UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE ZOOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

ASPECTOS ECOLÓGICOS DE *Tityus pusillus* POCOCK, 1893 E *Ananteris mauryi* LOURENÇO, 1982 (SCORPIONES: BUTHIDAE) EM REMANESCENTES DE FLORESTA ATLÂNTICA NO NORDESTE DO BRASIL

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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, como exigência para a obtenção do grau de mestre em Biologia Animal.

Aluno: André Felipe de Araujo Lira

Orientadora: Dra. Cleide Maria Ribeiro de Albuquerque

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ANDRÉ FELIPE DE ARAUJO LIRA

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"Divided we're nothing, united we're breaking the rules"

(The voice commanding you, Angra)

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RESUMO

Redução e fragmentação de habitats naturais, e as modificações do ambiente são as principais ameaças da perda de biodiversidade levando a uma necessidade crítica de entender como a degradação do habitat afeta as comunidades animais. No presente trabalho foi investigada a influência relativa dos atributos ambientais (profundidade e peso seco da serapilheira, densidade de árvores, diâmetro a altura do peito do tronco, abertura do dossel e área do fragmento) sobre a estrutura populacional das espécies de escorpião *Tityus pusillus* Pocock, 1893 e *Ananteris mauryi* Lourenço, 1982 que co-habitam a serapilheira. As coletas foram realizadas em 11 fragmentos de Floresta Atlântica, pertencentes à Usina Trapiche, Mata Sul de Pernambuco. Em cada fragmento seis transectos de 30 m foram amostrados através de busca ativa noturna utilizando-se UV, nos meses de dezembro/2012 e janeiro/2013. No total 1.125 escorpiões pertencentes às espécies estudadas foram coletados durante o período analisado. A maioria (90%) pertencia a espécie *T. pusillus*, cuja variação de abundância nos diferentes fragmentos (2 a 177 indivíduos) mostrou-se positivamente correlacionada ao peso do seco da serapilheira. Não houve efeito significativo das demais variáveis sobre os parâmetros analisados para as espécies *T. pusillus* e *A. mauryi*. Em relação à estrutura populacional, uma proporção maior de machos em relação às fêmeas (1,5:1) foi registrada para *A. mauryi*, assim como observou-se a ausência de jovens dessa espécie no ambiente durante o período analisado. Esses resultados indicam que *T. pusillus* é a espécie de escorpião predominante na Floresta Atlântica e que apesar de viver no mesmo microhabitat, *T. pusillus* e *A. mauryi* podem ter diferentes exigências ecológicas. A maior quantidade de machos permite inferir uma possível diferenciação do período reprodutivo dessas espécies, podendo ser uma estratégia para evitar competição interespecífica.

Palavras-chave: Ecologia de populações, escorpiões neotropicais, fragmentação de habitat, espécies coexistentes.

ABSTRACT

Reduction and fragmentation of natural habitats and landscape modification are the main drivers of biodiversity loss leading to a critical need in understanding how habitat degradation affects animal communities. In the present study, we investigated the relative influence of environmental attributes (depth and dry weight of litter, tree density, diameter at breast height of tree, canopy openness and fragment area) on the population structure of co-habiting leaf litter scorpion' species, *Tityus pusillus* Pocock, 1893 and *Ananteris mauryi* Lourenço, 1982. Samples were collected in 11 fragments of the South Atlantic Forest of Pernambuco belonging to Trapiche Mill. In each fragment, six 30m transects were sampled by active search night using UV, in the months of December/2012 and January/2013. In total 1,125 scorpions belonging to the studied species were collected. The majority (90%) belonged to the *T. pusillus* species, whose abundance variation between different fragments (2-177 individuals) was positively correlated to the weight of dry litter. No significant effect of other variables on the analyzed parameters on *T. pusillus* and *A. mauryi* was observed. A higher proportion of males compared to females (1.5:1) were recorded for *A. mauryi*. In addition, no juveniles of this species were collected in the environment during the studied period. These results indicate that *T. pusillus* is the predominant species of scorpion in the Atlantic Forest and that, despite living in the same microhabitat, *T. pusillus* and *A. mauryi* may have different ecological requirements. The largest number of male infers a possible differentiation of reproduction of these species, and such behaviour may be a strategy to avoid interspecific competition.

Key words: Population ecology, Neotropical scorpions, habitat fragmentation, coexistent species

1. FUNDAMENTAÇÃO TEÓRICA

1.1. Fauna escorpiônica do Brasil

O Brasil é um país de tamanho continental que apresenta variada posição geográfica, estrutura física e climatologia, além de possuir uma vasta gama de ecossistemas com uma das maiores biodiversidades do mundo (MYERS, 1991; LOYOLA *et al*., 2007). Juntamente com o Equador, Colômbia e Peru, o Brasil possui o maior número de espécies de escorpiões do mundo (LOURENÇO; EICKSTEDT, 2009). Esses aracnídeos encontram-se distribuído em quase todos os continentes, excetuando-se a Antártida (POLIS, 1990).

Cerca de um século atrás, eram conhecidas apenas 40 espécies de escorpiões no país (MAURANO, 1915), número que praticamente quadruplicou em consequência da descrição de novas espécies no final dos anos 1970 e de extensos trabalhos de revisão (LOURENÇO; EICKSTEDT, 2009; PORTO *et al*., 2010). Atualmente são registradas mais de 130 espécies no país com ocorrência em praticamente todos os estados e biomas, sendo a maioria descritas para as regiões Norte e Nordeste do país, com os estados do Amazonas e Bahia como representantes com o maior número de espécies (Figura 1). Apesar disso, a fauna escorpiônica brasileira é considerada subestimada devido à falta de especialistas e amostragens em diversas áreas (BRAZIL; PORTO, 2010).

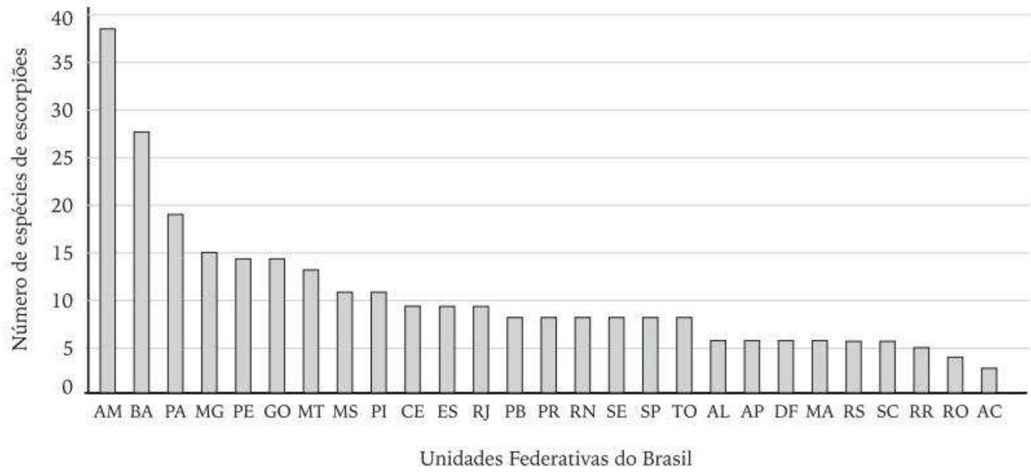

Figura 1. Número de espécies de escorpiões em cada estado brasileiro (BRAZIL; PORTO, 2010).

As 130 espécies catalogadas pertencem às famílias, Liochelidae Fet e Bechly, 2001, Chactidae Pocock, 1893, Bothriuridae Simon, 1880 e Buthidae C. L. Koch, 1837 (Figura 2) (BRAZIL; PORTO, 2010; PORTO *et al*., 2010). Essa última compreende a maior parte da fauna brasileira conhecida, ocorrendo em todos os estados (LOURENÇO, 2002) sendo representada pelos gêneros *Tityus* C. L. Koch, 1836; *Ananteris* Thorell, 1891; *Troglorhopalurus* Lourenço, Batista e Giupponi, 2004; *Physoctonus* (C. L. Koch, 1840); *Rhopalurus* Thorell, 1876; *Microtityus* Kjellesvig-Waering, 1966; *Isometrus* Ehrenberg, 1828 e *Zabius* Thorell, 1893. Destacam-se as espécies de butídeos do gênero *Tityus*, consideradas as únicas capazes de causar acidentes graves em humanos (BRASIL, 2009).

Figura 2. Representantes das famílias registradas para o Brasil. (A) Buthidae; (B) Bothriuridae; (C) Liochelidae e (D) Chactidae. Foto A-B por André F. A. Lira, C por Jave O. Rein e D por Arthur Anker.

A segunda família em representatividade de escorpiões no Brasil é a Bothriuridae, distribuída nas cinco regiões brasileiras, com a maior riqueza concentrada na parte leste do país (BRAZIL; PORTO 2010). Os bothriurídeos brasileiros são representados pelos gêneros *Bothriurus* Peters, 1861; *Thestylus* Simon, 1880; *Urophonius* Pocock, 1893; *Brazilobothriurus* Lourenço e Monod, 2000; *Brachistosternus* Pocock, 1893 (LOURENÇO, 2002). As demais famílias concentram a sua maior riqueza na região Norte, com Liochelidae contendo apenas duas espécies *Opistacanthus cayaporum* Vellard, 1932 e *Opistacanthus borboremai* Lourenço e Fé, 2003. Enquanto os chactídeos são representados pelos gêneros *Auyantepuia* (Gonzales-Sponga, 1978); *Broteochactas* Pocock, 1893; *Brotheas* C. L. Koch, 1837; *Chactas* Gervais, 1844; *Chactopsis* Kraepelin, 1912; *Neochactas* Soleglad e Fet, 2003; *Teuthraustes* Simon, 1878; *Vachoniochactas* Gonzales-Sponga, 1978 e *Hadrurochactas* Pocock, 1893

(LOURENÇO, 2002; BRAZIL; PORTO, 2010). Neste último gênero estão contidas as únicas espécies de chactídeos da região nordeste, *Hadrurochactas brejo* (Lourenço, 1988) e *Hadrurochactas araripe* Lourenço, 2010. De acordo com Lourenço (2010), estudos realizados por paleobiológos sugerem que a Amazônia e a Floresta Atlântica eram conectadas no passado. Desse modo, segundo esse autor, a presença de espécies da família Chactidae em ambas regiões seria mais um suporte à essa teoria.

