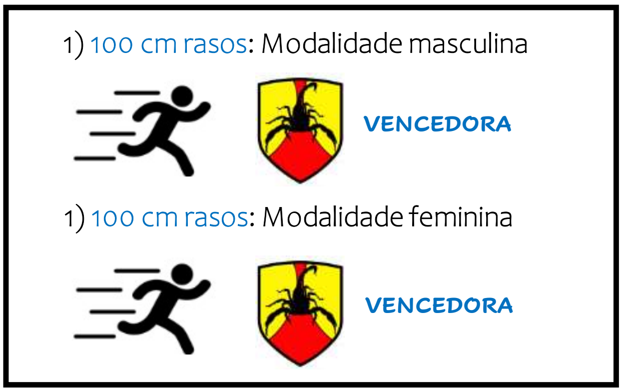

QUANDO SALAMANDRAS PERDEM A CAUDA E ESTRELAS-DO-MAR PERDEM UM DOS “BRAÇOS”, ELAS TAMBÉM TÊM MENOS FILHOTES. PORÉM, O MOTIVO É TOTALMENTE DIFERENTE.
ENERGIA É COMO UM COBERTOR CURTO. OU VOCÊ COBRE A CABEÇA OU COBRE O PÉ...
O QUE MINHA AMIGA ENGRAÇADINHA QUER DIZER É QUE OU USAMOS A ENERGIA PARA REGENERAÇÃO OU PARA A PRODUÇÃO DE FILHOTES. NÃO DÁ PARA FAZER AS DUAS COISAS.


APESAR DE SOBREVIVEREM AO ATAQUE DE UM PREDADOR, AS FÊMEAS DE ESCORPIÃO QUE PERDEM A CAUDA PAGAM UM PREÇO ALTO, POIS TÊM MENOS FILHOTES.

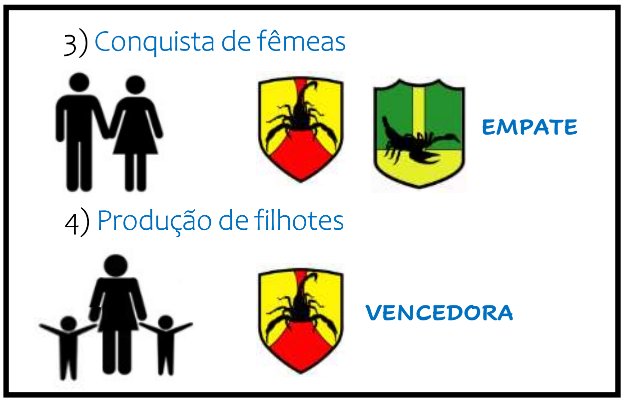

POIS É, AMIGA, PERDER A CAUDA É MUITO MELHOR DO QUE PERDER A VIDA. PORÉM, O MELHOR NEGÓCIO MESMO É NÃO PERDER NEM A CAUDA NEM A VIDA...


O ALTO CUSTO PAGO PELAS FÊMEAS TALVEZ EXPLIQUE PORQUE É TÃO DIFÍCIL ENCONTRÁ-LAS SEM CAUDA NA NATUREZA.


SENHORAS E SENHORES, VAMOS FINALMENTE DESCOBRIR QUEM GANHOU A GINCANA!


1) 100 cm rasos: Modalidade masculina
VENCEDORA
1) 100 cm rasos: Modalidade feminina
VENCEDORA


2) Caça ao grilo: Modalidade masculina
VENCEDORA
2) Caça ao grilo: Modalidade feminina
VENCEDORA


3) Conquista de fêmeas
EMPATE
4) Produção de filhotes
VENCEDORA


TEMOS UMA VITÓRIA DE LAVADA DA EQUIPE COM CAUDA!!!

