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Piter Kehoma Boll

INVESTIGANDO A ECOLOGIA TRÓFICA DE PLANÁRIAS TERRESTRES NEOTROPICAIS: ECOMORFOLOGIA, DESENVOLVIMENTO E COMPORTAMENTO

São Leopoldo
2019

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Tese apresentada como requisito parcial para a obtenção do título de Doutor em Biologia, área de concentração: Diversidade e Manejo da Vida Silvestre, pela Universidade do Vale do Rio dos Sinos.

Orientadora: Profª. Drª. Ana Maria Leal-Zanchet

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„Es ist auffallend, wie rasch sie sich auf die Beute stürzt.“

Josef Hauser, 1959

RESUMO

O papel de predadores na estruturação de comunidades é bastante estudado em grupos como vertebrados e aranhas, enquanto o papel de outros predadores de reconhecida importância, como as planárias terrestres, é pouco conhecido. Planárias terrestres formam um grupo diverso de platelmintos terrestres, especialmente em regiões tropicais, e são carnívoras, em sua maioria predadoras, mas sua grande sensibilidade a alterações ambientais as torna difíceis de serem mantidas em laboratório, o que reduz o número de estudos sobre seu comportamento. Por exemplo, os hábitos alimentares da maioria das espécies, incluindo a composição de sua dieta e seu comportamento predatório, são desconhecidos. Devido à relevância de estudos sobre a ecologia trófica de planárias terrestres para o adequado manejo de suas populações, este trabalho foi desenvolvido de maneira a enriquecer o conhecimento sobre as diversidades morfológica, fisiológica e comportamental das planárias terrestres em relação a sua dieta e seu comportamento predatório. Para facilitar a identificação de itens alimentares sem a necessidade de ofertas exaustivas de diversas possíveis presas, comparamos variáveis morfológicas de planárias de diversas espécies aos hábitos alimentares conhecidos. Os resultados indicaram que a espessura da musculatura cutânea é uma variável mais importante para determinar a dieta de planárias terrestres que a anatomia da faringe, sugerindo uma maior diversidade na adaptação morfológica para a captura e menor para o consumo. Em uma espécie de hábitos alimentares mais generalistas, que inclui tanto gastrópodes quanto outras planárias em sua dieta, examinamos a influência de diferentes dietas sobre a sobrevivência, o crescimento e a fecundidade. Espécimes que receberam uma dieta mista (planárias + lesmas) sobreviveram em média menos tempo que espécimes que se alimentaram apenas de planárias ou apenas de lesmas. No grupo misto, houve preferência por planárias. Não houve diferença significativa no crescimento entre os grupos. O maior número de cápsulas de ovos foi depositado por espécimes se alimentando apenas de lesmas, mas o grupo de dieta mista depositou cápsulas mais pesadas. A constante alternância entre diferentes itens alimentares parece ter efeitos negativos sobre as planárias, sugerindo a necessidade de grandes alterações fisiológicas para digerir diferentes presas. As cápsulas mais pesadas depositadas pelo grupo com a menor sobrevivência sugere investimento terminal, com aumento do investimento em reprodução ao final da vida. Por fim, examinamos o comportamento de detecção de presas e o comportamento de reconhecimento de predadores em uma planária terrestre que se alimenta de isópodes terrestres e é predada por outras planárias terrestres. A planária não foi capaz de detectar sinais químicos e mecânicos da presa à distância e não seguiu rastros químicos de presas, sugerindo um comportamento de emboscada para a captura de presas. Ao toque direto com predadores, a planária apresentou imediata resposta de fuga, a qual não ocorreu ao toque com não predadores. Espécies filogeneticamente próximas de predadores, mas que não são predadoras da planária, também desencadearam a resposta de fuga, sugerindo assinaturas químicas similares que não são distinguíveis pela planária. Secreções e rastros químicos do predador por si sós raramente desencadearam respostas de fuga, indicando a necessidade de estímulos táteis ou uma resposta de esquiva mais sutil que não foi detectada. O comportamento de emboscada para captura de isópodes e a rápida resposta de fuga diante de uma planária predadora parecem estratégias adequadas para capturar presas rápidas e escapar de predadores lentos. O conjunto de resultados evidencia a grande diversidade morfológica, ecológica e comportamental de planárias terrestres.

Palavras-chave: Comportamento. Ecomorfologia. Geoplanidae. Hábitos alimentares.

ABSTRACT

The role of predators in structuring communities is well studied in groups such as vertebrates and spiders, while the role of other admittedly important predators, such as land planarians, is little known. Land planarians for a diverse group of terrestrial flatworms, especially in tropical regions, and are carnivorous, mostly predators, but their sensitivity to environmental changes makes them difficult to keep in the laboratory, which reduces the number of studies on their behavior. For example, the feeding habits of most species, including the composition of their diet and their predatory behavior, is unknown. Due to the relevance of studies on the trophic ecology of land planarians for the adequate management of their populations, this work aimed to enrich the knowledge on the morphological, physiological and behavioral diversity of land planarians regarding their diet and their predatory behavior. To facilitate the identification of food items without the need to conduct exhaustive offers of possible prey, we compared morphological variables of several planarian species to their known feeding habits. The results indicate that the thickness of the cutaneous musculature is a more important variable to define the diet of land planarians than pharynx anatomy, suggesting a higher diversity in the morphological adaptation for capturing prey and lower for prey consumption. In a species of more generalist habits, which includes both gastropods and other planarians in its diet, we examined the influence of different diets on survival, growth and fecundity. Specimens receiving a mixed diet (planarians + slugs) survived, on average, less than specimens feeding only on planarians or only on slugs. In the mixed group, there was a preference for planarians. There was no significant difference in growth between the groups. The larger number of egg capsules was laid by specimens feeding only on slugs but the group receiving the mixed diet laid heavier capsules. The constant alternation between different food items seems to have negative effects on the planarians, suggesting the need for great physiological changes to digest different prey items. The heavier capsules laid by the group that had the lowest survival suggest terminal investment, with an increase in reproductive investment at the end of life. Finally, we examined the behavior of prey detection and the behavior of predator recognition in a land planarian that feeds on woodlice and is the prey of other land planarians. The planarian was unable to detect remote chemical and mechanical signals of the prey and did not follow chemical trails of the prey, suggesting an ambush behavior for prey capture. When touched directly by a predator, the planarian showed an immediate escape response, which did not occur when touched by non-predators. Species phylogenetically close to predators, but which are not themselves predators of the planarians, also triggered an escape response, suggesting similar chemical signatures that cannot be distinguished by the planarian. Chemical secretions and trails alone rarely triggered an escape response, indicating the need for tactile stimuli or a subtler avoidance response that was not detected. The ambush behavior for the capture of woodlice and the quick escape response in the presence of a predator planarian seem adequate strategies for the capture of fast-moving prey and the escape from slow-moving predators. In overall, the results highlight the great morphological, ecological and behavioral diversity of land planarians.

Keywords: Behavior. Ecomorphology. Feeding habits. Geoplanidae.

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APRESENTAÇÃO

A presente tese, intitulada “Investigando a ecologia trófica de planárias terrestres neotropicais: ecomorfologia, desenvolvimento e comportamento” é apresentada na forma de artigos científicos.

O primeiro capítulo, intitulado “Can morphology help us predict the diet of land planarians?”, investiga a relação entre variáveis morfométricas de planárias terrestres e suas dietas. Sua formatação segue as normas da revista Invertebrate Biology.

O segundo capítulo, intitulado “Survival, growth and fecundity of a Neotropical land planarian (Platyhelminthes, Geoplanidae) under different diets” analisa o desenvolvimento e a fecundidade de espécimes de *Obama anthropophila* Amaral, Leal-Zanchet & Carbayo,2015 submetidas a diferentes dietas. Sua formatação segue as normas da revista Invertebrate Biology.

O terceiro capítulo, intitulado “Lazy to prey and eager to run: behaviour of a Neotropical land planarian (Platyhelminthes: Geoplanidae) in the presence of its prey and predators”, analisa os métodos de detecção de presa e o reconhecimento de predadores pela planária *Luteostriata abundans* (Graff, 1899). Este capítulo foi publicado na revista Biological Journal of the Linnean Society (doi: 10.1093/biolinnean/bly114), estando, portanto, de acordo com as normas dessa.

INTRODUÇÃO GERAL

O papel de predadores na estruturação e na estabilidade de ecossistemas, atuando como reguladores do tamanho populacional, é bastante reconhecido (Spiller & Schoener 1994, Williams et al. 2004) e vem sendo estudado em diversos grupos, como vertebrados e aranhas (Belovsky & Slade 1993, Spiller & Schoener 1994, Wyman 1998, Williams et al. 2004). No entanto outro grupo de predadores de reconhecida importância, as planárias terrestres, tem sido pouco estudado (Ogren 1995, Cseh et al. 2017).

Planárias terrestres compreendem a família Geoplanidae de platelmintos da ordem Tricladida (conhecidos como planárias), tendo evoluído de ancestrais de água-doce (Carranza et al. 1998). Apesar de sua reconhecida importância ecológica como predadores, poucos estudos são focados em seus aspectos fisiológicos, tróficos ou comportamentais. Isso ocorre em especial devido à fragilidade destes animais e à dificuldade de mantê-los vivos em laboratório por longos períodos (Ogren 1995, Winsor et al. 2004), bem como à dificuldade de conduzir experimentos em campo devido aos seus hábitos noturnos e fotofóbicos que comprometem observações *in situ* (Ogren 1956). Um fator adicional que leva à ausência de planárias terrestres em estudos ecológicos é o viés europeu desta área de estudo e, sendo planárias terrestres incomuns na região holártica, sua importância costuma ser subestimada (Winsor et al. 1998).

Hábitat de planárias terrestres

Apesar de terem colonizado o ambiente terrestre há milhões de anos, provavelmente durante o período Cretáceo (Carranza et al. 1999), as planárias terrestres não apresentam mecanismos eficientes para retenção de água, sendo muito sensíveis à desidratação, mas também não suportam umidade excessiva, necessitando, portanto, de condições bem específicas em relação à umidade em seu habitat (Sluys 1998). Um local frequente onde as condições são ideais é a camada de serapilheira no solo de florestas, de forma que planárias terrestres são frequentemente encontradas sob folhas caídas, troncos e pedras, ou no interior de troncos ou galhos caídos, de maneira a se manterem úmidas e abrigadas da luz (Sluys 1999, Winsor et al. 2004). Eventualmente, algumas espécies podem ser encontradas sobre a vegetação, especialmente abrigadas no interior de bromélias epífitas (Beauchamp 1913, Froehlich 1955).

Devido a tal limitação fisiológica, planárias terrestres são animais com baixa capacidade de dispersão e, consequentemente, sua distribuição geográfica costuma ser restrita a pequenas áreas (Amaral et al. 2014). Todo este conjunto de peculiaridades também torna as comunidades de planárias terrestres muito sensíveis a alterações no ambiente, de forma que compreendem bons indicadores do estado de preservação de um ecossistema, especialmente em áreas tropicais, onde ocorre o pico de diversidade deste grupo (Sluys 1999).

Classificação e distribuição geográfica de planárias terrestres

Atualmente há cerca de 900 espécies descritas de planárias terrestres (Tyler et al. 2006), mas este número certamente está muito abaixo da riqueza total no planeta devido ao baixo número de pesquisadores dedicados a este grupo (Sluys 1999, Carbayo & Froehlich 2008). Ao se observar uma curva de acumulação de espécies descritas (Fig. 1), fica evidente que estamos bem longe de atingir uma assíntota que indica o conhecimento da maior parte das espécies do planeta.

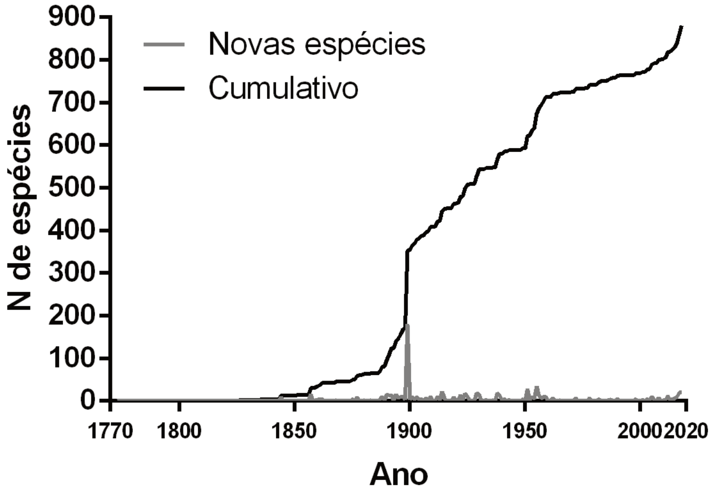

Fig. 1. Número de espécies novas de planárias terrestres descritas por ano no mundo e número cumulativo de espécies. Fonte: Elaborado pelo autor.

A atual posição filogenética das planárias terrestres, corroborada por dados moleculares, é como grupo-irmão da família Dugesiidae, de espécies de água doce. A família Geoplanidae é dividida em quatro subfamílias: Bipaliinae, Microplaninae, Geoplaninae e Rhynchodeminae, sendo esta última subdividida em cinco tribos: Rhynchodemini, Caenoplanini, Pelmatoplanini, Eudoxiatopoplanini, Argaplanini e Anzoplanini (Sluys et al. 2009) (Fig. 2).

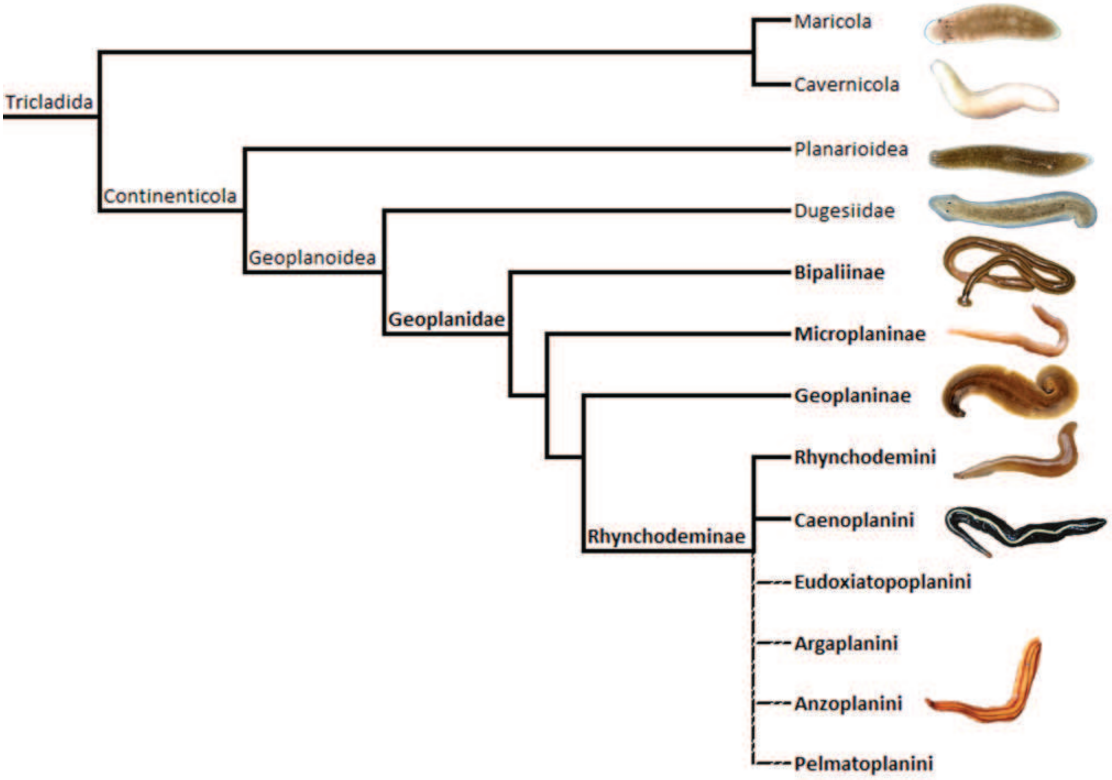

Fig. 2. Classificação atual *da ordem Tricladida (planárias) com ênfase na classificação da família Geoplanidae (planárias terrestres). Linhas tracejadas indicam clados de posição ainda não confirmada por estudos moleculares. Fonte: Elaborada pelo autor. Créditos das imagens: Miquel Vila Farré (Maricola), Leal-Zanchet et al. (2014) (Cavernicola), Brandl et al. (2016) (Planarioidea), Eduard Solà (Dugesiidae, Microplaninae, Rhynchodemini), Justine et al. (2018) (Bipaliinae), Jean-Lou Justine (Geoplaninae), Doug Beckers (Caenoplanini), Reiner Richter (Anzoplanini).