1.2. Escorpiões da floresta atlântica brasileira

Este bioma abriga em torno de 34 espécies de escorpiões pertencentes a sete gêneros (*Tityus, Ananteris, Bothriurus, Hadrurochactas, Isometrus, Thestylus* e *Zabius*) e três famílias (Buthidae, Bothriuridae e Chactidae) (LOURENÇO, 2002; YAMAGUTI; PINTO DA ROCHA, 2003; ACOSTA *et al*., 2008; ALMEIDA, 2010; LOURENÇO, 2010; PORTO *et al*., 2010; LOURENÇO *et al*., 2013). Os gêneros com maior representatividade são *Tityus* e *Ananteris,* contendo 13 e 5 espécies respectivamente. Nesse ambiente, os escorpiões podem ser encontrados em bromélias, folhiço, sob troncos caídos e na vegetação (LOURENÇO, 1982; LOURENÇO; EICKSTEDT, 1988; LIRA *et al*., 2013; LIRA; DE SOUZA, 2014). A degradação da Floresta Atlântica pode afetar a população de escorpiões como sugerido para a extinção de *T. anneae* Lourenço, 1997, descrito através de exemplares encontrados em Pernambuco na década de 1960, não sendo mais registrada a sua ocorrência desde então (BRAZIL; PORTO, 2010). A degradação desse bioma teve início com a chegada e ocupação dos europeus, a partir do século XVI, restando atualmente, pouco mais de 10% (194.524 km^2) de sua cobertura original (DE ANGELO, 2009; RIBEIRO *et al*., 2009) que era de mais de 3.300 km da costa constando de um grande bloco de florestas perenes e sazonalmente secas (RIBEIRO *et al*., 2009; TABARELLI *et al*., 2010).

No nordeste do Brasil, os remanescentes compreendem pequenos fragmentos isolados um do outro e imersos em uma matriz de campos de cana de açúcar (SILVA; TABARELLI, 2000). Apesar desse fato, a Floresta Atlântica permanece como um bioma que abriga uma elevada riqueza de espécies e taxas de endemismo do mundo (RIBEIRO *et al*., 2009). Levando em consideração o atual estado de degradação da Floresta Atlântica e o fato deste bioma conter um pouco mais de um quarto de todas as espécies de escorpiões do Brasil, é surpreendente a falta de estudos ecológicos sobre estes organismos na Floresta Atlântica brasileira. Dentre esses estudos destaca-se o de Yamaguti e Pinto da Rocha (2006) descrevendo a fenologia de *Thestylus aurantiurus* Yamaguti e Pinto da Rocha, 2003 no Parque Estadual da Serra da Cantareira em São Paulo. A abundância dessa espécie mostrou-se correlacionada com a sazonalidade, aumentando na estação seca, possivelmente associada ao período reprodutivo. Os autores observaram ainda uma seleção de habitat diferenciada entre as estações, com migração dos escorpiões para regiões mais elevadas a fim de evitar os alagamentos de suas tocas.

Em Pernambuco, Costa (2011) registrou a ocorrência de seis espécies (*Tityus stigmurus* (Thorell, 1876), *Tityus neglectus* Mello-Leitão, 1932, *Tityus brazilae* Lourenço e Eickstedt, 1984, *Tityus pusillus* Pocock, 1893, *Ananteris mauryi* Lourenço, 1982, *Bothiurus asper* Pocock, 1893) no Campo de Instrução Marechal Newton Cavalcante (CIMNC), maior fragmento de Floresta Atlântica ao norte do rio São Francisco. O autor comparou a riqueza e abundância dessas espécies em áreas com diferentes históricos de atividades de uso não encontrando diferenças entre elas. Em outro trabalho realizado no CIMNC foi constatado que *T. pusillus* e *A. mauryi* predominaram nesse ambiente compreendendo cerca de 90% da abundância de escorpiões na área (LIRA *et al*., 2013). Mais recentemente, *T. pusillus, T. brazilae* e *T. neglectus* foram também registradas em um brejo de altitude em Pernambuco (LIRA; DE SOUZA, 2014). Dias et al. (2006) registraram a ocorrência de quatro espécies (*T. pusillus, T. stigmurus, T. neglectus* e *A. mauryi*) na Mata do Buraquinho, na Paraíba com o aumento na abundância na estação seca em relação à chuvosa.

1.3. *Tityus pusillus* Pocock, 1893

O gênero *Tityus* destaca-se com cerca de 120 espécies (REIN, 2013), representando a maior riqueza de espécies de butídeos. Representantes deste táxon apresentam uma ampla distribuição nas Américas, podendo ser encontrados desde a Costa Rica (FRANCKE; STOCKWELL, 1987) até a Argentina Central (OJAGUREN-AFFILASTRO, 2005) e Republica Dominicana (ARMAS; ABUD ANTUN, 2004). O gênero *Tityus* foi dividido em cinco subgêneros: *Archaeotityu*s, *Brazilotityus*, *Atreus*, *Tityus* e *Caribetityus* (LOURENÇO, 2006), dos quais os quatro primeiros são registrados para o Brasil (ALMEIDA, 2010).

Espécies do gênero *Tityus* são largamente distribuídas no território nacional, podendo ser encontradas praticamente em todos os biomas brasileiros (LOURENÇO, 2002). No país já foram descritas 47 espécies de escorpiões pertencentes a esse gênero (ALMEIDA, 2010). Em Pernambuco, acidentes com sintomatologia moderada foram descritos para a espécie *T. pusillus* (Figura 3) por Albuquerque *et al*. (2009). Esta espécie apresenta um tamanho entre 25-34 mm e é caracterizada por uma coloração amarelada indo até o marrom-avermelhado com uma pigmentação variegada negra por todo o corpo e com os dois últimos segmentos do metassoma e o télson avermelhados

(LOURENÇO, 1982a). Essas características incluem *T. pusillus* no subgênero *Archaeotityus*, juntamente com as espécies dos grupos *clathratus* e *colombianus* (LOURENÇO, 2006).

Figura 3. Espécime de *Tityus pusillus* Pocock, 1893. Foto do autor

Tityus pusillus foi descrito a partir de dois exemplares fêmeas coletados na região de Igarassu, Pernambuco (LOURENÇO, 1982a). Acreditava-se que esta espécie era endêmica da região subgeográfica do Centro de Endemismo Pernambuco, no entanto ela apresenta uma extensa distribuição na região nordeste do Brasil, podendo ser encontrada nos estados do Sergipe, Bahia, Alagoas, Pernambuco, Paraíba e o Rio Grande do Norte (LOURENÇO, 2002; DIAS *et al*., 2006; PORTO *et al*., 2010), ocorrendo registros para a Floresta Atlântica e a Caatinga (DIAS *et al*., 2006; PORTO *et al*., 2014; LIRA *et al*., 2013).

Os primeiros dados acerca da biologia reprodutiva e ecologia de *T. pusillus* foram descritos por Lourenço (1982a), a partir de uma fêmea e 10 filhotes no dorso da mãe e o provável habitat sob pedra. Esses dados foram obtidos em coleta realizada na Estação Ecológica de Tapacurá (PE). Apesar de sua distribuição e limitada importância médica, não foram encontrados na literatura estudos sobre a biologia de *T. pusillus* posteriores ao realizado por Lourenço (1982a), exceto duas comunicações de congressos indicando a variação no tamanho da ninhada (4-12 filhotes) e a falta de correlação entre o tamanho da fêmea e a quantidade de juvenis (LIRA *et al*., 2010a). Os autores utilizaram 18 fêmeas grávidas com tamanho do prossoma variando de 3,55 a 4,19 mm, coletadas no CIMNC, Aldeia-PE. Além disso, foi registrada a iteroparidade para *T. pusillus*, podendo uma fêmea gerar até três ninhadas ao longo do ano (LIRA *et al*., 2010b).

Nesse mesmo local, foi realizado um estudo no âmbito ecológico, observando-se a influência de fatores abióticos, como a pluviometria, sobre a abundância de *T. pusillus*, com expressivo aumento da população na estação seca (LIRA *et al*., 2013). Além disto, foi registrado para o ambiente de Floresta Atlântica o uso diferencial de microhabitat pela espécie, com os adultos colonizando as camadas superiores do folhiço e os indivíduos jovens, as camadas inferiores. De acordo com os autores, este uso diferencial do ambiente pode estar relacionado com uma estratégia de evitar competição intra-especifica. Os autores encontraram também diferenças comportamentais relacionadas ao estágio de desenvolvimento de *T. pusillus*, com os indivíduos adultos apresentando o comportamento de senta e espera, enquanto os jovens apresentaram-se como forrageadores ativos.

1.4. *Ananteris mauryi* Lourenço, 1982

Ananteris é um gênero Neotropical com registro para todos os países da América do Sul (LOURENÇO, 2004). Dentre as 70 espécies descritas para esse gênero (REIN, 2013), 24 são conhecidas no Brasil (LOURENÇO *et al*., 2013) distribuindo-se em praticamente todos os biomas (Amazônia, Cerrado, Caatinga e Floresta Atlântica) (LOURENÇO, 2004). Segundo o Manual de Controle de Escorpiões do Ministério da Saúde (2009), as principais espécies no país são: *Ananteris balzani* Thorell, 1891; *Ananteris franckei* Lourenço, 1982; *Ananteris mauryi* (Figura 4) e *Ananteris luciae* Lourenço, 1984 (BRASIL, 2009).