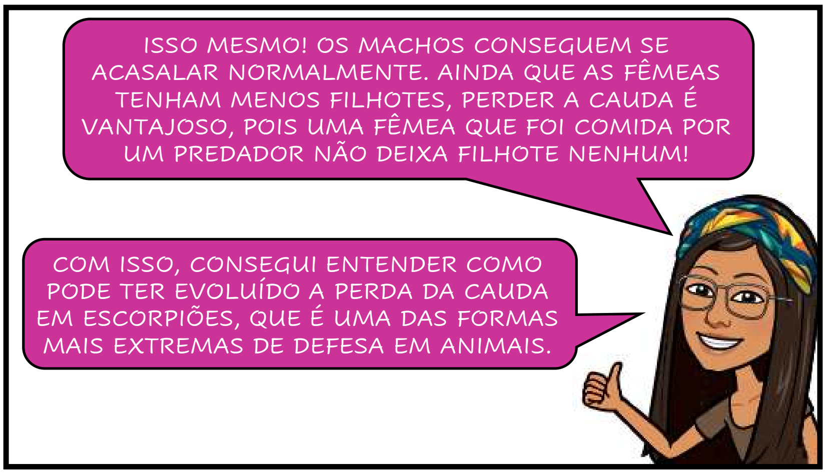

E O QUE ESSA VITÓRIA DE LAVADA QUER DIZER?

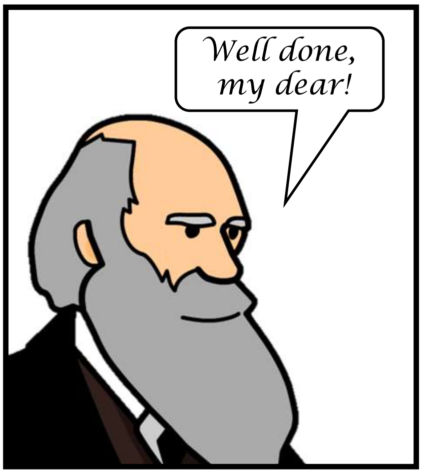

QUER DIZER QUE PERDER A CAUDA É UMA DECISÃO MUITO DIFÍCIL NA VIDA DOS ESCORPIÕES. ELES FICAM MAIS LENTOS, MENOS EFICIENTES EM CAÇAR E AS FÊMEAS TÊM MENOS FILHOTES.
ENTÃO PERDER A CAUDA É UMA BAITA BURRICE!


ESSE CARA NÃO ESTÁ PRESTANDO ATENÇÃO NA HISTÓRIA!


CALMA, SOLY. PODE DEIXAR QUE EU EXPLICO. SALVAR A VIDA É UMA PRIORIDADE, EMBORA DEPOIS DE PERDER A CAUDA, O ESCORPIÃO ESTEJA COM OS DIAS CONTADOS.
2:03

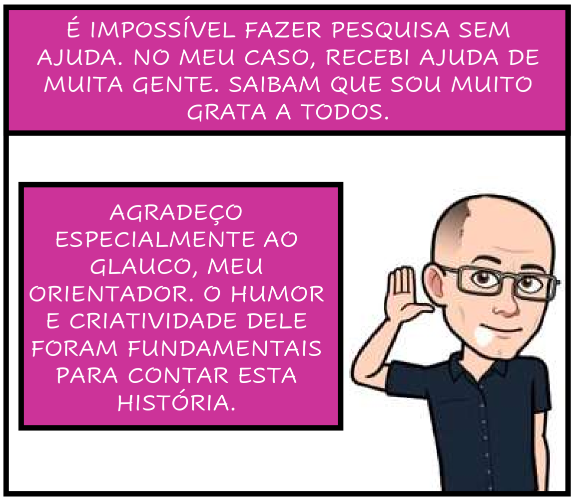

MAS ANTES DA BOMBA RELÓGIO EXPLODIR, OS ESCORPIÕES CONSEGUEM SE REPRODUZIR.