A subfamília Bipaliinae apresenta distribuição original predominantemente indo-malaia, alcançando também Madagascar e o sudeste da região Neártica (China, Coreia e Japão) (Ogren & Kawakatsu 1987) (Fig. 3). Entretanto diversas espécies foram introduzidas, através do comércio de plantas, em outras localidades, de maneira que sua distribuição é atualmente cosmopolita (Álvarez-Presas et al. 2014, Justine et al. 2018). As principais espécies introduzidas desta subfamília são: *Bipalium adventitium* Hyman, 1943, *Bipalium pennsylvanicum* Ogren, 1987, *Bipalium vagum* Jones & Sterrer, 2005, *Diversibipalium multilineatum* (Makino & Shirasawa, 1983) e *Bipalium kewense* Moseley, 1878, sendo esta última encontrada em todos os continentes (exceto a Antártica) e diversas ilhas (Winsor 1983).

A subfamília Microplaninae é predominante nas regiões Paleártica e Afrotropical, com algumas espécies encontradas nas Américas e no Subcontinente Indiano (Ogren & Kawakatsu 1989) (Fig. 3). Apenas uma espécie, *Microplana terrestris* (Müller, 1774), nativa da Europa, é eventualmente registrada na América do Norte (Ogren 1984), mas não parece ser ecologicamente relevante como espécie introduzida.

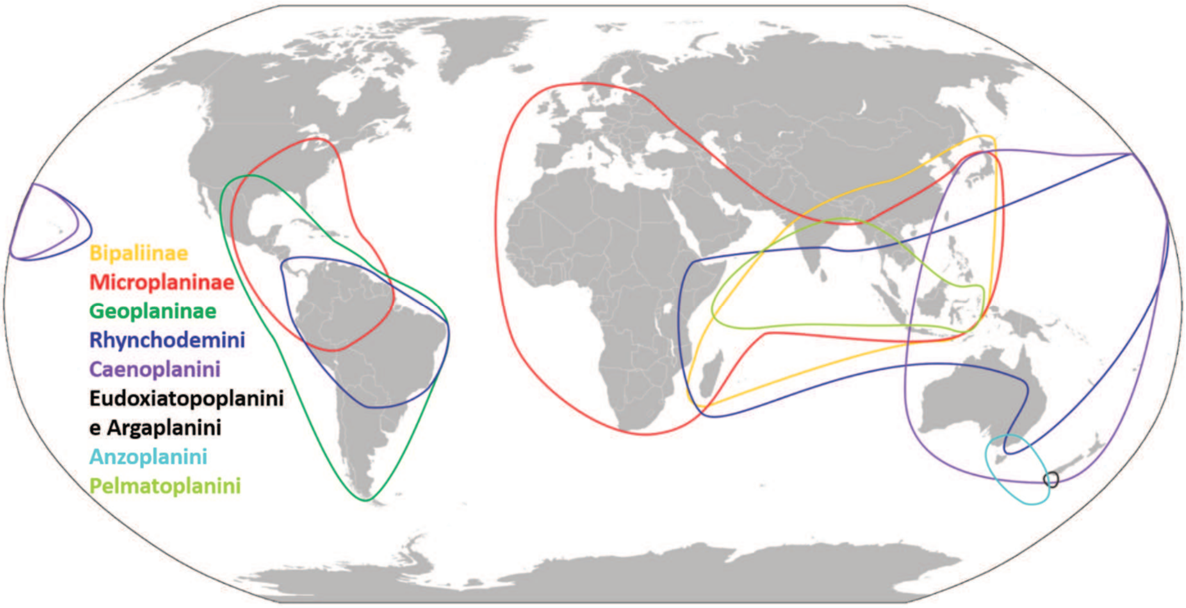

Fig. 3. Provável distribuição geográfica original (sem considerar introduções por causas antrópicas) dos diferentes subgrupos de planárias terrestres. Fonte: Elaborada pelo autor.

A subfamília Geoplaninae é originalmente restrita à região Neotropical (Ogren & Kawakatsu 1990) (Fig. 3). Atualmente apenas uma espécie é reconhecida como introduzida, *Obama nungara* Carbayo, Álvarez-Presas, Jones & Riutort, 2016, nativa do sul do Brasil e norte da Argentina e introduzida na Europa (Carbayo et al. 2016, Lago-Barcia et al. 2018).

A subfamília Rhynchodeminae possui duas tribos cuja relação é suportada por dados moleculares: Rhynchodemini e Caenoplanini, além de três tribos de posição incerta: Anzoplanini, Eudoxiatopoplanini e Pelmatoplanini (Sluys et al. 2009). A maior parte das espécies parece ser originária das regiões Australásia e Indo-Malaia (Fig. 3), mas há registros, especialmente de Rhynchodemini, de todos os continentes (Ogren & Kawakatsu 1988, 1991), contudo seu status como espécies nativas ou introduzidas é incerto. Diversas espécies desta subfamília são invasoras de maior ou menor importância, entre as quais podemos mencionar *Caenoplana coerulea* Moseley, 1877, *Arthurdendyus triangulatus* (Dendy, 1896), *Kontikia ventrolineata* (Dendy, 1892), *Endeavouria septemlineata* (Hyman, 1939), *Dolichoplana striata* Moseley, 1877 e *Platydemus manokwari* De Beauchamp, 1963, todas introduzidas na Europa e muitas também em outros continentes (Jones & Boag 1996, Jones 1998, Justine et al. 2014a, 2015, Álvarez-Presas et al. 2014).

Dieta de planárias terrestres

As planárias terrestres são carnívoras, sendo em sua maioria predadoras (Winsor et al. 2004), mas algumas espécies são primariamente necrófagas (McDonald & Jones 2007, Boll et al. 2015) (Fig. 4). Como habitam o solo de regiões florestais, suas principais presas incluem outros animais que habitam o mesmo ambiente (Cseh et al. 2017).

Fig. 4. Diversos indivíduos da espécie *Endeavouria septemlineata* se alimentando de um espécime morto do caracol *Bradybaena similaris*. Fonte: Elaborada pelo autor.

Minhocas e gastrópodes terrestres parecem ser as presas mais frequentes de planárias terrestres (Fig. 5), constituindo a dieta das mais importantes espécies invasoras. Algumas espécies dos gêneros *Arthurdendyus, Bipalium*, *Dolichoplana* e *Obama*, introduzidas em diversos países, alimentam-se exclusiva ou preferencialmente de minhocas (Winsor et al. 2004, Carbayo et al. 2016). Gastrópodes constituem a presa preferencial, mas não exclusiva, de *Platydemus manokwari*, a mais problemática de todas as espécies invasoras deste grupo por ter levado populações de caracóis à extinção (Sugiura 2010, Justine et al. 2015). Gastrópodes também compõem toda ou parte da dieta de várias planárias neotropicais do gênero *Obama*, algumas das quais, devido aos seus hábitos sinantrópicos, são invasoras em potencial (Boll & Leal-Zanchet 2016, Cseh et al. 2017).

Artrópodes são outro item comum na dieta de planárias terrestres. A espécie *Luteostriata abundans*, do sul do Brasil, alimenta-se unicamente de isópodes terrestres (Hauser & Maurmann 1959, Prasniski & Leal-Zanchet 2009, Boll & Leal-Zanchet 2016) (Fig. 6). Já *Microplana termitophaga*, do sudeste da África, alimenta-se de cupins do gênero *Odontotermes* que captura em aberturas dos cupinzeiros (Cumming 1995). Outros artrópodes registrados na dieta de planárias terrestres incluem opiliões, diplópodes, colêmbolos e larvas de lepidópteros (Terrace & Baker 1994, Nakamori & Suzuki 2012, Cseh et al. 2017, Silva et al. 2018).

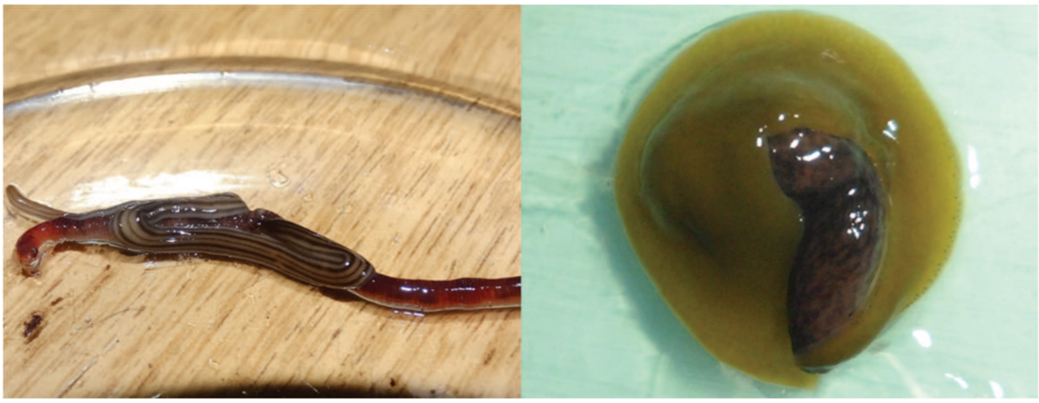

Fig. 5. Indivíduo de *Dolichoplana carvalhoi* se alimentando de uma minhoca *Eisenia andrei* (esquerda) e indivíduo de *Obama ladislavii* se alimentando de uma lesma *Deroceras laeve* (direita). Fonte: Elaborada pelo autor.

Fig. 6. Indivíduo de *Luteostriata abundans* se alimentando de isópode terrestre. Fonte: Elaborada pelo autor.

Além de minhocas, gastrópodes e artrópodes, outros grupos já registrados na dieta de algumas espécies são outras planárias terrestres (Boll & Leal-Zanchet 2016), onicóforos (Steel 1900) e nemertíneos (Ohbayashi et al. 2005, Cseh et al. 2017).

Ao se analisar os hábitos alimentares das mais frequentes presas de planárias terrestres, nota-se que grande parte delas é constituída de invertebrados detritívoros que se alimentam de matéria orgânica morta ou de fungos decompositores de matéria orgânica (Boll & Leal-Zanchet 2016, Cseh et al. 2017). Assim, planárias terrestres parecem ser predadores especialmente importantes em teias tróficas de detritos, ou seja, aquelas baseadas em matéria orgânica morta oriunda, direta ou indiretamente, da fixação por organismos autotróficos. Teias de detritos são menos estudadas que teias tróficas de pastagem, mas a maior parte da produção primária de autótrofos entra para a teia de detritos e apenas uma pequena fração é consumida por herbívoros, entrando na teia de pastagem (Edwards et al. 1973).

As extinções locais de grupos utilizados como presas, causadas por algumas espécies invasoras, deixam clara a importância ecológica destes animais (Iwai et al. 2010a). Mesmo assim, a dieta da maior parte das espécies ainda é desconhecida, o que impede uma melhor compreensão de seu papel nos ecossistemas em que estão inseridas.

Detecção de presas por planárias terrestres

Exceto por algumas espécies adaptadas à vida subterrânea, planárias terrestres apresentam olhos simples (ocelos) que podem variar de dois frontais (em Rhynchodemini e Microplaninae) a dezenas ou centenas distribuídos ao longo do corpo (nos demais táxons) (Ball & Reynoldson 1981). Comparado a outros filos de animais, os olhos de planárias terrestres são bastante simples, mas em relação a outros platelmintos são consideravelmente complexos (Rieger et al. 1991). O olho é composto de um cálice de células pigmentadas dentro do qual desembocam projeções fotossensíveis de neurônios bipolares (células da retina). Ao contrário dos outros grupos de planárias, onde as projeções penetram o cálice por sua abertura, em planárias terrestres eles entram por entre as células pigmentadas (Carranza et al. 1998). O cálice é coberto por um epitélio que forma uma espécie de córnea, a qual pode permitir certo poder de refração (Hyman 1951). Assim, supõe-se que planárias terrestres possuam uma capacidade visual maior em relação a planárias aquáticas, permitindo que, além de determinarem intensidade e direção de luz, também gerem imagens difusas (Kawaguti 1932). A maioria das espécies, no entanto, apresenta hábitos noturnos e comportamento fotofóbico, de maneira que a principal função dos olhos, na maioria das espécies, continua sendo evitar a luz, não sendo adequados para a procura de alimento (Ogren 1956).

Já se sabe que espécies que consomem gastrópodes e minhocas são capazes de detectar a presa através de sinais químicos, como rastros de secreções, deixados pela presa no meio (Fiore et al. 2004, Iwai et al. 2010b, Boll & Leal-Zanchet 2015). Tal detecção química ocorre através de invaginações ciliadas presentes na margem anterior do corpo, denominadas fossetas sensoriais (Hyman 1951). Por outro lado, não há na literatura dados sobre como as espécies que se alimentam de artrópodes detectam suas presas. Em geral, predadores que fazem busca ativa se baseiam em sinais químicos, enquanto predadores de emboscada utilizam sinais mecânicos, tal como vibrações do substrato (Horridge & Boulton 1967, Stubbs 1980, Svensen & Kiørboe 2000), de forma que este se mostra um ponto interessante a ser investigado.

Captura e ingestão de presas por planárias terrestres

Uma vez encontrada a presa, planárias terrestres podem fazer uso de diversas técnicas de captura, as quais parecem estar relacionadas com o tipo e o tamanho da presa a ser subjugada (Boll & Leal-Zanchet 2018). A captura consiste em imobilização da presa e posterior reposicionamento corporal da planária para que a presa possa ser ingerida (Boll & Leal-Zanchet 2018). A ingestão ocorre através da faringe, um órgão tubular que é evertido através da boca, a qual se encontra na região ventral do corpo, aproximadamente na metade da distância entre as extremidades anterior e posterior da planária (Ball & Reynoldson 1981).

A captura de minhocas, gastrópodes ou outras planárias de pequeno porte muitas vezes é feita com a planária apenas cobrindo a presa com seu próprio corpo, assim impedindo-a de fugir (Boll & Leal-Zanchet 2018). Minhocas e lesmas de tamanho maior geralmente são imobilizadas com a planária se deslocando por cima de parte do corpo da presa e se prendendo firmemente ao substrato por ambos os lados, pressionando a presa contra o substrato (Jennings 1959, Boll & Leal-Zanchet 2018). A captura de nemertíneos terrestres, registrada para algumas espécies (Ohbayashi et al. 2005, Cseh et al. 2017), provavelmente segue um padrão similar ao de captura de outras planárias.

A captura de minhocas e gastrópodes muito maiores que a planária também pode ser eficiente em espécies que são capazes de se fixar firmemente ao corpo da presa com suas faringes. Quando isso ocorre, mesmo movimentos bruscos da presa dificilmente conseguem desalojar a planária, que eventualmente perfura o corpo da presa com a faringe e o processo de alimentação se inicia (Pfitzner 1958, Dindal 1970). Este tipo de captura já foi registrado em algumas espécies de hábitos gregários, nas quais o ataque simultâneo de diversos indivíduos facilita a captura de presas maiores (Mead 1963, Barker 1989, Sugiura 2010). Em caracóis, a planária pode subir sobre a presa pela concha e ser arrastada com ela, facilitando seu posicionamento para o início do processo de alimentação (Boll & Leal-Zanchet 2016, 2018).

Gastrópodes também podem ser capturados por uma abordagem na qual a planária se aproxima por um lado da presa e a circunda em frente à cabeça, impedindo sua fuga enquanto a imobiliza com um “abraço muscular” (Froehlich 1955, Boll & Leal-Zanchet 2018). Algumas espécies também demonstram a capacidade de segurar pequenos gastrópodes e outras planárias com a região anterior enquanto curvam as laterais do corpo para o ventre ou se enrolam numa espiral, formando um tubo com o corpo pelo qual conduzem a presa em direção à boca (Boll & Leal-Zanchet 2018).