Figura 4. Espécime de *Ananteris mauryi* Lourenço, 1982. Foto do autor

Ananteris mauryi é um escorpião de pequeno porte (20-25 mm), caracterizado pela coloração amarela avermelhada com uma pigmentação variegada negra por todo o corpo e com os dois últimos segmentos do metassoma e o télson avermelhados (LOURENÇO, 1982b; LOURENÇO, 2002). Essa espécie foi originalmente descrita para João Pessoa no complexo da Mata do Buraquinho em um fragmento no Campus da Universidade Federal da Paraíba (LOURENÇO, 1982b). Até o ano 2000, a espécie havia sido registrada apenas nos estados da Paraíba e do Rio Grande do Norte (FET *et al*., 2000). Posteriormente, foi descrita para os estados de Pernambuco (LOURENÇO, 2002), Sergipe (DIAS *et al*., 2006) e Bahia (PORTO *et al*., 2010). Lourenço (1986b) sugeriu que esta espécie teria sido isolada durante um recuo da Floresta Atlântica durante um período seco histórico, sendo restrita ao Centro de Endemismo Pernambuco.

No entanto, a nova distribuição sugere a necessidade de revisão da 'hipótese de endemismo' para o grupo *Ananteris* do Nordeste brasileiro (DIAS *et al*., 2006).

Ananteris mauryi está adaptado ao ecossistema da Floresta Atlântica (LOURENÇO, 2002) utilizando a serapilheira como microhabitat (LIRA *et al*., 2013). Nesse ambiente, coloniza o sub-folhiço (camada inferior) durante a estação seca, migrando para o epi-folhiço (camada superior) na estação chuvosa possivelmente devido ao alagamento das porções inferiores da serapilheira. Essa espécie de escorpião é classificada como uma forrageadora ativa (LIRA *et al*., 2013).

A abundância de *A. mauryi* tem se mostrado associada à pluviometria com aumentos significativos no tamanho da população durante a estação seca (DIAS *et al*.. 2006; LIRA *et al.*, 2013). Essa estação corresponde também ao período reprodutivo da espécie, resultando em aparente elevação do número de machos em coletas de campo, devido ao comportamento de procura das fêmeas durante a estação reprodutiva (DIAS *et al*., 2006). Segundo Barbosa *et al*. (2011), o número de filhotes varia entre 16-21, considerado alto para o pequeno porte da espécie. Num estudo recente, Lira *et al*. (2014) registraram a ocorrência da autotomia para *A. mauryi*. Este fenômeno somente foi registrado para indivíduos adultos, sendo mais rápido em machos do que em fêmeas. Os autores levantaram a hipótese de que isso provavelmente está relacionado ao elevado custo no valor adaptativo das fêmeas, uma vez que o indivíduo autotomizado perde o seu télson, podendo reduzir drasticamente a sua capacidade predatória.

2. OBJETIVOS

2.1. Geral

Avaliar a influência de atributos ambientais sobre aspectos da estrutura populacional das espécies de escorpiões *T. pusillus* e *A. mauryi*, que co-habitam em serapilheira na Floresta Atlântica

2.2. Específicos

1. Determinar o efeito da qualidade do ambiente (Área do fragmento, profundidade e peso seco da serapilheira, densidade de árvores, DAP, luminosidade) sobre a abundância de escorpiões das espécies *T. pusillus* e *A. mauryi*.

2. Determinar a proporção de jovens/adultos e a razão sexual de *T. pusillus* e *A. mauryi* nas populações estudadas.

3. Correlacionar o tamanho dos escorpiões adultos das espécies estudadas com os atributos ambientais.

3. HIPÓTESES

- Se a abundância de indivíduos e o tamanho corporal dos adultos estão ligados à estrutura do habitat, espera-se que locais com menor profundidade de serapilheira, densidade de árvores e maior luminosidade, reduza a disponibilidade de habitats e recursos, e consequentemente a abundância das espécies analisadas.

- Por se tratarem de escorpiões com reprodução sexuada a razão esperada entre machos e fêmeas é 1:1 para ambas as espécies.

- Considerando-se a iteroparidade de *T. pusillus* e a elevada taxa de reprodução de *A. mauryi* espera-se que a abundância de jovens dessas espécies sejam maior do que a de adultos.

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OS RESULTADOS SERÃO APRESENTADOS E DISCUTIDOS NA FORMA DE CAPÍTULOS.

How important are environmental factors for the population structure of co-occurring scorpion species in a tropical forest?

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Abstract

Understanding scorpion responses to environmental disturbances in forest remnants is important because, as generalist predators, they exert pressure on a wide variety of arthropod populations that contribute to forest health. In this study, we investigate the drivers of *Tityus pusillus* and *Ananteris mauryi* scorpion abundance in 11 Brazilian Atlantic Forest remnants. Six environmental factors, litter dry weight, remnant area, leaf litter depth, diameter at breast height of tree, canopy openness, and tree density, were assessed. Field surveys were conducted at night using ultraviolet lamps. From a sample of 1,125 captured scorpion specimens, approximately 90% were *T. pusillus* and 7% were *A. mauryi*. The abundance of *T. pusillus,* but not *A. mauryi,* was positively correlated with litter dry weight. Other variables had no effect on the abundance of either species. Our results suggest that, despite living in the same microhabitat, *T. pusillus* and *A. mauryi* may have different ecological requirements since most of the environmental factors were not associated with either population.

Key words: *Ananteris mauryi*, Arachnida, Brazilian Atlantic Forest, habitat fragmentation, population biology, *Tityus pusillus*

Introduction

Arthropods are the most diverse and abundant group of animals in tropical forests (Stork 1988, Giller 1996), and they contribute to several important ecosystem services, such as the maintenance of soil structure, regulation of water resources, nutrient cycling, and decomposition rates of litter (reviewed in Laurance et al. 2002). In tropical forests, soil arthropods and litter-dwelling arthropods may constitute three-quarters of the total animal biomass (Wilson 1990), leading to differential vulnerability to habitat disturbance (Cagnolo et al. 2009). Most habitat disturbances are related to fragmentation (particularly due to habitat loss) and its consequences, and fragmentation is defined as the most remarkable effect that influences arthropod richness and abundance in the environment (Fahrig 2003, Filgueiras et al. 2011, Leal et al. 2012).

By reducing remaining areas increases changes in vegetation structure of the ecosystem and microclimatic conditions (e.g., temperature, humidity, and solar radiation) (Saunders *et al.* 1991, Chen et al. 1999). In tropical forests, vegetal structure hardly influences the amount of sunlight exposure of the soil surface and heterogeneity and physical structure of the litter, directly impacting the populations of litter-dwelling arthropods (Medianero et al. 2007). The abundance of such fauna depends on the manner in which these animals respond to changes in environmental factors, depending on trophic level, dispersion capacity, and habitat specialization (Ewers and Didham 2006). For instance, the relative abundance of wolf spiders, which belong to the family Lycosidae, reduced over a gradient of litter depth, contrasting with the increased abundance of ambush spiders that belong to the families Clubionidae, Thomisidae, and Gnaphosidae (Uetz 1979). Such a difference was attributed to significant changes in prey species richness, litter complexity, and microclimate found across the gradient. In litter manipulation experiments, litter removal, but not litter addition, significantly reduced the abundance of ants, mites, staphylinid beetles, and diplurans in the soil, suggesting a bottom-up effect on soil invertebrates (Ashford et al. 2013).

Amongst litter-dwelling arthropods in tropical forests, scorpions influence energy flow through predation of different invertebrates (Polis 1990, Lourenço 2008). The order Scorpiones is composed of approximately 2,000 species, of which around 50% inhabit tropical forests (Lourenço 2002a, Stockman and Ythier 2010). Despite their widespread distribution and ecological importance, scorpion ecology remains relatively unknown, particularly in tropical forests (Cala-Riquelme and Colombo 2011). As scorpions are highly adapted, their habitat requirements are very specific (Polis 1990); some species, such as *Tityus pusillus* Pocock, 1893 and *Ananteris mauryi* Lourenço, 1982 (Lira et al. 2013), cohabit in the same microhabitat, sharing prey and shelter. Both species inhabit different levels of the leaf litter in remnants of the Atlantic Forest in northeastern Brazil. However, the relative roles of habitat changes and microhabitat requirement in determining species abundance remain unknown. Therefore, studies on scorpion populations in disturbed forest patches may help us understand the biota's responses to environmental changes.

Here, we compared the relative abundance of *T. pusillus* and *A. mauryi* and investigated how these species respond to habitat changes (remnant areas, understory density, diameter at breast height of tree, canopy openness) and microhabitat requirement (litter depth and dry weight). Since different remnants can vary with respect to their environmental attributes, we predicted that a smaller remnant area, shallow leaf litter, low litter weight, low understory density, smaller diameter at breast height, and high canopy openness would negatively affect the litter structure and abundance of *A. mauryi* and *T. pusillus*. Knowing the response of scorpions to these environmental attributes, in addition the particularities of each microhabitat, will contribute to understand the consequences of anthropogenic impacts on the biota.

Materials and methods

Study Area

The biogeographic sub-region of the Pernambuco Center of Endemism (PECE) in the Atlantic forest is one of the most species-rich areas and has high levels of local endemism (Tabarelli et al. 2010). PECE is one of the most important ecological hotspots and has high priority for biodiversity conservation.

Our study was conducted in the municipality of Sirinhaém (8°35'27" S, 35°06'58" W), Pernambuco State, Northeastern Brazil on eleven remnants of Atlantic forest (hereafter F1-F11) (Fig. 1). Remnants ranged in size from 6 to 469 ha and were inserted in a hyper-fragmented landscape of 5,202 ha of hundreds of small, irregular, and isolated patches belonging to Trapiche mill (Leite et al. 2011). The remnants are situated on a low altitude plateau (20 – 120 m above sea level) (Fig. 2A, B), and the predominant soils are composed of latosols, podzolics, and gleysol (Beltrão et al. 2005). Latosols are on flat top sand are deep and well drained. Podzolics are on steep slopes and are moderately deep and well drained. Gleysols are in narrow valley bottoms at lower altitudes and are organic, waterlogged soils (Beltrão et al. 2005). The vegetation is classified as Ombrophilous Dense Forest and is dominated by tree species such as

Bowdichia virgilioides (Leguminosae), *Virola gardneri* (Myristicaceae), *Didymopanax morototoni* (Araliaceae), and *Parkia pendula* (Leguminosae) (Ferraz 2002). All remnants studied are embedded in a matrix of sugar cane *Saccharum officinarum* (Poaceae). The average annual rainfall and temperature for the study site (Trapiche landscape) are 172.66 mm and 25°C, respectively (APAC 2013). According to the Trapiche staff, forest remnants of this area have been under the landowners' protection over the last 10 years.