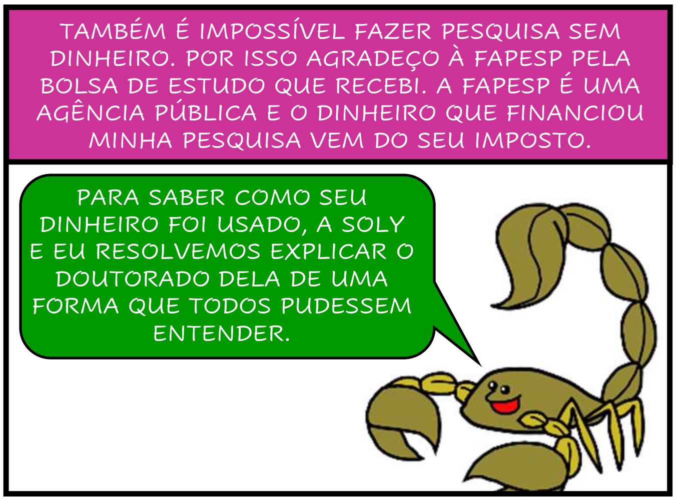

ISSO MESMO! OS MACHOS CONSEGUEM SE ACASALAR NORMALMENTE. AINDA QUE AS FÊMEAS TENHAM MENOS FILHOTES, PERDER A CAUDA É VANTAJOSO, POIS UMA FÊMEA QUE FOI COMIDA POR UM PREDADOR NÃO DEIXA FILHOTE NENHUM!
COM ISSO, CONSEGUI ENTENDER COMO PODE TER EVOLUÍDO A PERDA DA CAUDA EM ESCORPIÕES, QUE É UMA DAS FORMAS MAIS EXTREMAS DE DEFESA EM ANIMAIS.

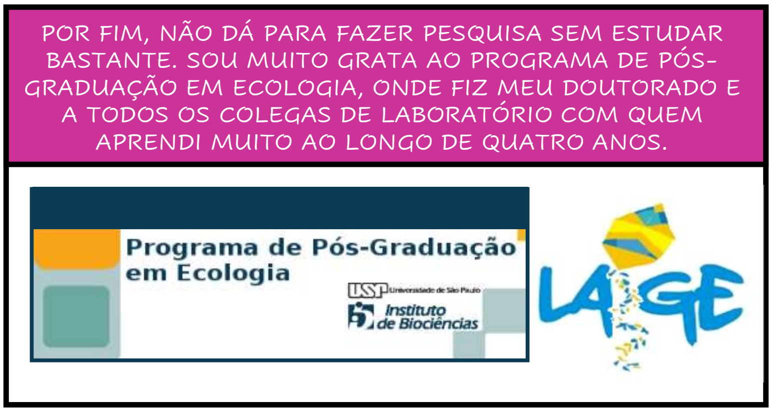

Well done, my dear!


AGORA TUDO FAZ SENTIDO!!!


E PARA ENCERRAR ESTA HISTÓRIA, QUERO AGRACEDER.


É IMPOSSÍVEL FAZER PESQUISA SEM AJUDA. NO MEU CASO, RECEBI AJUDA DE MUITA GENTE. SAIBAM QUE SOU MUITO GRATA A TODOS.
AGRADEÇO ESPECIALMENTE AO GLAUCO, MEU ORIENTADOR. O HUMOR E CRIATIVIDADE DELE FORAM FUNDAMENTAIS PARA CONTAR ESTA HISTÓRIA.


TAMBÉM É IMPOSSÍVEL FAZER PESQUISA SEM DINHEIRO. POR ISSO AGRADEÇO À FAPESP PELA BOLSA DE ESTUDO QUE RECEBI. A FAPESP É UMA AGÊNCIA PÚBLICA E O DINHEIRO QUE FINANCIOU MINHA PESQUISA VEM DO SEU IMPOSTO.
PARA SABER COMO SEU DINHEIRO FOI USADO, A SOLY E EU RESOLVEMOS EXPLICAR O DOUTORADO DELA DE UMA FORMA QUE TODOS PUDESSEM ENTENDER.


POR FIM, NÃO DÁ PARA FAZER PESQUISA SEM ESTUDAR BASTANTE. SOU MUITO GRATA AO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, ONDE FIZ MEU DOUTORADO E A TODOS OS COLEGAS DE LABORATÓRIO COM QUEM APRENDI MUITO AO LONGO DE QUATRO ANOS.
Programa de Pós-Graduação em Ecologia
USP Universidade de São Paulo
Instituto de Biociências
LAGE


ESPERAMOS QUE VOCÊ TENHA GOSTADO E SE DIVERTIDO.
ADIÓS!