Espécies que se alimentam de artrópodes pequenos e ágeis, como cupins e isópodes terrestres, costumam capturá-los com movimentos rápidos onde a presa é rapidamente puxada para junto do corpo da planária após o toque inicial, geralmente com a extremidade anterior. A planária adere seu corpo à presa aparentemente por uma secreção pegajosa (Jones et al. 1990, Prasniski & Leal-Zanchet 2009, Boll & Leal-Zanchet 2018). Na captura de opiliões, o ataque costuma iniciar também com a aderência da extremidade anterior à presa, geralmente por uma das patas, o que a ancora à planária, que então se desloca sobre a presa, envolvendo-a com muco para impedir sua fuga (Silva et al. 2018).

A forma de captura de outros animais já registrados na dieta, como onicóforos (Steel 1900) e diplópodes (Terrace & Baker 1994), não foi investigada até o momento.

A ingestão da presa após a captura também varia dependendo do tipo e do tamanho da presa. Minhocas e lesmas pequenas, bem como nemertíneos e outras planárias, costumam ser ingeridos inteiros, sugados pela faringe (Ohbayashi et al. 2005, Boll & Leal-Zanchet 2018). Minhocas e lesmas grandes, assim como caracóis, geralmente são perfurados ou dilacerados com a faringe e ingeridos em porções menores, às vezes com digestão externa parcial (Dindal 1970, Boll & Leal-Zanchet 2018). No consumo de isópodes terrestres, a planária *Luteostriata abundans* perfura o corpo do animal numa área mais frágil do exoesqueleto, geralmente entre dois segmentos abdominais, e suga seu conteúdo (Prasniski & Leal-Zanchet 2009, Boll & Leal-Zanchet 2018). Já a planária *Cephaloflexa bergi* (Graff, 1899), que se alimenta de opiliões, arranca apêndices da presa, geralmente pedipalpos e quelíceras, e suga o conteúdo através da lesão causada (Silva et al. 2018).

Anatomia de planárias terrestres e sua relação com a captura e ingestão de presas

Para a captura de presas, planárias terrestres dependem da sua musculatura corporal, a qual é dividida em dois grupos: musculatura cutânea e musculatura mesenquimática.

A musculatura cutânea, também chamada subcutânea, encontra-se logo abaixo da epiderme e é geralmente composta por três camadas: uma externa de fibras circulares, uma intermediária de fibras diagonais e uma interna de fibras longitudinais (Winsor 1998) (Fig. 7). As camadas circular e diagonal podem ser extremamente finas e, em alguns casos, entrelaçadas numa só camada (Winsor 1998). A camada longitudinal tende a ser a mais desenvolvida e é bastante espessa, formando feixes de fibras, nas subfamílias Geoplaninae e Rhynchodeminae (exceto na tribo Pelmatoplanini, de posição filogenética incerta dentro da subfamília) (Ogren & Kawakatsu 1991, Winsor 2006, 2009, Grau 2010). Na tribo Pelmatoplanini e nas subfamílias Bipaliinae e Microplaninae, especialmente na última, a musculatura cutânea no geral é muito fina, mas a real situação na maioria das espécies em Bipaliinae não é conhecida (Winsor 1998).

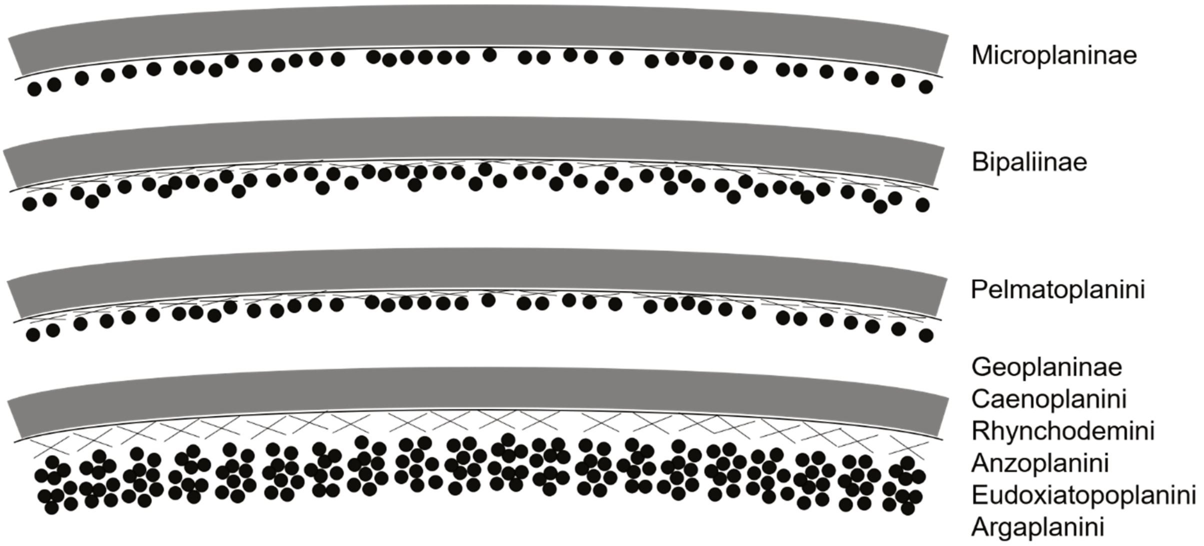

Fig. 7. Organização das camadas de musculatura cutânea em diferentes grupos de planárias terrestres como visto em corte transversal do corpo. Faixa cinza = epiderme; linha contínua = musculatura circular; linhas cruzadas = musculatura oblíqua; círculos pretos = musculatura longitudinal. Fonte: Elaborada pelo autor.

A musculatura mesenquimática localiza-se imersa no tecido conjuntivo de preenchimento (mesênquima) do corpo de planárias. Em praticamente todas as espécies, existe uma camada de fibras transversais acima do intestino (musculatura transversal supraintestinal) e uma abaixo do intestino (musculatura transversal subintestinal). Uma camada longitudinal subintestinal também é frequente, mas está ausente na tribo Eudoxiatopoplanini e na maior parte das espécies de Geoplaninae (Winsor 2009, Grau 2010), enquanto em Bipaliinae existe ainda uma camada longitudinal supraintestinal (Winsor 1983). Em Microplaninae, Bipaliinae e Pelmatoplanini ocorre um tubo muscular longitudinal bem desenvolvido circundando o intestino, os cordões nervosos dorsais e o aparelho copulador (Winsor 1983, Grau 2010, Sluys et al. 2017). Em Geoplaninae, Rhynchodemini, Caenoplanini e Anzoplanini, este tubo está ausente, mas há uma camada de fibras diagonais cruzadas na região dorsal, acima da camada transversal (Grau 2010). Em Eudoxiatopoplanini, as duas condições ocorrem, com a camada de fibras diagonais cruzadas entrelaçada na parte dorsal do tubo longitudinal (Winsor 2009). Em Argaplanini, o tubo muscular longitudinal é substituído por uma placa massiva dorsal e uma ventral, as quais são entrelaçadas por fibras diagonais cruzadas (WInsor 2011). Rhynchodemini, Caenoplanini e Anzoplanini possuem ainda uma camada ventral extra de fibras longitudinais entrelaçadas com fibras transversais, e apenas em Rhynchodemini e Anzoplanini há ainda mais uma camada de fibras diagonais cruzadas abaixo dessa camada ventral (Grau 2010). Além de toda esta diversidade de fibras em camadas, ocorrem também, em todos os grupos, fibras dorsoventrais cruzando o mesênquima (Winsor 2003, Grau 2010).

Todos estes diferentes conjuntos de musculatura ainda variam bastante no grau de desenvolvimento entre diferentes espécies de um mesmo grupo. Dentro da subfamília Geoplaninae, por exemplo, os feixes de musculatura subcutânea longitudinal são bem finos no gênero *Obama* (Álvarez-Presas et al. 2015, Marques et al. 2018) e bem espessos em gêneros como *Choeradoplana, Cephaloflexa, Supramontana* e *Luteostriata*, muitas vezes sendo divididos até em duas camadas distintas (Carbayo 2010, Carbayo & Froehlich 2012).

Para a ingestão de presas, o órgão utilizado é a faringe, que constitui um tubo alojado numa cavidade, a bolsa faríngea, e evertido pela boca. Num corte transversal da bolsa faríngea, pode-se notar que a inserção ventral da faringe se localiza sempre na região mais anterior da bolsa. Já a posição da inserção dorsal varia bastante e é utilizada para classificar as faringes em três tipos de acordo com seu formato aproximado quando evertida: (1) cilíndrica, quando a inserção dorsal se localiza no terço anterior da bolsa, (2) campanuliforme, quando se localiza no terço médio da bolsa, e (3) colarinho, quando se localiza no terço posterior da bolsa (Winsor et al. 1998). Estas categorias, no entanto, não são discretas, e a posição da inserção dorsal pode variar num contínuo quando diversas espécies são comparadas (confira Fig. 1 do capítulo 1, página 36). Apesar de haver a hipótese de que faringes cilíndricas perfuram a presa enquanto campanuliformes e colarinho as envolvem (Winsor et al. 1998), isso nunca foi investigado.

Predadores de planárias terrestres

Até o final do século XX, quase nenhum predador de planárias terrestres havia sido identificado. Como consequência, planárias terrestres como um todo eram e ainda são muitas vezes consideradas predadoras de topo (Ogren 1995, Sluys 1998).

Nas últimas décadas, este cenário mudou um pouco e alguns predadores foram identificados para algumas espécies, entre eles besouros carabídeos e estafilinídeos na Escócia (Gibson et al. 1997), um caracol carnívoro, *Rectartemon depressus*, no sul do Brasil (Lemos et al. 2012) e outras planárias terrestres, incluindo diversas espécies nativas da América do Sul e as invasoras *Platydemus manokwari* e *Obama nungara* (Ohbayashi et al. 2005, Boll et al. 2015, Boll & Leal-Zanchet 2016). Vertebrados, no entanto, parecem não ser predadores comuns de planárias, pois estas foram rejeitadas como presas por serpentes e salamandras (Ducey et al. 1999) e parecem não ser palatáveis a aves (Dendy 1890, Justine et al. 2014b).

Algumas espécies de planárias terrestres a princípio se enquadram na definição de predadores de topo por sua dieta variada e pelos baixos registros de seu uso como alimento por outros animais (Sugiura 2010, Boll & Leal-Zanchet 2016), mas outras possivelmente são presas importantes de outros organismos, incluindo as espécies de planárias verdadeiramente predadoras de topo (Boll & Leal-Zanchet 2016).

O impacto de planárias terrestres sobre ecossistemas

Baseados nos conhecimentos reunidos recentemente e comentados acima, o papel trófico de planárias terrestres parece mais diversificado do que inicialmente pensado. No entanto, muito pouco se sabe sobre o quanto a predação desempenhada por elas afeta o tamanho populacional de suas presas através de, por exemplo, estudos sobre a frequência de predação (Prasniski & Leal-Zanchet 2009). Os estudos existentes, quase exclusivamente direcionados a espécies invasoras, demonstram o quão importantes estes organismos são para a estabilidade dos ecossistemas, podendo causar prejuízos econômicos e ecológicos quando introduzidas em outros ambientes ou quando sua densidade em ecossistemas nativos é alterada por impactos antrópicos (Mead 1963, Sugiura et al. 2006, Chiba & Cowie 2016).

A influência antrópica em regiões florestais costuma impactar negativamente as populações de planárias (Carbayo et al. 2008, Oliveira et al. 2014), mas algumas espécies podem se beneficiar com as novas condições criadas e se tornar dominantes no ambiente (Oliveira et al. 2014). Muitas espécies se adaptam a ambientes antropizados e são encontradas em áreas urbanas e rurais, como parques e jardins, em maior número que em áreas preservadas (Froehlich 1955). A proximidade dessas espécies com humanos fez com que muitas fossem transportadas para outras regiões do planeta, especialmente através do comércio de plantas, situação em que frequentemente são levadas acidentalmente no substrato (Winsor et al. 2004, Justine et al. 2014b, Sluys 2016).

Dois interessantes exemplos de planárias terrestres invasoras que resultaram em relevantes impactos ambientais são *Arthurdendyus triangulatus* (Dendy, 1894) e *P. manokwari*.

Arthurdendyus triangulatus (syn. *Artioposthia triangulata*), conhecida popularmente em inglês como New Zealand flatworm ("planária da Nova Zelândia"), é uma espécie nativa da Nova Zelândia que se alimenta exclusivamente de minhocas (Blackshaw 1990, Blackshaw & Stewart 1992). Introduzida na Grã-Bretanha, a espécie rapidamente se estabeleceu em algumas regiões, especialmente na Escócia, e o impacto de sua predação sobre minhocas levou a um rápido declínio das populações destes animais, afetando a drenagem do solo devido à redução do número de galerias construídas por minhocas (Haria 1995, Haria et al. 1998, Murchie & Gordon 2013).

Platydemus manokwari, chamada em inglês de New Guinea flatworm ("planária da Nova Guiné"), foi inicialmente descoberta na cidade de Manokwari, na ilha de Nova Guiné, e considerada a responsável pelo declínio da população do caracol-gigante-africano *Achatina fulica* (Férussac, 1821), uma espécie invasora. Posteriormente, esta espécie foi deliberadamente introduzida em outras ilhas dos oceanos Pacífico e Índico, como Bugsuk, nas Filipinas, Honshu, no Japão, e as Maldivas (Muniappan et al. 1986, Justine et al. 2014b). Além disso, ela foi introduzida acidentalmente em diversas outras ilhas, como Guam, Palau, Havaí, Samoa, entre outras (Kawakatsu et al. 1992). No entanto, além de controlar o caracol-gigante-africano, a planária passou a se alimentar de espécies nativas de caracóis, levando diversas populações à extinção, sendo o caso mais grave registrado nas ilhas Ogasawara, Japão (Okochi et al. 2004, Ohbayashi et al. 2005, Sugiura et al. 2006). Atualmente, *P. manokwari* figura entre as "100 piores espécies invasoras do mundo" (Lowe et al. 2004) e recentemente foi registrada na França, nos Estados Unidos e em Porto Rico (Justine et al. 2015).

Justificativa

O conhecimento sobre as interações ecológicas de planárias terrestres possui relevância para o manejo adequado das populações e dos ecossistemas em que se encontram, além de poder auxiliar no controle populacional de espécies invasoras. A frequente presença de planárias terrestres em ambientes antropizados no Rio Grande do Sul as torna espécies relevantes para estudo devido ao grande potencial de se estabelecerem em novas localidades após transporte acidental e, a partir disso, se tornarem invasoras.

Objetivos

Diante da situação acima exposta, o presente trabalho foi desenvolvido de maneira a investigar a diversidade morfológica, fisiológica e comportamental de planárias terrestres em relação a suas interações tróficas, tendo como objetivos específicos:

- Analisar as morfologias gerais da faringe e da musculatura cutânea e buscar suas relações com a dieta e o comportamento predatório, de maneira a buscar um padrão que permita prever a dieta com base na morfologia;
- Determinar a influência de diferentes presas sobre o crescimento e a fecundidade em espécies de hábitos generalistas;
- Investigar os métodos de rastreio de presas em planárias terrestres que se alimentam de artrópodes;

- Analisar os métodos de reconhecimento de predadores por planárias terrestres que são presas de outras planárias terrestres.

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CAPÍTULO 1

Can morphology help us predict the diet of land planarians?

Piter Kehoma Boll*, Ana Maria Leal-Zanchet
Instituto de Pesquisa de Planárias and Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos – UNISINOS, Av. Unisinos, 950, Cristo Rei, São Leopoldo, RS 93022-750, Brazil

*Corresponding author. E-mail: piterkeo@gmail.com

Abstract

The use of morphometric data for taxonomic studies and to predict the diet of an organism based on related species has been applied to several groups. This methodology could be useful for land planarians, which are considered important predators of leaf-litter invertebrates but are difficult to keep in the laboratory due to their strict ecological requirements. In this study, we used morphometric data about the relative position of the mouth and dorsal insertion of the pharynx in the pharyngeal pouch, body shape (transverse circularity) and relative thickness of the cutaneous musculature to the body height to find patterns that could distinguish different genera and different feeding habits in 84 species of geoplaninid land planarians. Mouth position was partially explained by the position of the dorsal insertion of the pharynx, and transverse circularity was partially explained by the relative thickness of the cutaneous musculature. The character that best separated genera and diet was the thickness of cutaneous musculature. A principal component analysis recovered some patterns previously revealed in studies of molecular phylogenetics, with some closely related genera appearing close to each other in the biplot. The same analysis also showed two clearly distinct groups, one of species that feed on fast-moving arthropods and other of species that feed on soft-bodied prey. We conclude that the use of morphometry can help narrow down the potential prey of geoplaninid land planarians and, combined with descriptive morphology, can aid taxonomic studies as well.