Scorpion sampling

Six transects of 30 m each were used as sampling units in each forest fragment studied (N = 11, area range: 6-469 ha). Transects were 20 m apart and were established at least 200 m from the edge of each Trapiche landscape remnant. Scorpions up to 5 m away from each transect on either side were collected, resulting in a 300 m^2 sampling area per transect (or 1,800 m^2 per remnant). The abundance of *T. pusillus* and *A. mauryi* in each forest fragment was expressed as the sum of all individuals collected in the six transects. We actively searched scorpions on one night for each remnant between 19 h and 21 h during the new moon phase. Scorpions were captured manually using tweezers. Each transect was covered twice (round-trip) for 1 h by a pair of collectors equipped with ultraviolet lights. Scorpions were collected from December 2012 to January 2013 (the dry season). Each scorpion caught was individually stored in a vial containing 70% ethanol. The specimens were identified according to the methods described by Lourenço (2002b). Voucher specimens were deposited in the Arachnological Collection of the Universidade Federal de Pernambuco, Brazil.

Habitat structure

The six variables used to characterize the studied remnants were: i) Size of remnant: studied fragments were digitalized from the Google Earth® satellite images and forest remnant areas were calculated using the ArcGis 10.1.program (ESRI 2012); ii) Understory density: this was estimated by counting how many times live vegetation more than 1.3 m above ground touched the transect tape that was established to delineate the area for scorpion sampling; iii) Diameter at breast high of tree (DBH): DBH was estimated by measuring the diameters of all trees that touched the transects; iv) Canopy openness: this was measured using digital hemispherical photographs taken at the center of each transect and analyzed using Gap Light Analyzer 2.0. (Frazer et al. 1999); v) Depth of the leaf litter: three quadrats (25 × 25 cm) in each of the six transects (18 quadrats/remnant) 10 m apart from each other served as the basis for estimating the depth of the leaf litter, which was measured with a ruler at the corners of each quadrat; vi) Litter dry weight: in the same quadrat, dry litter was collected after the twigs were removed and desiccated in an oven at 50°C to 55°C for at least 24 h. Except for the size of the forest remnant, environmental variable values for each fragment were expressed as the mean of the measurements obtained from the six transects from each of the 11 fragments studied.

Data Analysis

Correlations between all the environmental factors and the relative abundance of *T. pusillus* and *A. mauryi* were analyzed using redundancy analysis ordination (RDA) by means of a “manual selection of environmental variables" in the program CANOCO v. 4.5 (ter Braak and Šmilauer 2002). This procedure allows accessing the effect of each environmental factor on the relative density of species as the only explanatory variable. The significance of each environmental variable in the RDA was tested by a partial Monte Carlo permutation test with 9,999 permutations (McCune and Grace 2002, Lepš and Šmilauer 2003). In this test, the environmental factors already selected in the RDA model are used as the co variables, which decrease the redundancy (multicollinearity) of the dataset (Lepš and Šmilauer 2003). To assess the degree of relationship between the density of *T. pusillus* and *A. mauryi* and the environmental factors selected by the RDA, a normal multivariate linear regression (Hidalgo and Goodman 2003, Johnson and Wichern 2007) was performed in the program Systat v. 12 (Wilkinson 2007).

Results

A total was 1,125 scorpion specimens were collected. From these approximately 90% were *T. pusillus* and 7% were *A. mauryi*. Both *T. pusillus* and *A. mauryi* individuals (Table 1) were collected in all 11 remnants averaging 92.0 ± 62.0 and 7.8 ± 5.8 individuals, respectively. *Tityus brazilae* Lourenço and Eickstedt, 1984, *Tityus neglectus* Mello-Leitão, 1932 and *Tityus stigmurus* (Thorell, 1876) were also collected comprising only 2.2% of the samples and were not analyzed.

Overall, environmental factors explained 82.4% of the variability of both species abundance, of which 69.5% was explained by Axis 1 and 30.5% by Axis 2 of the RDA.

Only the litter dry weight was correlated with the scorpions' abundance in the fragments (RDA: F = 6.43; P = 0.0027) and was higher for the *T. pusillus* population (Fig. 3). *T. pusillus* was predominant in all remnants except in F10. This remnant was characterized by having the lowest litter dry weight (Table 1). In contrast, *A. mauryi* showed no reduction in abundance in F10 but was predominant in F11 (Table 1), the largest remnant, although there was no relationship between fragment size and abundance (Table 2).

The relationship between the litter dry weight (the variable choose in the RDA) and the relative abundance was confirmed in the multivariate linear regression (Wilks's Lambda = 0.132; $F_{2,8}$= 26.35; p < 0.001), but only for *T. pusillus* ($F_{1,9}$ = 19.67; P = 0.002; R^2 = 0.651) , and not to *A. mauryi* ($F_{1,9}$ = 2.98; P = 0.118; R^2 = 0.166) in the univariate space.

Discussion

Tityus pusillus and *Ananteris mauryi* are two widespread scorpion species in the Brazilian Northeast Atlantic Forest (Lourenço 2002b, Dias et al. 2006; Porto et al. 2010) and they co-occur at a local scale (Dias et al. 2006, Lira et al. 2013). This study showed that they are the most abundant scorpion species in this biome and that *T. pusillus* is the predominant species. Except for *T. pusillus* something else abundance showed a positive correlation with leaf litter dry weight, both species were unresponsive to the environmental factors studied in this work. Variations in remnant size, DBH, leaf litter depth, and canopy openness had no effect on the abundance of both scorpion species. However, a greater number of *T. pusillus* occurred in remnants with higher litter dry weight, which suggests differences in the response of the species to environmental factors on a smaller scale.

Differential microhabitat use and predatory behavior displayed by these species (Lira et al. 2013) is likely to play an important role in such differential responses. Although both scorpion species colonize leaf litter, they exhibit microhabitat segregation related to their size and litter depth. Adults of the larger species, *T. pusillus*, usually inhabit the upper layer, while adults of the smaller species, *A. mauryi*, occupy both the bottom and upper layers, particularly during the wet season (Lira et al. 2013). Similar distribution patterns have been described in other arthropod groups such as spiders (Wagner et al. 2003) and centipedes (Gunther et al. 2013). Differences in habitat structure (amount of litter in the litter layer), density of prey species, and density of predators may be the probable causes of these distribution patterns in spiders (Uetz 1991). Sparse structure allows easier foraging by predators but generally contains fewer profitable prey, while dense structure contains abundant, highly profitable prey (Chen and Wise 1999). Thus, the positive correlation between *T. pusillus*, an ambush predator (Lira et al. 2013), and leaf litter biomass, may be influenced by litter structure, since low leaf litter dry weight may indicate low prey availability. This, in turn, may reduce the predatory efficiency of wandering foragers such as *A. mauryi* (Lira et al. 2013) that have to actively hunt for prey in the interstices between the leaves. Reduced prey capture performance due to difficulties in detecting collembolan prey species was attributed to increasingly complex habitat structure in laboratory studies using the centipede *Lithobius mutabilis* L. Koch, 1862 (Kalinkat et al. 2012). Further studies in the field found a lower consumption frequency of prey by *L. mutabilis* in higher amounts of litter (Gunther et al. 2013). Others laboratory studies in which spiders prey on collembolans (Vucic-Pestic et al. 2010) and tardigrades (Hohberg and Traunspurger 2005) prey on nematodes showed that habitat structure significantly affected hunting and was a regulating factor in predator–prey interactions in food webs (Schmitz and Suttle 2001).

Overall, our work suggests that environmental factors on a microhabitat scale rather than broad-scale variation in the forest fragments had more influence on the abundance of the leaf litter scorpions *T. pusillus* and *A. mauryi* in the Brazilian Atlantic Forest. Furthermore, differences related to hunting behavior (sit-and-wait or wandering) and microhabitat selection may explain the influence of dry litter weight on the abundance of *T. pusillus*, but not of *A. mauryi*.

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Table 1. Abundance of Tityus pusillus and Ananteris mauryi, and environmental factors (mean ± SD) measured in 11 Atlantic forests remnants.

	Scorpion abundance		Environmental Attributes					
Remnants	*Tityus pusillus*	*Ananteris mauryi*	Understory density	DBH (cm)	Cannopy opennes (%)	Leaf litter depth (cm)	Litter dry weigth (g)	Area (ha)
F1	155	3	3.66 ± 1.86	7.54 ± 6.06	2.63 ± 0.36	3.55 ± 2.25	0.216 ± 0.10	6.43
F2	37	16	5.66 ± 0.81	9.94 ± 2.66	2.93 ± 0.21	4.83 ± 2.64	0.207 ± 0.12	8.27
F3	37	3	6.66 ± 3.14	19.47 ± 50.53	2.85 ± 0.31	1.73 ± 1.21	0.144 ± 0.03	10.53
F4	86	5	3.50 ± 1.51	18.85 ± 3.78	4.32 ± 0.49	7.79 ± 3.58	0.252 ± 0.11	18.52
F5	47	1	6.50 ± 3.61	12.11 ± 4.62	3.28 ± 0.35	4.76 ± 5.23	0.181 ± 0.09	33.51
F6	173	9	6.66 ± 1.36	11.52 ± 9.58	3.07 ± 0.33	12 ± 5.14	0.416 ± 0.18	46.08
F7	50	6	6.66 ± 1.50	12.71 ± 1.28	3.43 ± 0.31	6.57 ± 3.72	0.184 ± 0.11	75.80
F8	96	10	6.66 ± 1.21	11.21 ± 13.08	3.77 ± 0.24	7.88 ± 4.55	0.336 ± 0.20	120.70
F9	177	4	10 ± 3.16	10.48 ± 11.69	3.05 ± 0.39	4.19 ± 2.78	0.396 ± 0.17	197.52
F10	2	9	6 ± 1.41	22.58 ± 2.07	4.11 ± 0.33	5.45 ± 2.87	0.114 ± 0.05	280.33
F11	153	20	4.66 ± 3.38	11.06 ± 6.62	4.29 ± 0.54	4.71 ± 2.92	0.489 ± 0.17	469.76

Table 2. Summary of Redundancy Analysis (RDA) between the correlation of environmental factors and the abundance of Tityus pusillus and Ananteris mauryi. The first and second canonical variates explained 82.4% of the variability in the scorpions' abundance caused by the environmental factors used in the RDA.