Keywords: behavior, ecomorphology, Geoplaninae, predation

Introduction

Ecomorphology is the study of the relationship between the morphology of an organism and its ecology. This relationship occurs especially through the organism's behavior, which is adapted to its ecology and depends on its anatomy (Wainwright 1991). Finding relationships between the morphology and the ecology of an organism allows predictions regarding the behavior and the ecological role of a species by studying its anatomy (Griffen & Mosblack 2011). This is especially useful in the study of fossil species, in which analogies with the functional morphology of current species allow us to infer the ecology of extinct species (Hickman 1988). In current ecosystems, ecomorphological studies can be useful to predict the ecological role of a species based on the morphology of similar species (Saunders & Barclay 1992).

The study of morphology to predict the behavior of a species, and as a consequence its ecological role, has been explored in certain groups such as fishes (Motta et al. 1995, Griffen & Mosblack 2011), birds (Leisler & Winkler 1985), arthropods (Patterson 1984, Freire et al. 1996) and several groups of parasites (Bush et al. 2006, Vignon et al. 2011, Hiller et al. 2018). Another group in which ecomorphology could be useful is that of land planarians.

Land planarians are terrestrial flatworms inhabiting the leaf litter of forest environments. They are considered important predators of invertebrates that share the same habitat, such as gastropods, earthworms, arthropods and even other land planarians (Ogren 1995, Boll & Leal-Zanchet 2016, Cseh et al. 2017, Silva et al. 2018). Due to the strict ecological requirements of many land planarians, it is difficult to monitor them *in situ* or in the laboratory (Kaneda et al. 1990, Boll & Leal-Zanchet 2015), especially those species restricted to highly conserved areas.

Having a very simple anatomy, land planarians show little morphological variation. The anatomical feature more directly related to feeding and that shows the greatest variation is the pharynx, a tubular organ everted through the mouth and used to ingest food (Seitenfus & Leal-Zanchet 2004). The shape of the everted pharynx is determined by the relative position of the dorsal insertion of the pharynx in the pharyngeal pouch. It is thus classified as cylindrical, bell-shaped or collar-shaped depending on the position of the dorsal insertion at the anterior, middle or posterior third of the pouch, respectively (Seitenfus & Leal-Zanchet 2004). It is sometimes assumed that cylindrical pharynges are used to perforate the prey's body, while bell- and collar-shaped pharynges are extended over soft-bodied prey (Winsor et al. 1998). However, currently there are no comparative studies regarding this relationship, and this categorical classification does not reflect the actual anatomical variation, which would be better defined as a continuous variable (Fig. 1). Similarly, the position of the mouth in the pharyngeal pouch also shows some variation, which could also be related to feeding habits.

Furthermore, the body musculature in land planarians is important for capturing prey (Ogren 1995) and the degree of development of different layers may be related to the type of consumed prey and the corresponding capture behavior (Boll & Leal-Zanchet 2018). For example, species that feed on gastropods usually capture prey by surrounding and immobilizing, while species feeding on earthworms press the prey against the substrate and those feeding on arthropods rely on sticky mucus and quick movements to pull the prey (Jones et al. 1990, Fiore et al. 2004, Boll & Leal-Zanchet 2018). Some genera have a cephalic retractor muscle that may be important for prey capture (Carbayo 2010).

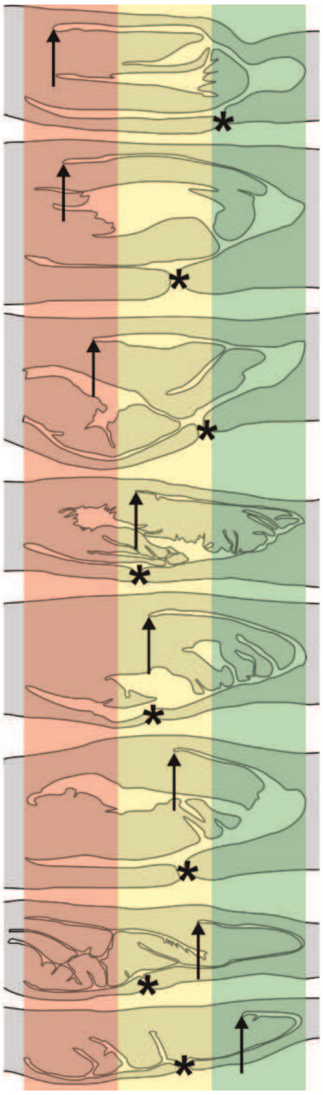

Fig. 1. The shape of the pharynx of land planarians is usually defined based on the position of the dorsal insertion (arrow) in the anterior (red), middle (yellow) or posterior (green) third of the pharyngeal pouch. However, as seen in these examples, such a categorical classification is not very useful. The position of the mouth (asterisk) is also somewhat variable.

Currently, the taxonomy of land planarians is mainly based on descriptive morphology, especially of the reproductive system, sometimes integrated with molecular phylogenetics (Carbayo et al. 2013, Amaral et al. 2018), although recently the importance of other aspects, such as body musculature, has also been recognized (Carbayo & Leal-Zanchet 2003, Carbayo 2010). The diet of land planarians seems to be at least partially related to phylogeny, with closely related species usually having similar feeding habits (Cseh et al. 2017). Thus, it is likely that morphometric data could also be applied to taxonomic studies, as already used in several other animal groups (Einsle 1988, Nadler et al. 2000, Moraes et al. 2004, Granados-Amores et al. 2014).

In this study, we gathered morphometric data of the position of the dorsal insertion of the pharynx, position of the mouth, body shape and thickness of the cutaneous musculature from several Neotropical land planarian species of known and unknown diet and compared them to find patterns that could allow us to distinguish genera and predict diet based on morphology. We expect that species with different diets have different pharynx morphologies and different degrees of muscle development, and that species in the same genus have similar values for these characters. By combining all characters, we expect to see species in the same genus forming defined clusters and species with different diets forming clearly separate groups.

Material and Methods

Morphometric data were collected from 230 preserved and histologically processed land planarian specimens deposited in the scientific collection of the Planarian Research Institute, Universidade do Vale do Rio dos Sinos (UNISINOS), São Leopoldo, Rio Grande do Sul, Brazil, making up 84 different species. All specimens belong to species in the subfamily Geoplaninae and include the following genera: *Cephaloflexa*, *Choeradoplana*, *Cratera*, *Imbira*, *Luteostriata*, *Matuxia*, *Notogynaphallia*, *Obama*, *Paraba*, *Pasipha*, *Supramontana* and *Xerapoa* plus a few specimens from undetermined genera.

We analyzed histological sections of two body regions: pharynx, sectioned sagittally, and pre-pharyngeal region, sectioned transversally.

In sections of the pharynx, we measured (1) the length of the pharyngeal pouch (LPP), determined as the distance from its ventral insertion to the posterior end of the pouch at the point in which it reaches its maximum length; (2) the distance from the dorsal insertion to the posterior end of the pouch (DDI); (3) the distance from the mouth opening to the posterior end of the pouch (DMO). From these measurements, we calculated (a) the relative position of the dorsal insertion (RDI) dividing DDI by LPP; and (b) the relative position of the mouth opening (RMO) dividing DMO by LPP. Values of RDI closer to 1 indicate a more cylindrical pharynx and those closer to 0 a more collar-shaped pharynx, with bell-shaped pharynges having intermediate values.

In sections of the pre-pharyngeal region, we measured (1) body height; (2) body width; (3) thickness of the dorsal cutaneous musculature (TDM); and (4) thickness of the ventral cutaneous musculature (TVM). From these measurements, we calculated (a) the transverse circularity (TC) by dividing body height by body width; (b) the ratio of the TDM to the body height (RDM); (c) the ratio of the TVM to the body height (RVM); and (d) the ratio of the total thickness of the cutaneous musculature to the body height (RCM, usually called CMI or mc:h in taxonomic works of land planarians).

Of the 280 analyzed specimens, 51 had only data of the pharynx available and 18 only data of the pre-pharyngeal region, resulting in 161 specimens with all measurements available (Supplementary Table S1).

Additional to the measurements described above, we also collected data regarding the diet of the analyzed specimens. Diet was classified, according to what is known for the corresponding species, into: only gastropods (G), only planarians (P), gastropods and planarians (GP), gastropods, planarians and earthworms (GPE), woodlice (W), harvestmen (H) and unknown (U). Additionally, G, P, GP and GPE were grouped in a single category of soft prey, and W and H in a category of arthropod prey.

We performed linear regressions to verify whether RMO was related to RDI and whether TC was related to RCM. We also performed analyses of variance (ANOVA) followed by Tukey test for pairwise comparisons to verify whether there were differences in the mean values of RDI, RMO, TC and RCM according to genus and diet, when diet was known. For these analyses, we included only one specimen of each of the sampled species. The specimen was randomly chosen from the available specimens. This was done to avoid that the oversampling of one species could bias the data of a genus, since a species with many specimens would contribute more strongly to the genus' mean values. After this selection, only genera with more than three remaining samples were used in the analyses.

We ran a principal component analysis (PCA) using the values of RDI, RMO, TC, RDM, RVM and RCM for the analyzed specimens. Only principal components (PCs) with eigenvalues greater than 1 were extracted and the factor matrix was rotated by a varimax rotation. The rotated factor matrix was used to determine which variables were more correlated to the PCs. We displayed the result graphically according to genus and diet to determine whether there was a significant separation related to phylogeny or diet.

All analyses were performed by means of the program IBM SPSS Statistics 20.

Results

The relative position of the mouth varied between about 0 and 0.8 and was partially explained by the relative position of the dorsal insertion of the pharynx, which varied between about 0 and 1 ($R^2 = 0.260$, $F_{1,82} = 28.743$, $p < 0.001$). Higher values of the position of the dorsal insertion are usually associated with lower values of the position of the mouth. However, as seen in the dispersion graph (Fig. 2), the position of the mouth is more variable in cylindrical pharynges (values closer to 1) than in collar-shaped pharynges (values closer to 0).

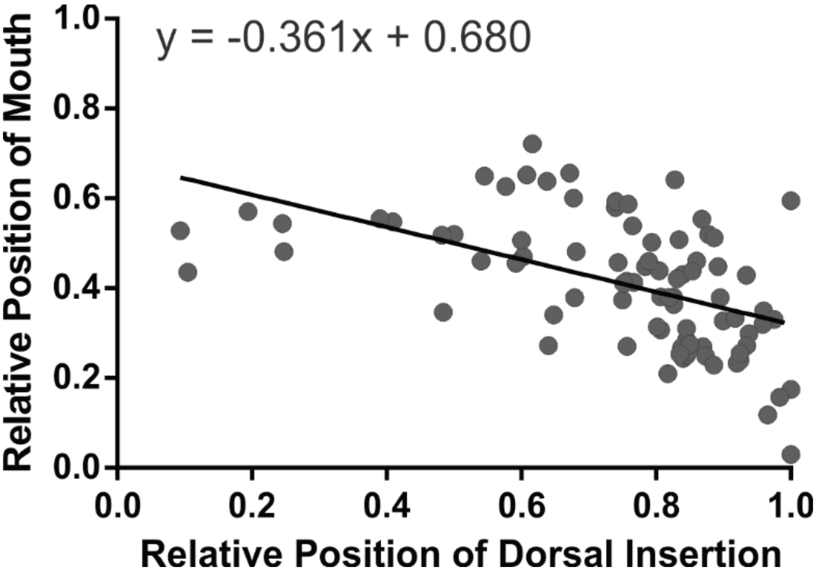

Fig. 2. Relationship between relative position of the dorsal insertion of the pharynx in the pharyngeal pouch and relative position of the mouth in the pharyngeal pouch.

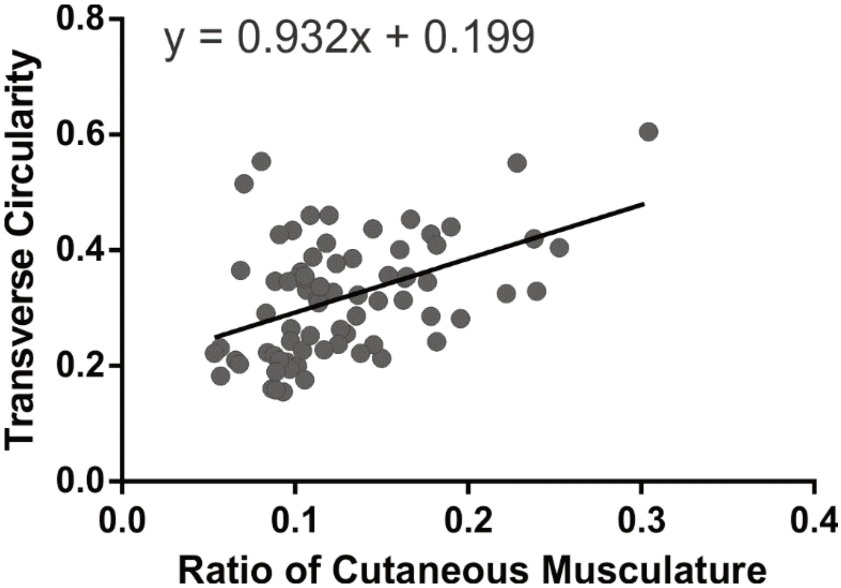

Fig. 3. Relationship between the ratio of the height of the cutaneous musculature to the body height and the transverse circularity of the body.

Likewise, the ratio of the cutaneous musculature to the body height partially explained transverse circularity ($R^2 = 0.209$, $F_{1,72} = 18.727$, $p < 0.001$), with higher values of cutaneous musculature associated with higher values of circularity (Fig. 3).

Mean relative position of the dorsal insertion of the pharynx was significantly different between some genera (ANOVA, $F_{6,64} = 3.345$, $p = 0.006$), with *Paraba* showing higher values than *Choeradoplana*, *Luteostriata* and *Obama*. *Cratera, Pasipha* and *Notogynaphallia* had intermediate values (Fig. 4). Data on the other genera were too few to include in the analysis. Mean values of the dorsal insertion were not significantly different between species feeding on arthropods and species feeding on soft prey (ANOVA, $F_{1,16} = 0.504$, $p = 0.488$).

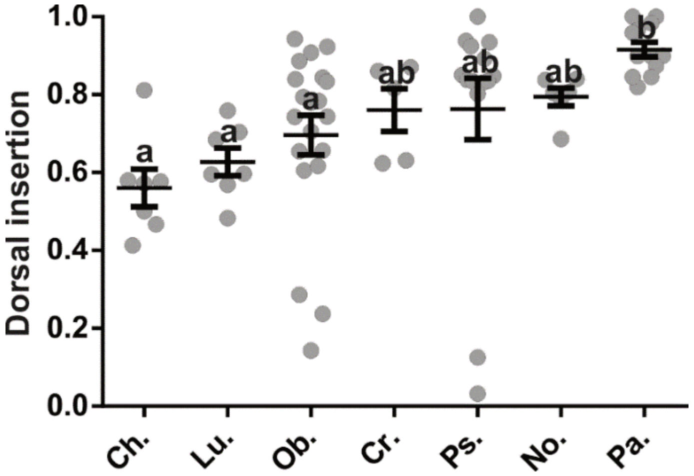

Fig. 4. Relative position of the dorsal insertion of the pharynx in the pharyngeal pouch in land planarians of different genera. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively. Groups that do not share the same letter are significantly different from each other. Ch. = *Choeradoplana*, Cr. = *Cratera*, Lu. = *Luteostriata*, No. = *Notogynaphallia*, Ob. = *Obama*, Pa. = *Paraba*, Ps. = *Pasipha*.