					Axis 1 (Eigenvalue = 0.81)		Axis 2 (Eigenvalue = 0.11)	
Environmental variable	Variation coefficient	Variance explained (%)	F	P	Correlation	Coefficient	Correlation	Coefficient
Litter dry weight	0.417	50.61	6.43	0.0027	0.708	-2.343	0.531	0.773
Understory density	0.198	24.03	4.10	0.061	-0.334	-0.079	0.506	0.120
Remnant area	0.122	14.81	3.25	0.086	0.619	2.1597	-0.092	0.2319
Canopy openness	0.069	8.37	2.14	0.1732	0.252	-0.311	-0.193	-0.245
Litter depth	0.017	2.06	4.79	0.58	0.168	1.179	0.307	-0.218
DBH	0.001	0.46	0.09	0.8191	-0.423	-0.6187	-0.350	-0.1976

Figure captions

Figure 1. Study area showing the spatial position of the 11 Trapiche remnants.

Figure 2. A. Remnant situated on a low altitude plateau and immersed on sugar cane matrix. B. Habitat occupied by *Tityus pusillus* and *Ananteris mauryi* in the remnants studied: Ombrophilous Dense Forest from Trapiche landscape.

Figure 3. RDA ordination diagram of the abundance of scorpions *Tityus pusillus* and *Ananteris mauryi*, and the litter dry weight (g) in 11 Atlantic Forest remnants. The litter dry weight (dashed line) explained 41.7% of the variability in the scorpions' abundance (solid lines).

Figure 4. Relationship between litter dry weight and abundance of scorpions *Tityus pusillus* and *Ananteris mauryi* in 11 Atlantic forests remnants. *Significant linear relationship ($p<0.05$).

Figure 1.

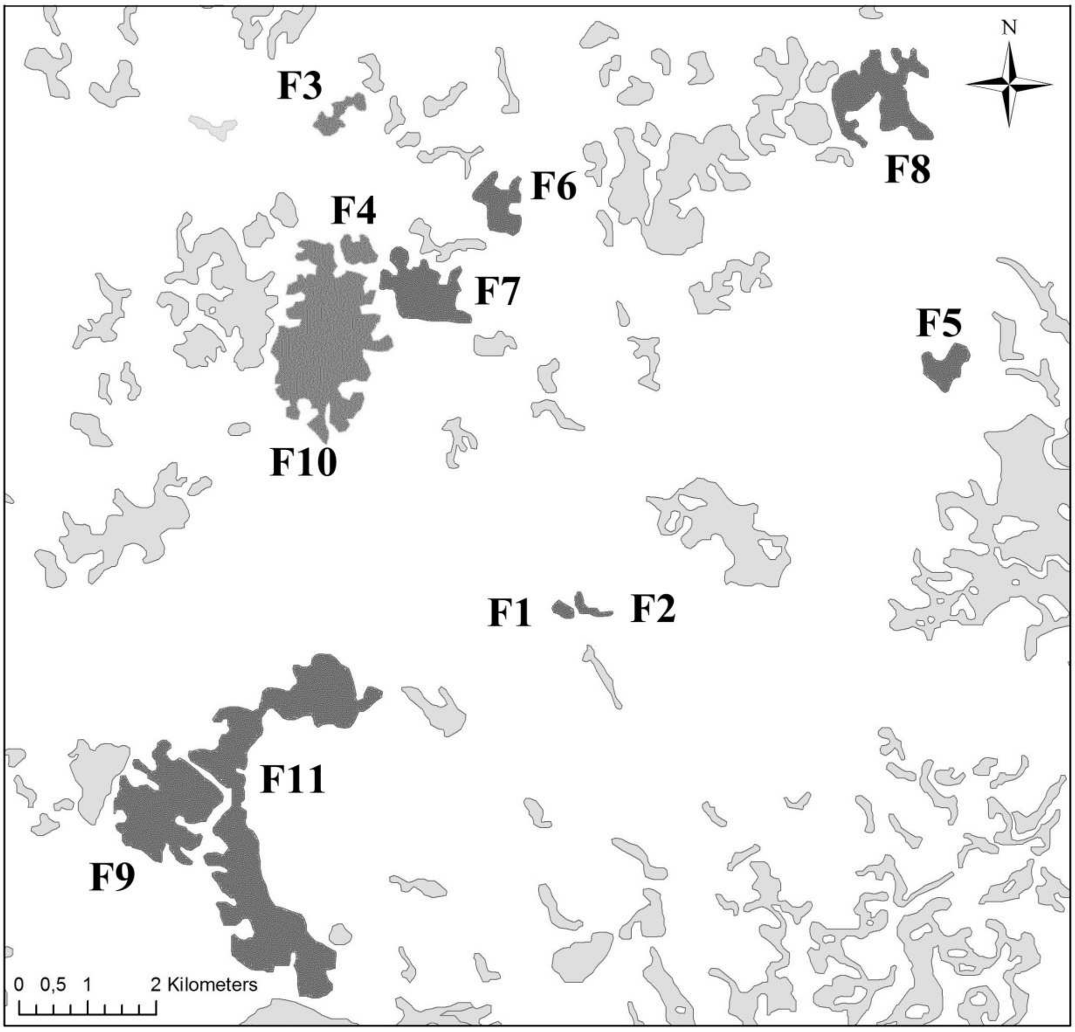


Figure 2.

Figure 3.

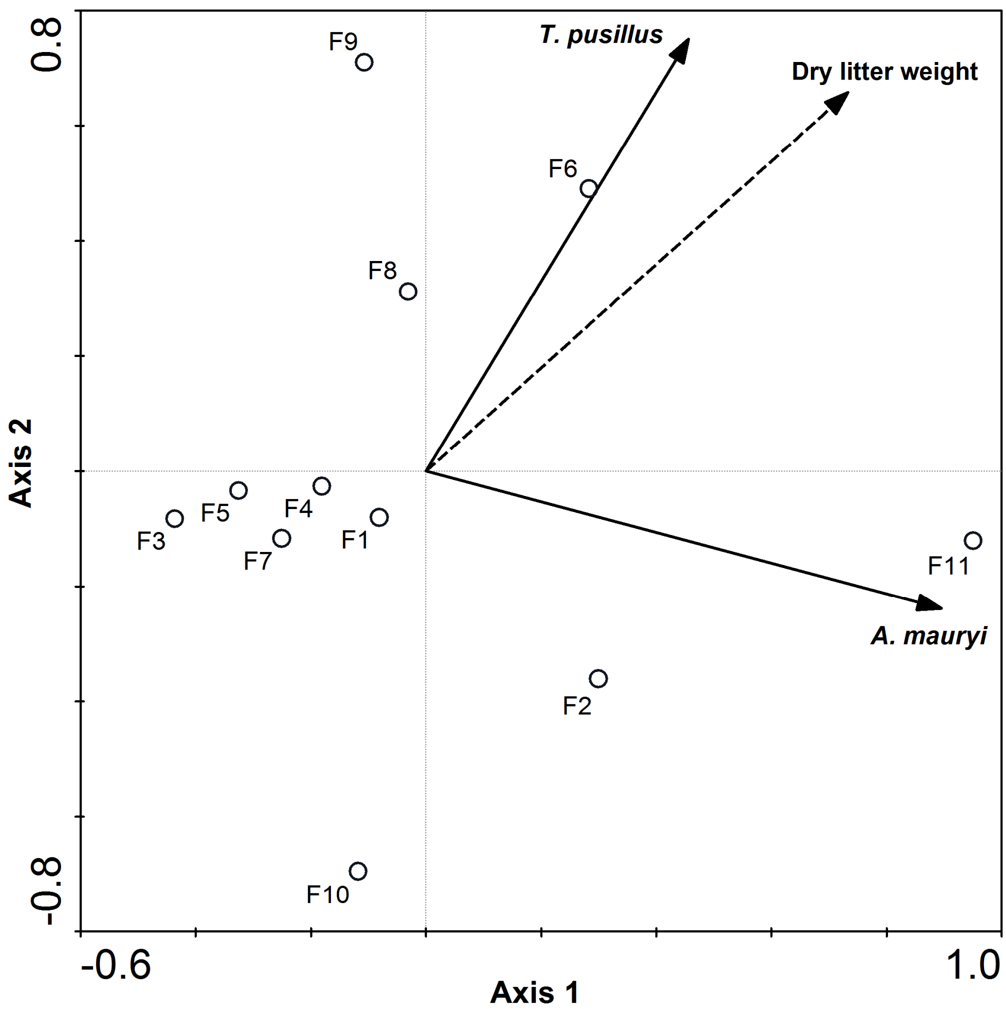
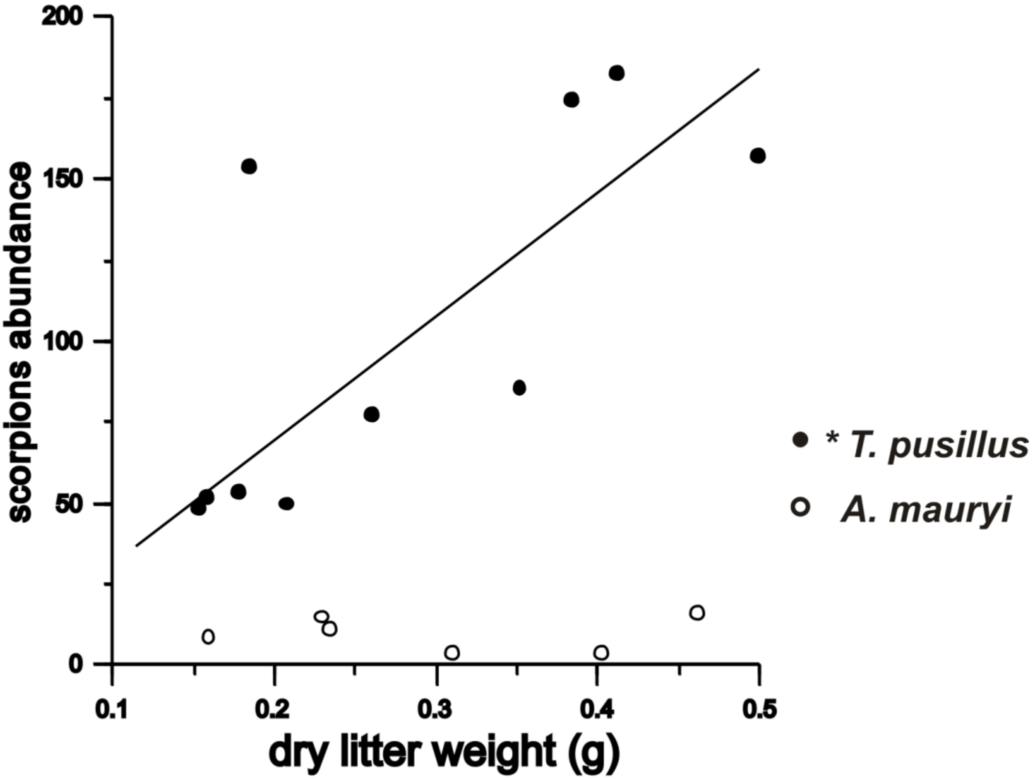

0.8
-0.8
Axis 2
-0.6
1.0
Axis 1
T. pusillus
Dry litter weight
A. mauryi
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11

Figure 4.


200
150
100
50
0
scorpions abundance
0.1
0.2
0.3
0.4
0.5
dry litter weight (g)
* T. pusillus
A. mauryi

CAPÍTULO 2: Parâmetros populacionais de *Tityus pusillus* e *Ananteris mauryi* em fragmentos de Floresta Atlântica de Pernambuco

A razão sexual das espécies *T. pusillus* e *A. mauryi* diferiu nos ambientes analisados, com predominância de machos na população de *A. mauryi* em oito dos 10 fragmentos amostrados, contrastando com *T. pusillus* com um número mais elevado de fêmeas na população estudada (Tabela 1). Esses resultados, contudo, não significam que os machos são mais frequentes nas populações de *A. mauryi*. De acordo com Polis (1990), a proporção sexual esperada de escorpiões com reprodução sexual é 1:1. Contudo diferentes fatores podem influenciar o número de indivíduos capturados durante as coletas como, por exemplo, estação reprodutiva das espécies. Nesse período, os machos costumam ter maior mobilidade uma vez que saem à procura de fêmeas facilitando sua captura. A maior quantidade de machos de *A. mauryi* encontrado nos fragmentos analisados, particularmente XA2 e BA (Tabela 1), pode ser um indicativo da possível estação reprodutiva desta espécie. Ao contrário, os valores encontrados abaixo do 1:1 para *T. pusillus* (Tabela 1) sugerem que essa espécie não se encontra no período de acasalamento, sendo provável que *T. pusillus* e *A. mauryi* apresentem estação reprodutiva diferenciada. Essa possível diferença entre as estações reprodutivas das duas espécies pode ser interpretada como um mecanismo adaptativo para evitar possíveis encontros, uma vez que essas espécies colonizam o mesmo microhabitat, competindo por alimento e abrigo, além da predação da espécie de maior tamanho *T. pusillus* sobre *A. mauryi* (LIRA *et al*., 2013).

Durante o período de coleta, a presença de jovens de *A. mauryi* só foi registrada em dois fragmentos com um individuo em cada. Desse modo, não foi possível verificar a proporção entre adultos e imaturos para essa espécie. No mesmo período, a presença de imaturos de *T. pusillus* foi observada em todos os fragmentos analisados, embora em abundância do que os adultos (Tabela 1). Essa observação reforça a hipótese de período reprodutivo diferencial para as espécies de escorpiões estudadas. Mais estudos sobre a ecobiologia e comportamento dessas espécies são necessários para se obter conclusões mais precisas sobre as interações entre elas e de como essas interações podem afetar a dinâmica populacional de espécies co-especificas que habitam em ambiente de Floresta Atlântica.

Fragmentos	Espécies		
	Tityus pusillus		*Ananteris mauryi*
	Razão sexual (machos/fêmeas)	**Proporção jovens/adultos**	**Razão sexual (machos/fêmeas)**
F1	0,13:1	0,48:1	1,28:1
F2	0,34:1	0,51:1	0,5:1
F3	0,36:1	0,42:1	3:1
F4	0,75:1	0,75:1	1:1
F5	1,16:1	0,80:1	-
F6	0,63:1	1,26:1	1,25:1
F7	0,78:1	-	2:1
F8	0,62:1	0,37:1	1:1
F9	0,29:1	0,31:1	3:1
F10	-	-	1,5:1
F11	0,60:1	0,48:1	0,81:1

- ausência de adultos

Tabela 1. Razão sexual de *Tityus pusillus* e *Ananteris mauryi* e proporção jovens/adultos da primeira espécie em fragmentos de Floresta Atlântica de Pernambuco.

Nos fragmentos amostrados o tamanho de adultos de *T. pusillus* variou entre 29,3 e 43,6 mm, enquanto *A. mauryi* foi de 16,2 a 25,9 mm. Dentre os atributos analisados, a análise de RDA evidenciou relação apenas entre a profundidade da serapilheira e tamanho dos adultos de *T. pusillus* e *A. mauryi* (RDA: F=14,7; P=0,0112) (Figura 1). No entanto essa relação não se manteve quando as populações dessas espécies foram analisadas individualmente através do GLM para verificar diferenças entre as médias de *T. pusillus* ($F_{1,7}$=0.001; p=0.978, R^2<0.0001) e *A. mauryi* ($F_{1,7}$=0.014; p=0.910, R^2 = 0.046). Possivelmente na primeira análise ocorreu um erro tipo I pela RDA, ocasionado pelo baixo n (9) amostral. Para estas análises dois fragmentos foram excluídos devido à falta de indivíduos adultos, Tauá e Pedra do Cão, para *T. pusillus* e *A. mauryi* respectivamente.

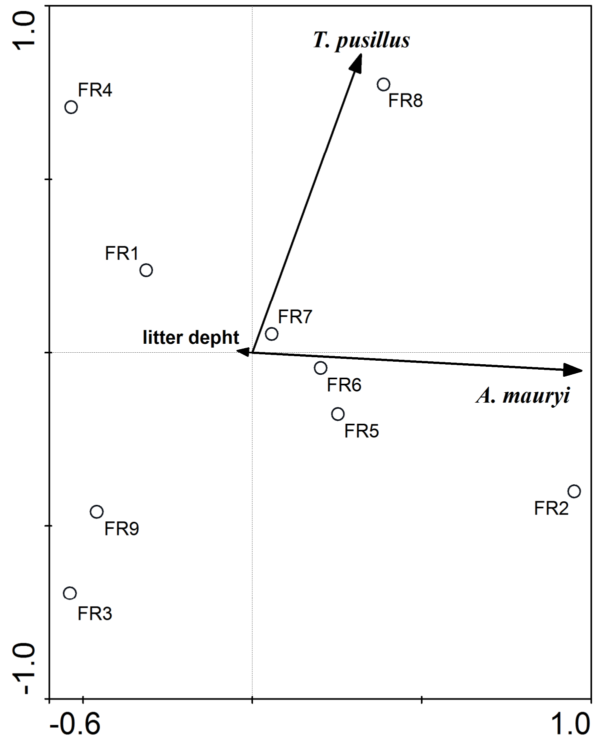


Figura 1. Resultado da RDA, mostrando a relação entre a profundidade da serapilheira e a abundânciade *Tityus pusillus* e *Ananteris mauryi* em 11 fragmentos da Floresta Atlantica de Pernambuco.

Portanto, não encontramos apoio para a nossa previsão de que o tamanho corporal do adulto seria influenciado pela área do remanescente e atributos ambientais, tais como a profundidade da serapilheira, o que está associado a uma alta abundância de presas (SAYER *et al*., 2010; SILVEIRA *et al* ., 2010). Na maioria dos artrópodes, a qualidade e quantidade do alimento durante o desenvolvimento inicial tem demonstrado que afetam o crescimento e, em última análise, o tamanho do corpo adulto (SCRIBER; SLANSKY, 1981; TAMMARU, 1998). É possível que os predadores generalistas, como *T. pusillus* e *A. mauryi* tendem a ser menos suscetíveis à variação na abundância de presas e diversidade associada a condições ambientais. Por outro lado, a redução do tamanho corporal em rola bostas tem demonstrado estar relacionado com uma diminuição na disponibilidade de alimentos (FILGUEIRAS *et al*., 2011), como consequência da extinção de grandes mamíferos e a diminuição drástica nas populações de aves devido a fragmentação florestal (LOVEJOY *et al*., 1986; STOUFFER; BIERGAARD, 1995). Os valores mais baixos de excrementos e carcaças utilizadas como recursos por escaravelhos seria a principal causa da redução do tamanho do corpo (FILGUEIRAS *et al*., 2011).

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CONCLUSÕES FINAIS

1. *Tityus pusillus* é a espécie de escorpião predominante na Floresta Atlântica Sul de Pernambuco.
2. Apesar das espécies *T. pusillus* e *A. mauryi* compartilharem o mesmo microhabitat, elas apresentam diferentes requerimentos ecológicos.
3. A profundidade da serapilheira é o único atributo ambiental que exerce influência na abundancia de *T. pusillus*, mas não afeta *A. mauryi*.
4. Os atributos ambientais analisados não influenciam o tamanho dos adultos de *T. pusillus* e *A. mauryi*
5. A proporção entre jovens e adultos e a razão sexual das espécies estudadas nesse trabalho indica uma diferenciação em relação à estação reprodutiva.

ANEXO

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Format and organization

The manuscript should be **double spaced**, on paper 21.5 cm × 28 cm (8.5 in. × 11 in. or ISO A4) **with continuous numbered lines**. Each page should be numbered, beginning with the title page. For material that is to be set in italics, use an italic font; do not underline. Use capital letters only when the letters or words should appear in capitals.