Mean relative position of the mouth was significantly smaller in *Paraba* compared to *Choeradoplana, Luteostriata, Obama, Cratera* and *Pasipha*. *Notogynaphallia* showed intermediate values (ANOVA, $F_{6,55} = 8.012$, $p < 0.001$) (Fig.5). Likewise, mean relative position of the mouth was smaller in species feeding on soft prey than in those feeding on arthropods (ANOVA, F1,40 = 17.503, $p < 0.001$) (Fig. 6).

Mean transverse circularity was significantly smaller in *Obama* compared to *Choeradoplana*, *Notogynaphallia*, *Luteostriata*, *Pasipha* and *Paraba*, with *Cratera* having intermediate values (ANOVA, $F_{6,52} = 12.385$, $p < 0.001$) (Fig. 7). Similarly, mean transverse circularity was significantly smaller in planarians feeding on soft prey than in planarians feeding on arthropods (ANOVA, $F_{1,28} = 50.885$, $p < 0.001$) (Fig. 8).

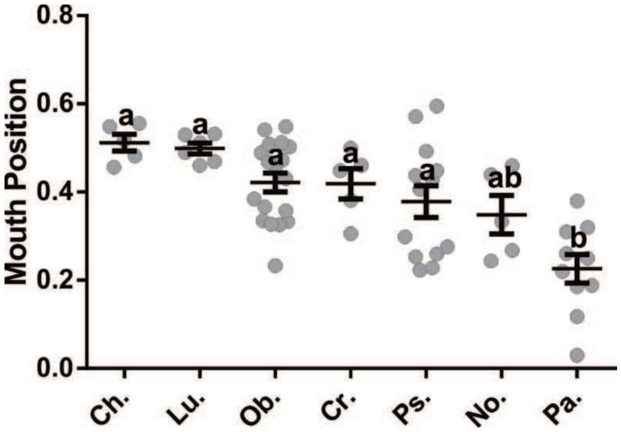

Fig. 5. Relative position of the mouth in the pharyngeal pouch in land planarians of different genera. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively. Groups that do not share the same letter are significantly different from each other. Ch. = *Choeradoplana*, Cr. = *Cratera*, Lu. = *Luteostriata*, No. = *Notogynaphallia*, Ob. = *Obama*, Pa. = *Paraba*, Ps. = *Pasipha*.

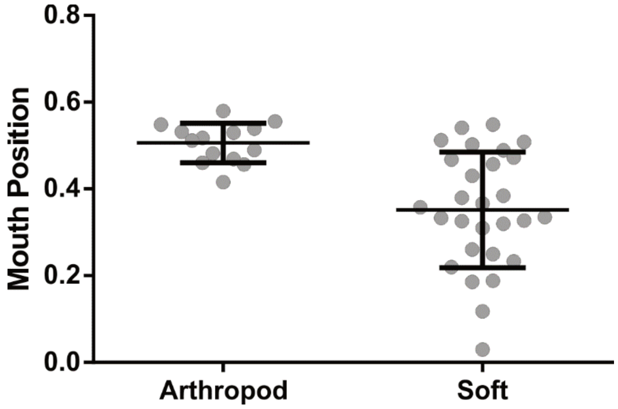

Fig. 6. Relative position of the mouth in the pharyngeal pouch in land planarians that feed on arthropods or soft prey. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively.

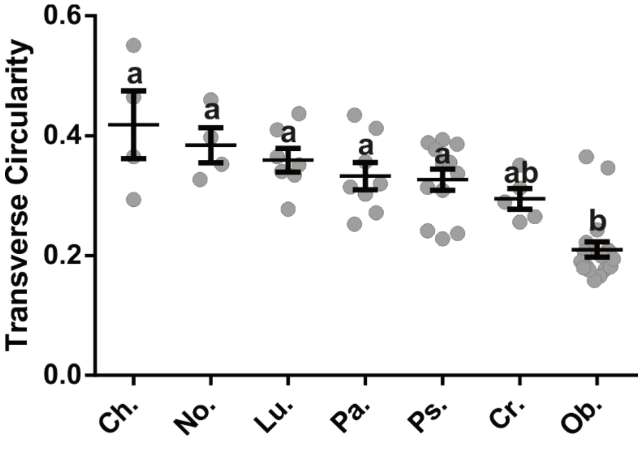

Fig. 7. Transverse circularity in land planarians of different genera. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively. Groups that do not share the same letter are significantly different from each other. Ch. = *Choeradoplana*, Cr. = *Cratera*, Lu. = *Luteostriata*, No. = *Notogynaphallia*, Ob. = *Obama*, Pa. = *Paraba*, Ps. = *Pasipha*.

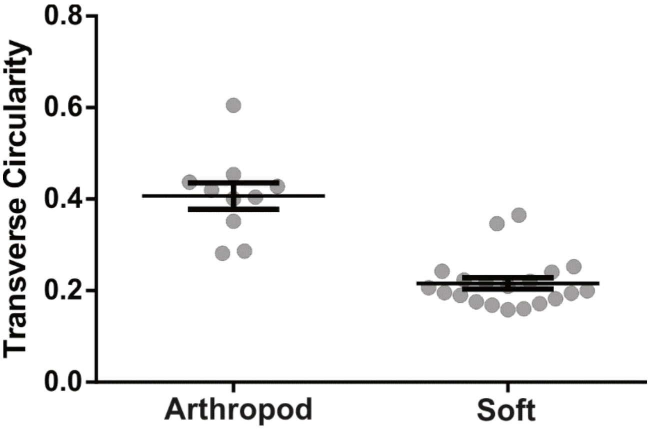

Fig. 8. Transverse circularity in land planarians that feed on arthropods or soft prey. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively.

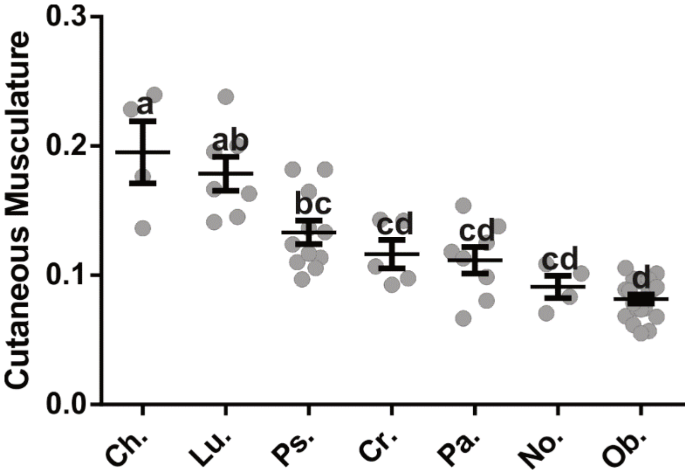

Fig. 9. Ratio of the thickness of the cutaneous musculature to the body height in land planarians of different genera. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively. Groups that do not share the same letter are significantly different from each other. Ch. = *Choeradoplana*, Cr. = *Cratera*, Lu. = *Luteostriata*, No. = *Notogynaphallia*, Ob. = *Obama*, Pa. = *Paraba*, Ps. = *Pasipha*.

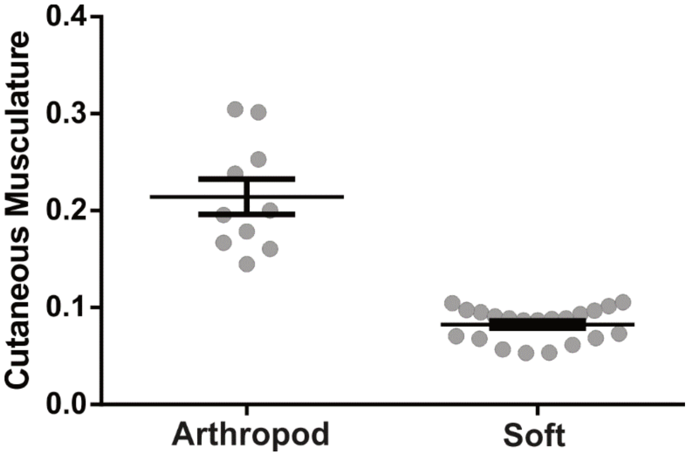

Fig. 10. Ratio of the thickness of the cutaneous musculature to the body height in land planarians that feed on arthropods or soft prey. Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively.

Mean ratio of the cutaneous musculature to the body height was significantly different between some genera (ANOVA, $F_{6,51} = 18.771$, $p < 0.001$), with *Choeradoplana* showing the highest values and *Obama* the lowest (Fig. 9). Species that feed on arthropods have significantly higher values of cutaneous musculature than species that feed on soft prey (ANOVA, $F_{1,29} = 98.789$, $p < 0.001$) (Fig. 10).

In the PCA, two PCs had eigenvalues greater than 1 (3.315 for PC1 and 1.295 for PC2). PC1 explained more than half of the variation (55.28%) and PC2 explained 21.59%. Variables of cutaneous musculature and transverse circularity were more related to PC1, while variables of the relative position of the mouth and the dorsal insertion of the pharynx were more related to PC2 (Table 1).

Table 1. Rotated component matrix showing the relation of the six variables to the first two principal components.

Variable	**Component**	
	1	**2**
Relative position of the dorsal insertion of the pharynx (RDI)	0.046	-0.886
Relative position of the mouth (RMO)	0.288	0.784
Height of dorsal cutaneous musculature to body height (RDM)	0.884	0.254
Height of ventral cutaneous musculature to body height (RVM)	0.910	0.199
Height of total cutaneous musculature to body height (RCM)	0.950	0.238
Transverse circularity (TC)	0.653	-0.164

Plotting the results of the two first PCs did not show a complete separation of specimens according to genus (Fig. 11), although some tendencies are seen. *Cephaloflexa*, *Choeradoplana*, *Luteostriata* and *Supramontana* are highly overlapped and show a separation from another group composed of *Cratera*, *Matuxia*, *Notogynaphallia*, *Paraba* and most specimens of *Obama* and *Pasipha*. The genus *Imbira*, as well as some specimens of *Obama* and *Pasipha* form clearly separate groups. According to diet (Fig. 13), specimens showed a better separation, with specimens of species that feed on arthropods (woodlice and harvestmen) well-separated from specimens that feed on soft prey (gastropods, planarians and earthworms) (Fig. 14).

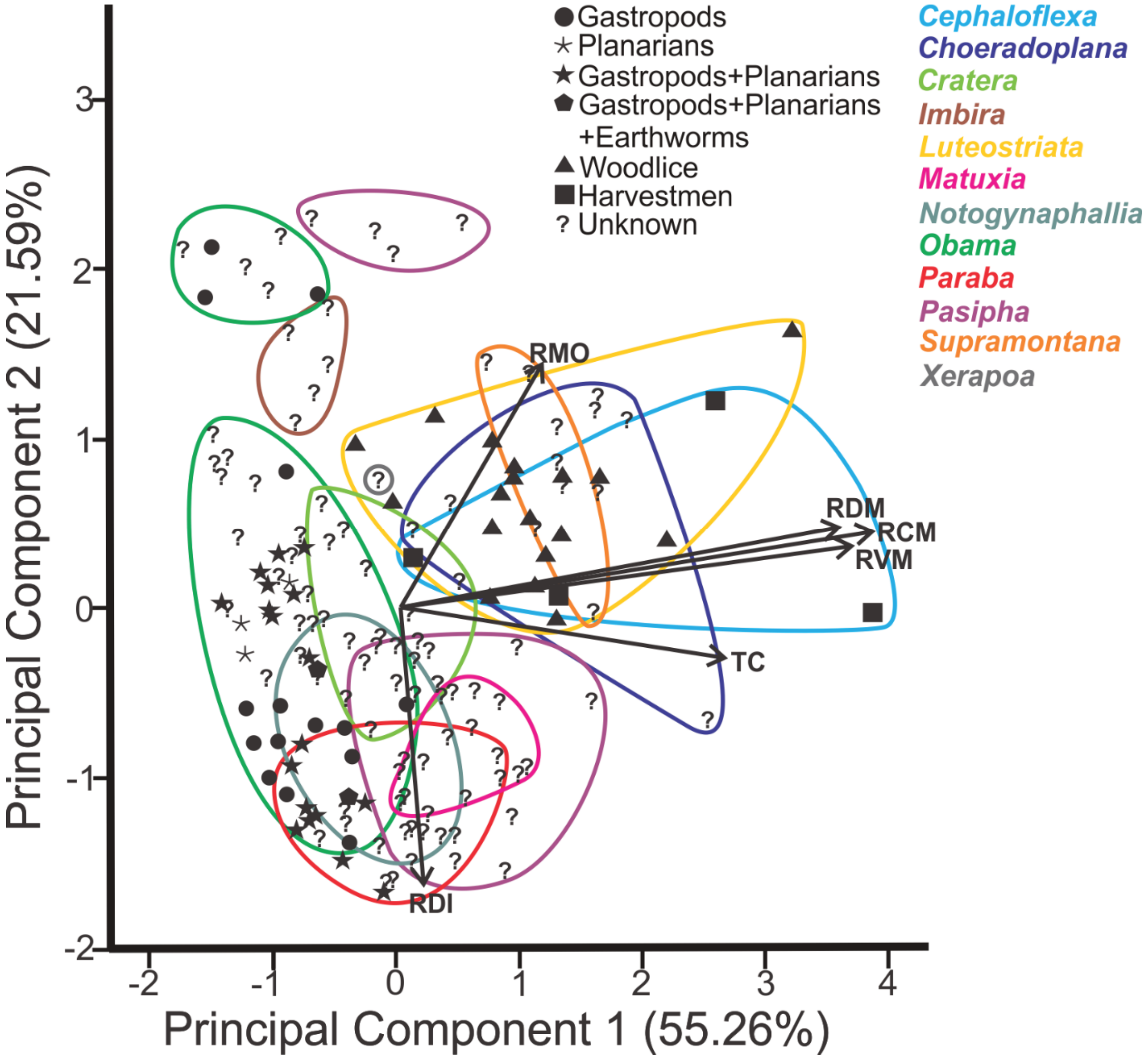

Fig. 11. Biplot of variables and the distribution of the examined land planarian specimens according to the first and second principal components. Specimens are marked by diet and distribution of each genus is outlined.

Discussion

This is the first morphometry study to address behavior in land planarians and relate it to diet. Some previous studies measured weight and position of the mouth and the gonopore, but only for intraspecific comparisons (Mather & Christensen 1996, 2003).

Our results indicate that, at least in the subfamily Geoplaninae, the position of the mouth in the pharyngeal pouch is partially influenced by the position of the dorsal insertion of the pharynx, although there is a greater variation in mouth position in cylindrical pharynges than in bell-shaped ones, a tendency that had already been noticed by Graff (1899). In species that show a negative relation between the position of the mouth and the position of the dorsal insertion of the pharynx, it may be an adaptation to facilitate the eversion of the pharynx. Collar-shaped pharynges are directed ventrally while cylindrical pharynges are directed backward, so a mouth opening respectively at the center of the pouch or at its posterior end is directly in front of the pharynx.

The relation between the thickness of the cutaneous musculature and the shape of the body in transversal section had also been examined by Graff (1899), who concluded that there is no correlation. Our results, which include only species of the subfamily Geoplaninae, indicate that the transverse circularity is at least partially explained by the cutaneous musculature. However, this probably does not apply to all land planarians. Species in the subfamily Microplaninae, for example, have a very weak cutaneous musculature (Sluys et al. 2016) and usually a very cylindrical body (with high values of transverse circularity), a tendency that goes on the opposite direction of what we observed in Geoplaninae. It is worth mentioning, though, that microplaninid land planarians have a very well-developed mesenchymal musculature (Mateos et al. 2017), which does not occur in geoplaninid land planarians. Thus, while the cutaneous musculature may not be a good predictor of body shape in land planarians in general, it seems to have a considerable influence in species of the subfamily Geoplaninae.

The shape of the everted pharynx, although usually considered to be somewhat related to the diet (Winsor et al. 1998), did not show significant differences between species feeding on soft-bodied prey and species feeding on arthropods. Between species of the same genus, the shape of the pharynx and the mouth position are usually similar, although at least in the genera *Obama* and *Pasipha* there are species lying on both extremes regarding the shape of the pharynx. In *Obama*, it is known that both species with collar-shaped pharynges and species with cylindrical pharynges can have a similar diet composed of gastropods (Boll & Leal-Zanchet 2017). The mouth position in arthropod-eating species showed to be located more centrally in the pharyngeal pouch, while the position was more variable in species eating soft-bodied prey.