All articles must contain a title page (p. 1) and an abstract (p. 2), followed by Introduction (p. 3), Materials and methods, Results, Discussion, and Acknowledgements sections, plus references, tables, figure captions, and appendices. (See descriptions of each part of the manuscript, below.) Tables and captions for illustrations should be on separate pages. Supplementary material should be clearly denoted and uploaded as part of the electronic submission process. Note that Notes, Reviews, and Comments do not necessarily have Introductions, Material and methods, Results, and Discussions.

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Authors can set up their manuscript to maximize its online discoverability by following a few simple guidelines. Because the Title and Abstract are free to all readers and because most search engines give extra weight to keyword phrases in headings and to repeated phrases, wording of the Title and Abstract is especially important to increase the chance your paper will be found. Follow the instructions below when writing your Title and Abstract; include key phrases you feel a reader would use when conducting a literature search in the area of your paper.

Title

Titles not only provide information for alerting and information retrieval services, they are also the most heavily weighted element of a paper for online search engines. Therefore titles should contain important descriptive phrases that relate to the topic, stating information such as the experimental organism, specific behaviour, modifying agent, and key result. Titles should be brief and clear. Common names and correct taxonomic names should both be included in the title, as in the example "The cuticle of tephritid fruit flies (Urophora spp.)". Do not include authorities in taxonomic names.

Title page

The title page should contain the following. (*i*) The full title of the paper. (*ii*) Authors' names (with initials only) listed in the order in which they are to appear at the head of the printed article. (*iii*) Affiliation and address (including e-mail address) for each author. This should reflect the affiliation and address at the time of the study. Indicate current affiliations and addresses (including e-mail addresses) that differ from those in the by-line in a footnote. (*iv*) Name, address, telephone number, fax number, and e-mail address of the author responsible for correspondence.

Abstract

An abstract is required for every contribution. Its content is particularly important for alerting services, search engines, and for readers, who scan the abstract to decide whether to download and read the rest of the paper. The abstract should be well written and contain three to four descriptive keyword phrases that will draw the reader to the content. Because search engines look for duplication of terms, repeating keyword phrases in the title and abstract increases the chance that a paper will be found during an online search; care should be taken, however, because excessive repetition of a term can cause a search engine to reject a Web page.

The abstract should state the academic rationale (purpose) of the work, the design and methods used in the study, key results and trends, and lastly implications and conclusions of your work. It should **not be more than 200 words** and appear on a separate page. **All authors' names and initials, as well as the complete title of the paper,** must be included at the top of the page (a copy of this page is sent separately to the translator). Authors able to submit abstracts in both fluent English and French are encouraged to do so. Abstracts submitted in one language will be translated into the other official language by the Journal translator. References should not be cited in the abstract unless they are absolutely essential, in which case full bibliographic information must be provided.

Key words

Authors must provide a minimum of five key words below the Abstract, two of which must be the scientific name of the study species and the common name of the study species.

Introduction

Limit the Introduction largely to the scope and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. The Introduction should finish with a clear statement of purpose for the work.

Materials and methods

The **degree of reproducibility of experiments** should be indicated either in general statements in Materials and methods and Results or, preferably, as statistical treatments of numerical data cited in tabular or graphic form.

The **experimental** or **computational** material must be sufficiently detailed to permit reproduction of the work, but must be concise and avoid lengthy descriptions of known procedures; the latter should be specified by appropriate references. **A reader's**

attention should be drawn to any new or unusual hazards encountered in the experimental work.

Sources of biological materials, experimental methods, geographical locations, and statistical methods should be described. Precise locations of rare and endangered organisms should not be divulged. Sources of commercially available laboratory of field equipment and fine chemicals should be indicated in parentheses; list the company name, city, and country.

Results

The Results section should contain only enough explanation and interpretation to allow the reader to understand why experiments or observations were carried out and what they mean. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Authors should ensure that the number of significant digits used to describe their data does not exceed the accuracy with which the measurement can be made. For numbers from –1 to 0 and from 0 to +1, the decimal must be preceded by a zero in text, tables, and figures. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text (see Supplementary material section).

Discussion

The Discussion section should not rehash results and **contain no new findings that have not already been mentioned under Results**. Conclusions should be put under Discussion, not as a separate section.

Acknowledgements

Acknowledgements should be written in the third person and kept to a concise recognition of relevant contributions.

Footnotes

Footnotes to material in the text should not be used unless they are unavoidable, but their use is encouraged in tables. Where used in the text, footnotes should be cited in the manuscript by superscript Arabic numbers (except in the tables, see below) and should be numbered serially beginning with any that appear on the title page. Each footnote should be typed on the manuscript page upon which the reference is made; **footnotes should not be included in the list of references**.

Equations and list of symbols

Equations should be clearly typed; triple-spacing should be used if superscripts and (or) subscripts are involved. Superscripts and subscripts should be legible and carefully placed. Distinguish between lowercase l and the numeral one, and between capital O and the numeral zero. A letter or symbol should represent only one entity and be used consistently throughout the paper. Each variable must be defined in the text or in a **List**

of symbols to appear after the reference list. Variables representing vectors, matrices, vector matrices, and tensors must be clearly identified. Numbers identifying equations must be in parentheses and placed flush with the **left margin**. In numbering, no distinction is made between mathematical and chemical equations.

References

The author is responsible for verifying each reference against the original article. Each reference must be cited in the text using the surnames of the authors and the year, for example, (Walpole 1985) or Green and Brown (1990). Depending on the sentence construction, the names may or may not be in parentheses, but the year always is. If there are three or more authors, the citation should give the name of the first author followed by et al. (e.g., Green et al. 1991). If references occur that are not uniquely identified by the authors' names and year, use *a*, *b*, *c*, etc., after the year (e.g., Green 1983*a*, 1983*b*; Green and Brown 1988*a*, 1988*b*) for the text citation and in the reference list.

Uniform reference locators (**URLs**) or digital object identifiers (**DOIs**) are useful in locating references on the Web, and authors are encouraged to include these; they should be added to the reference in the reference list (see example below).

Unpublished reports, private communications, and in-press references

References to unpublished reports, private communications, and papers submitted but not yet accepted are not included in the reference list but instead must be included as footnotes or in parentheses in the text, giving all authors' names with initials; for a private communication, year of communication should also be given (e.g., J.S. Jones (personal communication, 1999)). If an unpublished book or article has been**accepted for publication**, include it in the reference list followed by the notation "In press". Do not include volume or page number in an in-press reference, as these are subject to change before publication. Authors must confirm in their covering letter that papers cited as "In press" have been accepted for publication.

Presentation of the list

The **reference list** must be double-spaced and placed at the end of the text. References must be listed in alphabetical order according to the name of the first author and not numbered. References with the same first author are listed in the following order. (*i*) Papers with **one author only** are listed first in chronological order, beginning with the earliest paper. (*ii*) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (*iii*) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically. For example,

Wilson 2000
Wilson and Andrews 1999
Wilson and James 1987

Wilson, James, and Andrews 1986
Wilson, Andrews, and James 1988

General guidelines on references

References should follow the form used in current issues of the Journal. The names of serials are abbreviated in the form given in Chemical Abstracts Service Source Index (CASSI) (Chemical Abstracts Service, 2540 Olentangy River Road, P.O. Box 3012, Columbus, OH 43210-0012, USA). In doubtful cases, authors should write the name of the serial in full. The Journal encourages the inclusion of issue numbers which should be placed in parentheses after the volume number. References to **nonrefereed documents**(e.g., environmental impact statements, contract reports) must include the address where they can be obtained. The following bibliographic citations illustrate the punctuation, style, and abbreviations for references.

Examples of types of references, including electronic references

Journal article with DOI:

Sterling, J.T., and Ream, R.R. 2004. At-sea behavior of juvenile male northern fur seals (*Callorhinus ursinus*). Can. J. Zool. **82**(10): 1621–1637. doi:10.1139/Z04-136.

Journal article with URL:

Sterling, J.T., and Ream, R.R. 2004. At-sea behavior of juvenile male northern fur seals (*Callorhinus ursinus*). Can. J. Zool. **82**(10): 1621–1637. Available from http://pubs.nrc-cnrc.gc.ca/cgi-bin/rp/rp2_abst_e?cjz_z04-136_82_ns_nf [accessed 28 October 2005].

Journal article available online only (with DOI):

van der Sanden, J.J., and Hoekman, D.H. 2005. Review of relationships between grey-tone co-occurrence, semivariance, and autocorrelation based image texture analysis approaches [online]. Can. J. Remote Sens.**31**(3): 207–213. doi:10.1139/rs03-011.

Entire issue of journal:

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Report:

Sanders, W.W., Jr., and Elleby, H.A. 1970. Distribution of wheel loads in highway bridges. National Cooperative Highway Research Program Rep. No. 83, Transportation Research Board, National Research Council, Washington, D.C.

Book:

Williams, R.A. 1987. Communication systems analysis and design. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Book in a series:

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Part of book:

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. McNeil and D.C. Himsworth. Oregon State University Press, Corvallis. pp. 203–229.

Paper in conference proceedings:

Kemp, A.L.W. 1969. Organic matter in the sediments of Lakes Ontario and Erie. *In* Proceedings of the 12th Conference on Great Lakes Research, Ann Arbor, Mich., 5–7 May 1969. International Association for Great Lakes Research, Ann Arbor, Mich. pp. 237–249.

Institutional publications and pamphlets:

Dzikowski, P.A., Kirby, G., Read, G., and Richards, W.G. 1984. The climate for agriculture in Atlantic Canada. Available from the Atlantic Advisory Committee on Agrometeorology, Halifax, N.S. Publ. ACA 84-2-500. Agdex No. 070.

Corporate author:

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Thesis:

Keller, C.P. 1987. The role of polysaccharidases in acid wall loosening of epidermal tissue from young*Phaseolus vulgaris* L. hypocotyls. M.Sc. thesis, Department of Botany, The University of British Columbia, Vancouver, B.C.