Transverse circularity (or body shape) showed differences between genera and between species of different diets, and species feeding on soft-bodied prey tend to be flatter than species feeding on arthropods. This could be related to the fact that the capture of soft-bodied prey usually happens with the planarian covering or embracing the prey (Boll & Leal-Zanchet 2018), which is facilitated by a wider body.

The thickness of the cutaneous musculature was the character that better separated the genera and the diets. Genera with thicker cutaneous musculature, such as *Luteostriata*, are known to feed on arthropods (Prasniski & Leal-Zanchet 2009, Carbayo 2010), while some with a weaker musculature, such as *Obama*, feed on soft-bodied prey (Boll & Leal-Zanchet 2016). The thicker musculature of arthropod-eating species may help the capture of prey by pulling them quickly toward the body, and such species often have a cephalic retractor muscle that may facilitate the behavior (Carbayo 2010).

Although the PCA biplot did not clearly separate different genera, at least some of the clusters corroborate results from molecular phylogenetics, such as the close relationship of *Cephaloflexa* and *Choeradoplana*, and of *Luteostriata* and *Supramontana*, which do not seem to form clearly separate groups (Carbayo et al. 2013). On the other hand, the PCA biplot clearly separated species feeding on arthropods (i.e., woodlice and harvestmen) from species feeding on soft-bodied prey. The first group is characterized by a thick cutaneous musculature and bell-shaped pharynges and the latter is characterized by a thin cutaneous musculature and pharynges varying from collar-shaped to cylindrical. The diet of the species of *Imbira* used in this study is unknown, but they grouped with species that feed on soft-bodied prey, suggesting that their diet is likely composed of gastropods, earthworms or other planarians. Specimens of *Imbira marcusi* (not included in this study) have been observed feeding on earthworms in nature (Cseh et al. 2017), which supports our results. Similarly, there are no data on the diet of any of the species of *Cratera, Matuxia*, *Notogynaphallia* and *Pasipha* used in this study. These genera partially overlapped with the group of species feeding on soft-bodied prey but are more biased toward an intermediate position between the two defined diet groups. Field observations with other species of these genera suggest that species of *Matuxia* feed on insect larvae and species of *Pasipha* feed on millipedes, while *Notogynaphallia* may include both insect larvae and other land planarians in their diet (Cseh et al. 2017). While both millipedes and insect larvae are arthropods, their elongate bodies and slower movements perhaps make them easier to be captured using capture behaviors intermediate between those applied to slugs or earthworms and those used to capture fast-moving arthropods such as woodlice and harvestmen by combining the flexibility of the former and the agility of the latter. It is also worth noticing that their position in the biplot, when compared to woodlouse- and harvestman-eating planarians, indicates that these larva- and millipede-eating planarians have more cylindrical pharynges than the former. Although a cylindrical pharynx is likely used by this group to perforate the prey and suck their contents, as suggested by Winsor et al. (1998), the bell-shaped pharynx of *Luteostriata* is used similarly to perforate woodlice (Boll & Leal-Zanchet 2018), going against the idea that a bell-shaped pharynx would be extended over the prey rather than inserted into it (Winsor et al. 1998). Therefore, it is likely that bell-shaped pharynges are functionally more similar to cylindrical ones than to collar-shaped ones.

Although originally suggested to be related to the method of ingesting prey, the shape of the pharynx alone does not seem to be a good predictor of a planarian's diet. However, by combining information on musculature and pharynx shape, the potential prey of geoplaninid land planarians can be narrowed down, facilitating the investigation of their feeding habits.

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Supplementary Material

Supplementary Table S1. Morphometric data of land planarian specimens. BH: body height; BW: body width; DDI: distance of dorsal insertion; DMO: distance of mouth; LPP: length of pharyngeal pouch; RCM: ratio of TCM to BH; RDI: relative position of dorsal insertion; RDM: ratio of TDM to BH; RMO: relative position of mouth; RVM: ratio of TCM to BH; TC: transverse circularity (BH/BW); TCM: thickness of total cutaneous musculature; TDM: thickness of dorsal cutaneous musculature; TVM: thickness of ventral cutaneous musculature.

Genus	species	LPP (mm)	DDI (mm)	DMO (mm)	RDI	RMO	BH (mm)	TDM (mm)	TVM (mm)	MCT (mm)	RDM	RVM	RCM	BW (mm)	TC
Cephaloflexa	*araucariana*	3.08	2.33	1.28	0.76	0.42	-	-	-	-	-	-	-	-	-
Cephaloflexa	*araucariana*	0.88	0.54	0.27	0.61	0.31	-	-	-	-	-	-	-	-	-
Cephaloflexa	*araucariana*	1.85	0.85	0.85	0.46	0.46	0.73	0.11	0.11	0.22	0.15	0.15	0.30	3.23	0.23
Cephaloflexa	*araucariana*	1.89	1.19	0.77	0.63	0.41	0.96	0.07	0.06	0.13	0.07	0.06	0.14	3.35	0.29
Cephaloflexa	sp. 1	1.15	0.88	0.62	0.77	0.54	0.56	0.05	0.05	0.10	0.09	0.09	0.18	1.31	0.43
Cephaloflexa	sp. 2	1.19	0.88	0.69	0.74	0.58	0.92	0.14	0.14	0.28	0.15	0.15	0.30	1.52	0.61
Choeradoplana	*benyai*	2.81	1.15	1.54	0.41	0.55	1.39	0.19	0.11	0.30	0.14	0.08	0.22	3.35	0.41
Choeradoplana	*benyai*	1.96	0.81	0.96	0.41	0.49	1.08	0.10	0.12	0.22	0.09	0.11	0.20	2.96	0.36
Choeradoplana	*benyai*	1.92	0.92	0.96	0.48	0.50	-	-	-	-	-	-	-	-	-
Choeradoplana	*benyai*	2.35	1.19	1.27	0.51	0.54	0.96	0.12	0.11	0.23	0.13	0.11	0.24	2.92	0.33
Choeradoplana	*iheringi*	-	-	-	-	-	1.19	0.10	0.11	0.21	0.08	0.09	0.18	3.45	0.34
Choeradoplana	*iheringi*	2.88	1.65	1.62	0.57	0.56	2.17	0.14	0.14	0.28	0.06	0.06	0.13	4.73	0.46
Choeradoplana	*iheringi*	2.08	1.04	1.08	0.50	0.52	1.23	0.10	0.15	0.25	0.08	0.12	0.20	2.65	0.46
Choeradoplana	*iheringi*	1.35	0.92	0.65	0.68	0.48	-	-	-	-	-	-	-	-	-
Choeradoplana	*minima*	1.96	1.12	1.19	0.57	0.61	0.81	0.10	0.08	0.18	0.12	0.10	0.22	2.49	0.33
Choeradoplana	*minima*	1.00	0.58	0.42	0.58	0.42	0.44	0.03	0.03	0.06	0.07	0.07	0.14	1.50	0.29
Choeradoplana	*minima*	1.12	0.54	0.58	0.48	0.52	0.77	0.05	0.07	0.12	0.06	0.09	0.16	1.35	0.57
Choeradoplana	*minima*	-	-	-	-	-	0.44	0.03	0.03	0.06	0.07	0.07	0.14	1.44	0.31
Choeradoplana	sp. 1	1.38	1.12	0.63	0.81	0.46	0.92	0.08	0.13	0.21	0.09	0.14	0.23	1.67	0.55
Choeradoplana	sp. 2	2.46	1.42	1.54	0.58	0.63	-	-	-	-	-	-	-	-	-
Choeradoplana	sp. 2	2.69	1.54	1.19	0.57	0.44	-	-	-	-	-	-	-	-	-
Choeradoplana	sp. 2	3.04	1.42	1.69	0.47	0.56	-	-	-	-	-	-	-	-	-
Cratera	*aureomaculata*	2.50	2.15	1.15	0.86	0.46	1.23	0.05	0.07	0.12	0.04	0.06	0.10	4.65	0.26

Cratera	*cryptolineata*	2.50	1.96	1.12	0.78	0.45	1.00	0.05	0.07	0.12	0.05	0.07	0.12	2.85	0.35
Cratera	*cryptolineata*	2.12	1.73	0.73	0.82	0.34	1.08	0.04	0.06	0.10	0.04	0.06	0.09	2.96	0.36
Cratera	*cryptolineata*	2.46	1.69	0.88	0.69	0.36	1.31	0.05	0.09	0.14	0.04	0.07	0.11	3.96	0.33
Cratera	*nigrimarginata*	3.31	2.88	1.42	0.87	0.43	1.31	0.07	0.10	0.17	0.05	0.08	0.13	5.12	0.26
Cratera	*nigrimarginata*	3.73	3.08	1.42	0.83	0.38	1.12	0.08	0.08	0.16	0.07	0.07	0.14	4.96	0.23
Cratera	*ochra*	3.80	2.85	1.42	0.75	0.37	1.58	0.08	0.11	0.19	0.05	0.07	0.12	5.46	0.29
Cratera	*ochra*	3.08	1.92	0.88	0.62	0.29	1.69	0.09	0.15	0.24	0.05	0.09	0.14	7.42	0.23
Cratera	*ochra*	2.88	2.31	0.88	0.80	0.31	1.00	0.06	0.09	0.15	0.06	0.09	0.15	4.69	0.21
Cratera	*steffeni*	1.00	0.77	0.50	0.77	0.50	0.54	0.04	0.04	0.08	0.07	0.07	0.15	1.73	0.31
Cratera	*steffeni*	3.04	1.92	1.35	0.63	0.44	1.31	0.07	0.07	0.14	0.05	0.05	0.11	6.94	0.19
Cratera	*steffeni*	2.50	1.62	0.85	0.65	0.34	-	-	-	-	-	-	-	-	-
Imbira	*guaiana*	3.46	1.35	1.92	0.39	0.55	1.00	0.05	0.05	0.10	0.05	0.05	0.10	3.13	0.32
Imbira	*guaiana*	2.96	0.81	1.15	0.27	0.39	1.44	0.06	0.06	0.12	0.04	0.04	0.08	4.13	0.35
Imbira	*guaiana*	-	-	-	-	-	0.87	0.05	0.06	0.11	0.06	0.07	0.13	3.31	0.26
Imbira	sp.1	5.19	2.08	2.80	0.40	0.54	1.92	0.08	0.09	0.17	0.04	0.05	0.09	5.54	0.35
Imbira	sp.1	6.65	2.31	3.85	0.35	0.58	1.54	0.09	0.08	0.17	0.06	0.05	0.11	5.69	0.27
Imbira	sp.1	7.59	1.88	3.65	0.25	0.48	1.77	0.08	0.08	0.16	0.05	0.05	0.09	5.92	0.30
Luteostriata	*abundans*	2.50	1.35	1.15	0.54	0.46	1.06	0.05	0.08	0.13	0.05	0.08	0.12	3.23	0.33
Luteostriata	*abundans*	3.27	1.97	1.92	0.60	0.59	0.92	0.03	0.07	0.10	0.03	0.08	0.11	3.17	0.29
Luteostriata	*abundans*	1.73	0.81	0.77	0.47	0.45	-	-	-	-	-	-	-	-	-
Luteostriata	*abundans*	2.38	1.15	1.35	0.48	0.57	0.85	0.06	0.06	0.12	0.07	0.07	0.14	2.33	0.36
Luteostriata	*abundans*	3.46	2.23	2.08	0.64	0.60	0.81	0.05	0.08	0.13	0.06	0.10	0.16	2.02	0.40
Luteostriata	*abundans*	2.50	1.54	1.44	0.62	0.58	-	-	-	-	-	-	-	-	-
Luteostriata	*arturi*	-	-	-	-	-	0.87	0.12	0.10	0.22	0.14	0.11	0.25	2.15	0.40
Luteostriata	*arturi*	1.85	1.27	0.98	0.69	0.53	-	-	-	-	-	-	-	-	-
Luteostriata	*arturi*	1.54	0.92	0.69	0.60	0.45	0.77	0.08	0.06	0.14	0.10	0.08	0.18	1.88	0.41
Luteostriata	*arturi*	1.27	0.81	0.81	0.64	0.64	0.92	0.09	0.06	0.15	0.10	0.07	0.16	2.15	0.43
Luteostriata	*ceciliae*	2.04	1.23	0.96	0.60	0.47	1.00	0.09	0.10	0.19	0.09	0.10	0.19	2.33	0.43
Luteostriata	*ceciliae*	2.00	1.19	0.93	0.60	0.47	0.96	0.08	0.10	0.18	0.08	0.10	0.19	2.87	0.33
Luteostriata	*ceciliae*	1.73	1.15	0.92	0.66	0.53	0.85	0.09	0.07	0.16	0.11	0.08	0.19	2.12	0.40

Luteostriata	*ceciliae*	2.25	1.13	1.23	0.50	0.55	1.54	0.15	0.16	0.31	0.10	0.10	0.20	3.19	0.48
Luteostriata	*ceciliae*	2.31	1.50	1.11	0.65	0.48	1.08	0.08	0.10	0.18	0.07	0.09	0.17	2.38	0.45
Luteostriata	*ernesti*	1.85	1.19	1.15	0.64	0.62	-	-	-	-	-	-	-	-	-
Luteostriata	*ernesti*	1.79	0.90	0.84	0.50	0.47	-	-	-	-	-	-	-	-	-
Luteostriata	*ernesti*	2.31	1.46	1.15	0.63	0.50	0.84	0.11	0.09	0.20	0.13	0.11	0.24	2.00	0.42
Luteostriata	*ernesti*	1.97	1.12	1.15	0.57	0.58	1.23	0.10	0.10	0.20	0.08	0.08	0.16	2.77	0.44
Luteostriata	*ernesti*	2.19	1.35	1.58	0.62	0.72	0.46	0.08	0.07	0.15	0.17	0.15	0.33	1.66	0.28
Luteostriata	*graffi*	-	-	-	-	-	0.62	0.05	0.04	0.09	0.08	0.06	0.15	1.42	0.44
Luteostriata	*graffi*	3.00	2.22	1.78	0.74	0.59	-	-	-	-	-	-	-	-	-
Luteostriata	*graffi*	1.96	1.38	0.96	0.70	0.49	0.69	0.06	0.04	0.10	0.09	0.06	0.14	1.58	0.44
Luteostriata	*pseudoceciliae*	1.52	0.76	0.59	0.50	0.39	-	-	-	-	-	-	-	-	-
Luteostriata	*pseudoceciliae*	3.01	2.65	1.54	0.88	0.51	0.92	0.06	0.12	0.18	0.07	0.13	0.20	3.27	0.28
Luteostriata	*pseudoceciliae*	2.69	2.04	1.58	0.76	0.59	0.81	0.07	0.06	0.13	0.09	0.07	0.16	2.38	0.34
Luteostriata	sp. 1	3.13	2.12	1.88	0.68	0.60	0.85	0.08	0.09	0.17	0.09	0.11	0.20	2.54	0.33
Luteostriata	sp. 1	3.65	2.50	2.50	0.68	0.68	1.04	0.07	0.10	0.17	0.07	0.10	0.16	2.96	0.35
Matuxia	sp. 1	1.88	1.51	0.58	0.81	0.31	-	-	-	-	-	-	-	-	--
Matuxia	sp. 1	1.66	1.25	0.51	0.75	0.31	0.74	0.06	0.06	0.12	0.08	0.08	0.16	2.19	0.34
Matuxia	sp. 1	3.20	2.93	1.03	0.92	0.32	0.77	0.07	0.06	0.13	0.09	0.08	0.17	2.31	0.33
Matuxia	sp. 1	1.66	1.47	0.46	0.89	0.28	-	-	-	-	-	-	-	-	-
Matuxia	sp. 1	1.97	1.92	0.88	0.97	0.45	1.08	0.08	0.07	0.15	0.07	0.06	0.14	3.15	0.34
Matuxia	sp. 1	2.66	2.35	0.73	0.88	0.27	0.87	0.04	0.05	0.09	0.05	0.06	0.10	2.40	0.36
Matuxia	sp. 2	1.50	1.15	0.62	0.77	0.41	0.92	0.06	0.05	0.11	0.07	0.05	0.12	2.00	0.46
Notogynaphallia	*plumbea*	1.19	1.00	0.29	0.84	0.24	0.92	0.05	0.05	0.10	0.05	0.05	0.11	2.00	0.46
Notogynaphallia	sp. 1	1.80	1.54	0.60	0.85	0.33	-	-	-	-	-	-	-	-	-
Notogynaphallia	sp. 1	-	-	-	-	-	0.48	0.02	0.02	0.04	0.04	0.04	0.08	1.65	0.29
Notogynaphallia	sp. 1	1.85	1.27	0.62	0.69	0.34	0.85	0.03	0.03	0.06	0.04	0.04	0.07	2.60	0.33
Notogynaphallia	sp. 1	1.71	1.38	0.65	0.81	0.38	0.73	0.03	0.03	0.06	0.04	0.04	0.08	2.04	0.36
Notogynaphallia	sp. 2	2.46	1.98	1.08	0.80	0.44	-	-	-	-	-	-	-	-	-
Notogynaphallia	sp. 3	1.85	1.46	0.85	0.79	0.46	0.69	0.03	0.04	0.07	0.04	0.06	0.10	1.96	0.35
Notogynaphallia	sp. 4	1.42	1.19	0.38	0.84	0.27	0.85	0.03	0.03	0.06	0.04	0.04	0.07	1.65	0.52

Notogynaphallia	sp. 4	1.54	1.23	0.33	0.80	0.21	0.81	0.04	0.02	0.06	0.05	0.02	0.07	2.04	0.40
Obama	*"autumna"*	5.81	4.88	2.50	0.84	0.43	1.65	0.08	0.08	0.16	0.05	0.05	0.10	8.50	0.19
Obama	*"rubriventris"*	7.27	5.77	3.65	0.79	0.50	1.42	0.08	0.07	0.15	0.06	0.05	0.11	8.08	0.18
Obama	*anthropophila*	2.50	2.27	1.54	0.91	0.62	-	-	-	-	-	-	-	-	-
Obama	*anthropophila*	4.58	3.23	2.12	0.71	0.46	1.35	0.04	0.06	0.10	0.03	0.04	0.07	5.62	0.24
Obama	*anthropophila*	4.00	3.31	1.86	0.83	0.47	0.96	0.04	0.04	0.08	0.04	0.04	0.08	4.91	0.20
Obama	*anthropophila*	5.23	4.38	2.42	0.84	0.46	1.37	0.05	0.04	0.09	0.04	0.03	0.07	5.83	0.23
Obama	*anthropophila*	5.50	4.42	2.69	0.80	0.49	1.37	0.05	0.04	0.09	0.04	0.03	0.07	6.54	0.21
Obama	*anthropophila*	3.38	2.65	1.31	0.78	0.39	1.17	0.05	0.04	0.09	0.04	0.03	0.08	6.92	0.17
Obama	*anthropophila*	-	-	-	-	-	1.25	0.05	0.05	0.10	0.04	0.04	0.08	6.92	0.18
Obama	*anthropophila*	3.08	2.00	0.92	0.65	0.30	1.58	0.05	0.04	0.09	0.03	0.03	0.06	8.62	0.18
Obama	*anthropophila*	3.85	2.85	1.54	0.74	0.40	1.19	0.05	0.04	0.09	0.04	0.03	0.08	6.46	0.18
Obama	*anthropophila*	3.65	2.77	1.15	0.76	0.32	-	-	-	-	-	-	-	-	-
Obama	*anthropophila*	2.42	2.00	0.88	0.83	0.36	1.15	0.06	0.04	0.10	0.05	0.03	0.09	5.46	0.21
Obama	*anthropophila*	2.77	2.50	0.85	0.90	0.31	1.19	0.05	0.04	0.09	0.04	0.03	0.08	5.06	0.24
Obama	*anthropophila*	4.61	3.54	2.35	0.77	0.51	1.38	0.05	0.07	0.12	0.04	0.05	0.09	6.28	0.22
Obama	*baptistae*	7.04	2.88	2.58	0.41	0.37	1.76	0.04	0.06	0.10	0.02	0.03	0.06	7.62	0.23
Obama	*baptistae*	4.00	2.62	2.00	0.66	0.50	1.34	0.05	0.05	0.10	0.04	0.04	0.07	7.27	0.18
Obama	*baptistae*	5.77	2.65	1.92	0.46	0.33	2.13	0.07	0.08	0.15	0.03	0.04	0.07	13.88	0.15
Obama	*baptistae*	6.08	3.65	3.08	0.60	0.51	1.62	0.05	0.05	0.10	0.03	0.03	0.06	8.27	0.20
Obama	*carbayoi*	5.00	2.42	1.73	0.48	0.35	-	-	-	-	-	-	-	-	-
Obama	*carbayoi*	7.12	4.31	3.85	0.61	0.54	2.46	0.06	0.08	0.14	0.02	0.03	0.06	13.50	0.18
Obama	*decidualis*	3.50	2.77	1.92	0.79	0.55	1.19	0.05	0.05	0.10	0.04	0.04	0.08	5.33	0.22
Obama	*decidualis*	3.58	2.35	1.54	0.66	0.43	1.46	0.04	0.05	0.09	0.03	0.03	0.06	7.08	0.21
Obama	*decidualis*	3.46	2.35	1.31	0.68	0.38	1.12	0.05	0.04	0.09	0.04	0.04	0.08	5.44	0.21
Obama	*decidualis*	-	-	-	-	-	1.21	0.04	0.05	0.09	0.03	0.04	0.07	7.12	0.17
Obama	*ficki*	10.96	2.69	5.96	0.25	0.54	1.63	0.06	0.06	0.12	0.04	0.04	0.07	9.19	0.18
Obama	*ficki*	7.81	1.85	3.65	0.24	0.47	2.09	0.08	0.06	0.14	0.04	0.03	0.07	10.73	0.19
Obama	*ficki*	9.81	2.31	4.42	0.24	0.45	1.44	0.09	0.09	0.18	0.06	0.06	0.13	8.15	0.18
Obama	*ficki*	-	-	-	-	-	2.27	0.10	0.10	0.20	0.04	0.04	0.09	10.42	0.22

Obama	*josefi*	4.04	3.42	2.04	0.85	0.50	1.15	0.05	0.04	0.09	0.04	0.03	0.08	5.58	0.21
Obama	*josefi*	6.50	4.92	1.92	0.76	0.30	2.31	0.07	0.08	0.15	0.03	0.03	0.06	13.46	0.17
Obama	*josefi*	6.42	4.77	2.15	0.74	0.33	1.85	0.05	0.07	0.12	0.03	0.04	0.06	11.19	0.17
Obama	*josefi*	5.41	4.75	2.81	0.88	0.52	-	-	-	-	-	-	-	-	-
Obama	*josefi*	-	-	-	-	-	1.50	0.06	0.08	0.14	0.04	0.05	0.09	9.69	0.15
Obama	*ladislavii*	4.17	3.62	2.31	0.87	0.55	-	-	-	-	-	-	-	-	-
Obama	*ladislavii*	7.15	6.69	2.38	0.94	0.33	2.06	0.05	0.06	0.11	0.02	0.03	0.05	9.29	0.22
Obama	*ladislavii*	2.50	2.44	0.87	0.98	0.35	1.04	0.05	0.05	0.10	0.05	0.05	0.10	4.31	0.24
Obama	*ladislavii*	4.73	4.54	1.65	0.96	0.35	1.54	0.05	0.05	0.10	0.03	0.03	0.06	7.23	0.21
Obama	*ladislavii*	5.38	4.54	1.04	0.84	0.19	1.35	0.04	0.05	0.09	0.03	0.04	0.07	5.35	0.25
Obama	*ladislavii*	3.62	3.46	1.50	0.96	0.41	1.23	0.04	0.05	0.09	0.03	0.04	0.07	4.33	0.28
Obama	*ladislavii*	3.69	3.46	1.08	0.94	0.29	-	-	-	-	-	-	-	-	-
Obama	*ladislavii*	5.08	4.65	1.35	0.92	0.27	1.69	0.05	0.05	0.10	0.03	0.03	0.06	7.25	0.23
Obama	*ladislavii*	5.77	4.58	1.58	0.79	0.27	1.81	0.05	0.05	0.10	0.03	0.03	0.06	8.15	0.22
Obama	*ladislavii*	5.77	4.62	1.77	0.80	0.31	1.54	0.05	0.05	0.10	0.03	0.03	0.06	5.88	0.26
Obama	*maculipunctata*	3.38	3.12	0.50	0.92	0.15	1.62	0.09	0.07	0.16	0.06	0.04	0.10	7.38	0.22
Obama	*maculipunctata*	5.19	4.65	1.69	0.90	0.33	1.69	0.11	0.12	0.23	0.07	0.07	0.14	9.21	0.18
Obama	*maculipunctata*	4.15	3.88	1.35	0.93	0.33	1.54	0.07	0.09	0.16	0.05	0.06	0.10	8.50	0.18
Obama	*maculipunctata*	5.58	4.62	3.58	0.83	0.64	1.50	0.06	0.07	0.13	0.04	0.05	0.09	9.35	0.16
Obama	*nungara*	2.69	2.42	0.88	0.90	0.33	-	-	-	-	-	-	-	-	-
Obama	*nungara*	1.62	1.00	0.27	0.62	0.17	0.69	0.04	0.03	0.07	0.06	0.04	0.10	3.46	0.20
Obama	*polyophthalma*	1.81	1.46	0.46	0.81	0.25	-	-	-	-	-	-	-	-	-
Obama	*polyophthalma*	1.84	1.58	0.38	0.86	0.21	-	-	-	-	-	-	-	-	-
Obama	*polyophthalma*	1.74	1.64	0.36	0.94	0.21	-	-	-	-	-	-	-	-	-
Obama	*polyophthalma*	1.78	1.56	0.36	0.88	0.20	1.54	0.07	0.08	0.15	0.05	0.05	0.10	6.35	0.24
Obama	*polyophthalma*	1.50	1.38	0.35	0.92	0.23	-	-	-	-	-	-	-	-	-
Obama	sp. 1	2.69	2.00	1.23	0.74	0.46	1.46	0.05	0.05	0.10	0.03	0.03	0.07	4.00	0.37
Obama	sp. 2	-	-	-	-	-	2.31	0.10	0.12	0.22	0.04	0.05	0.10	11.19	0.21
Obama	sp. 3	5.00	4.17	2.54	0.83	0.51	1.35	0.06	0.06	0.12	0.04	0.04	0.09	8.54	0.16
Obama	sp. 4	4.81	3.08	1.31	0.64	0.27	1.54	0.07	0.07	0.14	0.05	0.05	0.09	7.35	0.21

Obama	sp. 4	8.35	1.19	3.85	0.14	0.46	1.77	0.06	0.05	0.11	0.03	0.03	0.06	9.77	0.18
Obama	sp. 4	4.42	3.13	1.58	0.71	0.36	1.88	0.05	0.05	0.10	0.03	0.03	0.05	9.12	0.21
Obama	sp. 5	6.60	5.85	3.38	0.89	0.51	1.35	0.05	0.07	0.12	0.04	0.05	0.09	7.12	0.19
Obama	sp. 6	-	-	-	-	-	2.04	0.10	0.08	0.18	0.05	0.04	0.09	12.11	0.17
Obama	sp. 6	8.38	0.88	3.65	0.11	0.44	1.50	0.09	0.08	0.17	0.06	0.05	0.11	8.65	0.17
Obama	sp. 6	6.19	1.77	3.27	0.29	0.53	1.62	0.08	0.07	0.15	0.05	0.04	0.09	7.42	0.22
Obama	sp. 6	-	-	-	-	-	2.08	0.12	0.11	0.23	0.06	0.05	0.11	11.61	0.18
Obama	sp. 6	6.92	1.54	3.27	0.22	0.47	1.27	0.07	0.05	0.12	0.06	0.04	0.09	8.27	0.15
Obama	sp. 6	3.88	3.12	2.19	0.80	0.56	1.15	0.07	0.05	0.12	0.06	0.04	0.10	5.08	0.23
Obama	sp. 7	4.65	3.73	1.46	0.80	0.31	1.77	0.06	0.06	0.12	0.03	0.03	0.07	8.71	0.20
Obama	sp. 7	2.81	2.19	1.08	0.78	0.38	1.27	0.05	0.04	0.09	0.04	0.03	0.07	3.67	0.35
Paraba	*franciscana*	2.00	2.00	0.35	1.00	0.18	-	-	-	-	-	-	-	-	-
Paraba	*franciscana*	-	-	-	-	-	1.02	0.04	0.05	0.09	0.04	0.05	0.09	2.62	0.39
Paraba	*franciscana*	1.62	1.54	0.46	0.95	0.28	0.80	0.05	0.04	0.09	0.06	0.05	0.11	1.77	0.45
Paraba	*franciscana*	2.15	2.04	0.38	0.95	0.18	1.08	0.05	0.06	0.11	0.05	0.06	0.10	3.38	0.32
Paraba	*franciscana*	2.69	2.46	0.50	0.91	0.19	0.87	0.06	0.06	0.12	0.07	0.07	0.14	3.92	0.22
Paraba	*franciscana*	-	-	-	-	-	1.19	0.05	0.06	0.11	0.04	0.05	0.09	3.19	0.37
Paraba	*multicolor*	2.00	1.73	0.44	0.87	0.22	1.04	0.05	0.05	0.10	0.05	0.05	0.10	3.44	0.30
Paraba	*multicolor*	2.08	1.77	0.31	0.85	0.15	0.87	0.04	0.03	0.07	0.05	0.03	0.08	3.54	0.25
Paraba	*multicolor*	3.23	2.96	0.42	0.92	0.13	1.08	0.05	0.05	0.10	0.05	0.05	0.09	4.46	0.24
Paraba	*multicolor*	2.13	1.90	0.27	0.89	0.13	1.04	0.05	0.05	0.10	0.05	0.05	0.10	3.01	0.35
Paraba	*multicolor*	1.42	1.21	0.23	0.85	0.16	-	-	-	-	-	-	-	-	-
Paraba	*multicolor*	2.12	1.73	0.50	0.82	0.24	-	-	-	-	-	-	-	-	-
Paraba	*multicolor*	1.77	1.58	0.31	0.89	0.18	0.88	0.03	0.03	0.06	0.03	0.03	0.07	3.42	0.26
Paraba	*multicolor*	-	-	-	-	-	1.04	0.03	0.03	0.06	0.03	0.03	0.06	3.15	0.33
Paraba	*multicolor*	2.92	2.58	0.58	0.88	0.20	-	-	-	-	-	-	-	-	-
Paraba	*multicolor*	1.08	1.00	0.23	0.93	0.21	-	-	-	-	-	-	-	-	-
Paraba	*multicolor*	1.88	1.69	0.38	0.90	0.20	1.35	0.04	0.05	0.09	0.03	0.04	0.07	5.35	0.25
Paraba	*multicolor*	1.38	1.23	0.35	0.89	0.25	0.85	0.03	0.02	0.05	0.04	0.02	0.06	2.35	0.36
Paraba	*multicolor*	2.85	2.50	0.69	0.88	0.24	1.23	0.05	0.04	0.09	0.04	0.03	0.07	5.58	0.22