Web site citation:

Quinion, M.B. 1998. Citing online sources: advice on online citation formats [online]. Available fromhttp://worldwidewords.org/articles/citation.htm [accessed 20 October 2005].

Translation:

Koike, A., and Ogura, B. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. [Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.]

Tables

Tables must be typed on separate pages, placed after the list of references, and numbered with Arabic numerals in the order cited in the text. The title of the table should be a concise description of the content, no longer than one sentence, that allows the table to be understood without detailed reference to the text, and should include the common name and binomen of the focal organism. Column headings should be brief, but may be amplified by footnotes. Vertical rules should not be used. A copy of the Journal should be consulted to see how tables are set up and where the lines in them are placed. Footnotes in tables should be designated by symbols (in the order *, †, ‡, §, ||, ¶, #) or superscript lowercase italic letters. Descriptive material not designated by a footnote may be placed under a table as a **Note**. Numerous small tables should be avoided, and the number of tables should be kept to a minimum.

Figure captions

Figure captions should be listed on a **separate page** and placed after the tables. The caption should informatively describe the content of the figure, without need for detailed reference to the text, and should include the common name and binomen of the focal organism. Experimental conditions should not be included, but should be adequately covered in the Methods. For graphs, captions should not repeat axis labels, but should describe what the data show. A single caption can be provided for multipart (composite) figures, with necessary details on the separate parts, identified by their individual labels. If the separate parts require enough information to warrant separate captions, then the composite should be separated into individual figures.

Appendices

An appendix should be able to stand alone, as a separate, self-contained document. Figures and tables used in an appendix should be numbered sequentially but separately from those used in the main body of the paper, for example, Fig. A1, Table A1, etc. If references are cited in an appendix, they must be listed in an appendix reference list, separate from the reference list for the article. If there is more than one appendix, label as follows: Appendix A, Appendix B, etc.

Supplementary material

Supplementary material (or data) consists of extra tables, figures (maps), detailed calculations, and data sets produced by the authors as part of their research, but not essential for understanding or evaluating the paper, and not published with the article in the print edition of the journal. This material is never edited, converted, or scanned, and therefore will appear exactly as submitted. This is to prevent any errors from being inadvertently introduced during file manipulation or printing. Tables and figures should be numbered in sequence separate from those published with the paper (e.g., Fig. S1, Table S1), and all supplementary material should be referred to in the manuscript by footnotes.

Supplementary material must be submitted with the article, in electronic format. During Web submission (ScholarOne), relevant files should be attached under "Supplementary data”. The electronic copy will be made available in its native file format on the Journal Web site at no cost to readers.

Illustrations

Each figure or group of figures should be planned to fit, after appropriate reduction, into the area of either one or two columns of text. The maximum finished size of a one-column illustration is 8.6 cm × 23.7 cm (3.4 in. × 9.3 in.) and that of a two-column illustration is 18.2 cm × 23.7 cm (7.2 in. × 9.3 in.). **Illustrations that exceed the maximum two-column size will not be accepted.** The figures (including halftones) must be numbered consecutively in Arabic numerals, and each one must be referred to in the text and must be self-explanatory. All terms, abbreviations, and symbols must correspond with those in the text. Only essential labeling should be used, with detailed information given in the caption.

Line drawings

All lines must be sufficiently thick (0.5 points minimum) to reproduce well, and all symbols, superscripts, subscripts, and decimal points must be in good proportion to the rest of the drawing and large enough to allow for any necessary reduction without loss of detail. Avoid small open symbols; these tend to fill in upon reproduction. **Lettering produced by dot matrix printers or typewriters, or by hand, is not acceptable.** The same font style and lettering sizes should be used for all figures of similar size in any one paper. Original recorder tracings of **NMR**, **IR**, **ESR spectra**, etc., are not acceptable for reproduction; they must be redrawn. For **hard-copy versions**, line drawings should be made with black ink or computer-generated in black on high-quality white paper or other comparable material; laser prints should be created at the highest resolution available.

Maps

Maps must have very **clear, bold patterns** and must show longitudes and latitudes (or UTM coordinates) and a scale, to ensure proper identification of study locations.
On **maps of Quebec**, the official name of municipalities must be used (e.g., Québec, Montréal, Clarke City) and physical features must be in French (e.g., Lac Bienville) except for those that are considered of pan-Canadian significance. Areas of pan-Canadian significance have an official form in English and French (e.g., Atlantic Ocean and Océan Atlantique) and should appear in the language of the paper. Quebec (the province) must also appear in the language of the paper. For a complete list of names of areas of pan-Canadian significance, see pp. 236–237 of Le guide du rédacteur (2nd ed., 1996), published by Public Works and Government Services Canada, Ottawa, ON K1A 0S5, Canada.

Photographs

Photographs should be continuous tone, of high quality, and with strong contrast. Only essential features should be shown. A photograph, or group of them, should be planned to fit into the area of either one or two columns of text with **no further reduction**. Electron micrographs or photomicrographs should include a scale bar directly on the print. The best results will be obtained if the authors match the contrast and density of all figures arranged as a single plate. **Hard-copy versions** must be printed on glossy paper and be trimmed and mounted on **thin** flexible white bristol board with no space between those arranged in groups.

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Colour illustrations will be at the author's expense. Further details on prices are available from the Editorial Office (email: cjz@nrcresearchpress.com).

Preparation of electronic graphic files

See the electronic graphics list at http://nrcresearchpress.com/page/authors/information/graphics for accepted file formats.

PC or Macintosh versions of True Type or Type 1 fonts should be used. **Do not use bitmap or nonstandard fonts.**

All figures should be submitted at the desired published size. For figures with several parts (e.g., *a*, *b*, *c*, *d*, etc.) created using the same software application, assemble them into one file rather than sending several files.

Remember that the more complex your artwork becomes, the greater the possibility for problems at output time. Avoid complicated textures and shadings, especially in vector illustration programs; this increases the chance for a poor-quality final product.

Bitmap (raster) files are image files produced using a grid format in which each square (or pixel) is set to one level of black, colour, or grey. A bitmap (rasterized) file is broken down into the number of pixels or picture elements per inch (ppi). Pixels per inch is sometimes referred to as dots per inch (dpi). The higher the resolution of an image, the larger the number of pixels contained within the rectangular grid. The proper resolution should be used when submitting bitmap artwork. The minimum requirements for resolution are 600 dpi for line art, 1200 dpi for finelines (line art with fine lines or shading), 300 dpi for halftones and colour, and 600 dpi for combinations (halftones with lettering outside the photo area).

All **colour** files submitted must be as CMYK (cyan, magenta, yellow, and black). These colours are used in full-colour commercial printing. RGB graphics (red, green, and blue; colours specifically used to produce an image on a monitor) will not print correctly.

Vector files are image files produced using elements such as lines and shapes. Typically these files are used for line drawings.

Bitmaps can be imported into vector/draw applications only for the purpose of adding and overlaying information, lines, text, etc. Bitmaps should not be resized, cropped, rotated, or otherwise manipulated after importing.

Multimedia files

The Journal allows authors to incorporate audio and video clips into their paper; these are published in the online version of the Journal, adding a dimension to the paper that cannot be achieved in the printed version. For submission guidelines and accepted formats, see the List of Accepted Graphic Files at http://nrcresearchpress.com/page/authors/information/graphics..

Manuscript guidelines

Style guides

As a general guide for biological terms, *The CSE Manual for Authors, Editors, and Publishers: Scientific Style and Format* (7th ed., 2006) published by the Council of Biology Editors, Reston, VA 20190, USA, is recommended.

Spelling

Spelling should follow *Webster's Third New International Dictionary* or the *Oxford English Dictionary*. Authors are responsible for consistency in spelling.

Nomenclature

Authors are required to follow the *International Code of Zoological Nomenclature* (4th ed., 1999), published by The International Trust for Zoological Nomenclature, c/o The Natural History Museum, Cromwell Road, London SW7 5BD, UK. Specifically, authors should provide the authority name(s) and date for all genus- and species-level taxa at the first mention within the Abstract and within the text (but not in the title). In subsequent mentions of the same species, the genus is abbreviated to the first letter and no author name(s) or date is listed (e.g., *S. plicata*). Genera are always written in full at the beginning of a sentence. In the case of changed combinations, the authorship and date should be set in parentheses. For example,*Ascidia plicata* Lesueur, 1823 is cited as *Styela plicata* (Lesueur, 1823) when transferred to the genus*Styela*.

Biochemical nomenclature should follow the rules recommended by the International Union of Biochemistry (IUB) Committee of Editors of Biochemical Journals with support of IUPAC. As a **general guide for biological terms**, *The CSE Manual for Authors, Editors, and Publishers: Scientific Style Format* (7th ed., 2006), published by the Council of Biology Editors, Reston, VA 20190, USA, is recommended. For **enzyme nomenclature**, *Enzyme Nomenclature (1992): Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology* (Academic Press, San Diego, Calif.) should be followed.

Abbreviations and acronyms

Abbreviations and acronyms that are standard in the discipline need not be defined. All others must be defined when they are first mentioned in the text and those with more than one meaning should be avoided.

Units of measurement

SI units (Système international d'unités) should be used or SI equivalents should be given. This system is explained and other useful information is given in the *Metric Practice Guide* (2000) published by CSA International (5060 Spectrum Way, Suite 100, Mississauga, ON M9W 1R3, Canada). For practical reasons, some exceptions to SI units are allowed.

Statistical analyses

The assumptions and (or) the model underlying any statistical analysis should be clearly stated. Symbols such as * and **, denoting levels of significance, should not be used except in conjunction with the actual values of the associated test statistic;
actual *p* values are preferred.

Writing numbers

In writing long numbers the digits should be separated into groups of three, counted from the decimal marker to the left and right. The separator should be a space and not a comma, period, or any other mark, for example, 25 562 987 and not 25,562,987. In English text, the decimal marker should be a point, for example, 0.1 mL and not 0,1 mL. The decimal point in all numbers between 1 and –1, except 0, must be preceded by a 0. The sign × should be used to indicate multiplication, e.g., 3×10^6 and not $3 \cdot 10^6$.