Paraba	*Pamu1*	1.46	1.35	0.35	0.92	0.24	0.98	0.03	0.04	0.07	0.03	0.04	0.07	3.73	0.26
Paraba	*rubidolineata*	2.31	2.12	0.77	0.92	0.33	-	-	-	-	-	-	-	-	-
Paraba	*rubidolineata*	2.12	1.73	0.23	0.82	0.11	0.87	0.03	0.04	0.07	0.03	0.05	0.08	3.21	0.27
Paraba	*rubidolineata*	1.58	1.42	0.27	0.90	0.17	0.83	0.04	0.04	0.08	0.05	0.05	0.10	3.58	0.23
Paraba	*rubidolineata*	2.00	1.85	0.73	0.93	0.37	0.85	0.05	0.04	0.09	0.06	0.05	0.11	2.42	0.35
Paraba	*rubidolineata*	1.19	1.04	0.31	0.87	0.26	-	-	-	-	-	-	-	-	-
Paraba	sp. 1	2.69	2.69	0.08	1.00	0.03	-	-	-	-	-	-	-	-	-
Paraba	sp. 2	1.00	0.82	0.38	0.82	0.38	-	-	-	-	-	-	-	-	-
Paraba	sp. 3	3.23	3.12	0.38	0.97	0.12	-	-	-	-	-	-	-	-	-
Paraba	sp. 4	3.08	2.77	0.58	0.90	0.19	0.96	0.06	0.06	0.12	0.06	0.06	0.13	3.35	0.29
Paraba	sp. 4	2.42	2.38	0.38	0.98	0.16	0.92	0.05	0.05	0.10	0.05	0.05	0.11	3.65	0.25
Paraba	sp. 5	2.00	1.69	0.50	0.85	0.25	1.42	0.05	0.09	0.14	0.04	0.06	0.10	3.27	0.43
Paraba	sp. 6	1.69	1.62	0.54	0.96	0.32	1.27	0.05	0.10	0.15	0.04	0.08	0.12	3.08	0.41
Paraba	sp. 7	2.69	2.35	0.67	0.87	0.25	1.15	0.04	0.09	0.13	0.03	0.08	0.11	3.66	0.31
Paraba	sp. 8	2.00	1.69	0.62	0.85	0.31	1.04	0.07	0.09	0.16	0.07	0.09	0.15	2.92	0.36
Pasipha	*albicaudata*	2.50	2.12	0.65	0.85	0.26	0.88	0.05	0.05	0.10	0.06	0.06	0.11	2.85	0.31
Pasipha	*backesi*	1.27	1.08	0.35	0.85	0.28	0.79	0.07	0.06	0.13	0.09	0.08	0.16	2.23	0.35
Pasipha	*backesi*	1.19	1.12	0.23	0.94	0.19	0.77	0.05	0.07	0.12	0.06	0.09	0.16	2.27	0.34
Pasipha	*brevilineata*	1.42	1.19	0.58	0.84	0.41	0.62	0.06	0.06	0.12	0.10	0.10	0.19	1.54	0.40
Pasipha	*brevilineata*	1.23	1.00	0.50	0.81	0.41	0.79	0.06	0.05	0.11	0.08	0.06	0.14	2.01	0.39
Pasipha	*brevilineata*	1.69	1.58	0.46	0.93	0.27	-	-	-	-	-	-	-	-	-
Pasipha	*brevilineata*	-	-	-	-	-	0.88	0.08	0.08	0.16	0.09	0.09	0.18	2.15	0.41
Pasipha	*hauseri*	3.35	0.42	1.65	0.13	0.49	0.77	0.07	0.08	0.15	0.09	0.10	0.19	3.58	0.22
Pasipha	*hauseri*	6.19	0.58	3.27	0.09	0.53	0.88	0.06	0.06	0.12	0.07	0.07	0.14	2.73	0.32
Pasipha	*hauseri*	-	-	-	-	-	0.80	0.05	0.05	0.10	0.06	0.06	0.13	3.38	0.24
Pasipha	*mesoxantha*	2.27	2.27	1.35	1.00	0.59	0.96	0.06	0.05	0.11	0.06	0.05	0.11	2.85	0.34
Pasipha	*paucilineata*	3.04	2.54	0.77	0.84	0.25	1.35	0.08	0.10	0.18	0.06	0.07	0.13	3.50	0.39
Pasipha	sp. 1	2.12	1.96	0.54	0.92	0.25	-	-	-	-	-	-	-	-	-
Pasipha	sp. 2	1.17	0.88	0.48	0.75	0.41	-	-	-	-	-	-	-	-	-
Pasipha	sp. 4	2.73	2.27	1.15	0.83	0.42	1.27	0.08	0.06	0.14	0.06	0.05	0.11	3.27	0.39

Pasipha	sp. 4	2.42	2.00	0.54	0.83	0.22	1.12	0.10	0.07	0.17	0.09	0.06	0.15	2.69	0.42
Pasipha	sp. 5	3.50	3.27	1.50	0.93	0.43	1.13	0.07	0.07	0.14	0.06	0.06	0.12	3.00	0.38
Pasipha	sp. 6	2.50	2.23	1.12	0.89	0.45	0.77	0.06	0.08	0.14	0.08	0.10	0.18	3.19	0.24
Pasipha	sp. 7	4.91	4.19	2.15	0.85	0.44	1.23	0.06	0.07	0.13	0.05	0.06	0.11	3.46	0.36
Pasipha	sp. 7	7.92	6.35	2.96	0.80	0.37	1.88	0.10	0.10	0.20	0.05	0.05	0.11	7.08	0.27
Pasipha	sp. 8	3.08	0.10	1.38	0.03	0.45	0.69	0.05	0.05	0.10	0.07	0.07	0.14	2.04	0.34
Pasipha	sp. 8	4.38	0.85	2.50	0.19	0.57	0.77	0.05	0.04	0.09	0.06	0.05	0.12	3.38	0.23
Pasipha	sp.3	2.58	2.42	0.77	0.94	0.30	-	-	-	-	-	-	-	-	-
Pasipha	*variistriata*	1.81	1.48	0.38	0.82	0.21	-	-	-	-	-	-	-	-	-
Pasipha	*variistriata*	2.50	2.00	0.42	0.80	0.17	1.65	0.08	0.08	0.16	0.05	0.05	0.10	3.65	0.45
Pasipha	*variistriata*	2.54	2.12	0.58	0.83	0.23	1.23	0.11	0.09	0.20	0.09	0.07	0.16	3.92	0.31
Pasipha	*variistriata*	2.92	2.58	0.58	0.88	0.20	1.58	0.08	0.10	0.18	0.05	0.06	0.11	5.04	0.31
Supramontana	*irritata*	5.15	3.46	3.38	0.67	0.66	0.84	0.07	0.11	0.18	0.08	0.13	0.21	5.46	0.15
Supramontana	*irritata*	4.04	2.04	2.50	0.50	0.62	1.12	0.10	0.10	0.20	0.09	0.09	0.18	3.92	0.29
Supramontana	sp. 1	2.04	1.73	1.23	0.85	0.60	1.00	0.06	0.13	0.19	0.06	0.13	0.19	2.27	0.44
Supramontana	sp. 1	1.46	0.81	0.73	0.55	0.50	0.79	0.07	0.09	0.16	0.09	0.11	0.20	2.27	0.35
Supramontana	sp. 1	3.42	2.08	2.23	0.61	0.65	1.38	0.10	0.15	0.25	0.07	0.11	0.18	3.04	0.45
Xerapoa	*hystrix*	0.77	0.42	0.50	0.55	0.65	0.62	0.02	0.03	0.05	0.03	0.05	0.08	1.12	0.55
Undetermined	sp. 1	1.15	1.00	0.31	0.87	0.27	-	-	-	-	-	-	-	-	-
Undetermined	sp. 2	1.00	0.96	0.35	0.96	0.35	-	-	-	-	-	-	-	-	-
Undetermined	sp. 3	3.27	3.19	1.08	0.98	0.33	-	-	-	-	-	-	-	-	-
Undetermined	sp. 4	1.48	1.12	0.40	0.76	0.27	-	-	-	-	-	-	-	-	-
Undetermined	sp. 5	1.23	1.04	0.35	0.85	0.28	0.90	0.05	0.06	0.11	0.06	0.07	0.12	2.75	0.33
Undetermined	sp. 6	2.96	2.65	1.12	0.90	0.38	1.10	0.06	0.10	0.16	0.05	0.09	0.15	4.65	0.24
Undetermined	sp. 7	0.70	0.62	0.16	0.89	0.23	-	-	-	-	-	-	-	-	-
Undetermined	sp. 7	0.34	0.15	0.12	0.44	0.35	-	-	-	-	-	-	-	-	-
Undetermined	sp. 8	1.69	1.00	0.77	0.59	0.46	0.88	0.04	0.04	0.08	0.05	0.05	0.09	2.06	0.43

CAPÍTULO 2

Survival, growth and fecundity of a Neotropical land planarian (Platyhelminthes, Geoplanidae) under different diets

Piter Kehoma Boll[1,*], Douglas Marques[2], Ana Maria Leal-Zanchet[1]

[1]Instituto de Pesquisas de Planárias and Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos – UNISINOS, Av. Unisinos, 950, Cristo Rei, São Leopoldo, RS, 93022-750, Brazil

[2]Instituto de Ciências Básicas da Saúde and Programa de Pós-Graduação em Neurociências, Universidade Federal do Rio Grande do Sul – UFRGS, Rua Sarmento Leite, 500, Farroupilha, Porto Alegre, RS, 90050-170, Brazil

*Corresponding author: piterkeo@gmail.com

Abstract

Although most land planarians are sensitive to environmental changes, some species are well adapted to human-disturbed areas and are easily transported to new places, having the potential to become a threat to native ecosystems. We investigated growth and survival in *Obama anthropophila*, a Neotropical land planarian common in human-disturbed areas in southern Brazil. Specimens were divided into three groups that received different diets: (1) only the land planarian *Luteostriata abundans* (N = 13), (2) only the slug *Deroceras leave* (N = 12), and (3) alternating both prey types (N = 13). We monitored the weight of the specimens for a month and counted and weighed produced egg capsules. Planarians receiving a mixed diet tended to survive less than the groups receiving a single prey type, the difference being significant in relation to those feeding on *D. laeve* only. Planarians with the mixed diet ate *L. abundans* more often than *D. laeve*, and those feeding only on *L. abundans* tended to eat more than the other groups. Most egg capsules were laid by specimens feeding only on *D. laeve* but the mixed-diet group laid heavier capsules. Both prey species are suitable as food for *O. anthropophila*, although it prefers planarians when both food items are available. The constant alternation between food items seems to have adverse effects, perhaps be caused by physiological changes to digest different food items. The heavier egg capsules of the mixed-diet group, considering its lower survival, suggest terminal investment, i.e., an increased reproductive effort when approaching death. The ability to feed on both snails and planarians, combined with its proximity to humans, make *O. anthropophila* a potentially invasive species.

Keywords: feeding habits, invasive species, Tricladida

Introduction

Land planarians (family Geoplanidae) are carnivorous flatworms that are often associated with the leaf litter of forest areas and feed on a variety of organisms such as gastropods, earthworms, arthropods or even other land planarians (Boll & Leal-Zanchet 2016, Cseh et al. 2017). Although most species are very sensitive to changes in humidity and temperature and are considered good indicators for characterizing the degree of disturbance of their environments (Sluys 1999, Carbayo et al. 2002), other species are well adapted to human-disturbed areas (Froehlich 1955, Álvarez-Presas et al. 2015). Such tolerant species are often associated with cultivated plants, being common in gardens and greenhouses and, therefore, are easily transported to new places via plant trade (Justine et al. 2014b). As a consequence, several land planarians were introduced in areas outside of their native range, many of which became invasive, posing a threat to the ecosystems or to some human activities, such as vermiculture (Jones et al. 2001, Murchie et al. 2003, Okochi et al. 2004, Sugiura & Yamaura 2009, Murchie & Gordon 2013).

Most invasive land planarians are species originally found in the Indomalayan and Australasian ecozones and belong to the subfamilies Bipaliinae and Rhynchodeminae. Notable examples include *Bipalium kewense* Moseley, 1878, *Bipalium adventitium* Hyman, 1943, *Platydemus manokwari* De Beauchamp, 1963, and *Arthurdendyus triangulatus* (Dendy, 1896) (Winsor 1983, Fiore et al. 2004, Murchie 2010, Iwai et al. 2010). Recently, the Neotropical species *Obama nungara* Carbayo, Álvarez-Presas, Jones & Riutort, 2016 has been identified in Europe. This is the first recorded Neotropical land planarian to be introduced outside of the Neotropical ecozone and is currently widespread in Great Britain, France and the Iberian Peninsula (Justine et al. 2014a, Lago-Barcia et al. 2015, Carbayo et al. 2016). In its original region, which includes southern Brazil and Argentina, *O. nungara* is commonly found in human-disturbed areas (Lago-Barcia et al. 2015, 2018, Boll & Leal-Zanchet 2016), this being the most likely reason for its accidental transportation to Europe.

Another species of *Obama* that is very common in urban-disturbed areas in southern Brazil is *Obama anthropophila* Amaral, Leal-Zanchet & Carbayo, 2015. Studies on its diet indicated that it feeds on land gastropods, land nemerteans and other land planarians (Boll & Leal-Zanchet 2016, Cseh et al. 2017). Due to its close association with humans and its broad diet, *O. anthropophila* has the potential to become an invasive species as well. However, it is currently unknown whether different prey items have different nutritional values for *O.*

anthropophila, thus affecting its development and fecundity. In order to shed some light on the subject, we investigated the growth and survival of *O. anthropophila* under different diets.

Since *O. anthropophila* seems to accept both land planarians and slugs equally as food and both are commonly found in its natural environment (Boll & Leal-Zanchet, 2016), our hypothesis is that specimens receiving a mixed diet will have improved growth and survival rates because different food items may be the source of complementary nutrients needed by the planarian. Similarly, a mixed diet would increase fecundity, expressed as an increase in number or weight of egg capsules.

Material and Methods

We collected 38 adult specimens of *Obama anthropophila* in human-disturbed habitats of Rio Grande do Sul, southern Brazil in the winter and spring of 2016 and 2017. As the captured individuals were adults, age could not be determined. In the laboratory, we kept the planarians individually in closed plastic containers measuring 11 × 8 × 4 cm (length × width × height) with a wet cotton ball to retain humidity and a piece of wood bark for refuge. The container remained in the dark under a temperature of 22°C.

The animals received food three times a week (Mondays, Wednesdays, Fridays) during a month (31 days) and were weighed right before being fed. Food included the land planarian *Luteostriata abundans* (Graff, 1899) and the land slug *Deroceras laeve* (O. F. Müller, 1774).

The 38 planarians were divided into three groups, each receiving a different diet: (1) Luab group (N = 13): only *L. abundans*; (2) Dela group (N = 12): only *D. laeve*; (3) Mixed group (N = 13): *L. abundans* + *D. laeve*;

Planarians in the Luab and Dela groups received the same prey in every meal, while those in the Mixed group received one prey item in one meal and another in the following meal. Prey items were offered alive and weighed between half and two-thirds of the planarian's weight. If the prey had not been consumed until the next feeding day, it was replaced by a new prey item, which, in the Mixed group, was always the species previously assigned to that day, regardless of whether the previous prey item (of the other species) had been eaten or not.

We weighed any egg capsule laid during the experiment and added its weight to the planarian's weight, cumulatively, as we considered it an investment in growth and included matter coming from food.

We compared the survival of planarians in each treatment from Kaplan-Meier survival curves using a two-sided log-rank test. Additionally, we performed a Cox proportional hazards regression analysis using the initial weight of the specimens as an additional variable with the treatment. All specimens were monitored for a period of 31 days, so that information on specimens surviving up to this date is censored.

As the planarians did not necessarily eat every prey item offered to them, we compared the mean number of prey items ingested by the planarians in each group after 10, 20 and 31 days using a One-Way Analysis of Variance (ANOVA). For the Mixed group, we also compared the mean proportion that each of the two prey items was consumed using a paired t-test to verify whether there was a preference for either *L. abundans* or *D. laeve*. The proportion was calculated by dividing the number of ingested prey items of each type by the total number of times that the corresponding prey item was offered.

We compared the mean increase in the planarians' weight in each group after 10, 20 and 31 days from the beginning of the measurements using a One-Way ANOVA. Additionally, without considering the groups, we verified whether there was a relation between the weight gain and the initial weight of the planarians using simple linear regression.

All egg capsules laid during the experiment were weighed. We compared the mean weight of the egg capsules laid in each treatment using a One-Way ANOVA.

Statistical analyses were performed by means of the software IBM SPSS Statistics 20.

Results

In pairwise comparisons, planarians of the Dela group survived significantly more (mean = 26.833±9.318 days) than those of the Mixed group (20.923±8.704 days, p = .018), with the Luab group showing an intermediate value (24.077±10.074 days) (Fig. 1). In overall comparison, the survival of planarians in the different treatments showed a marginally significant difference (log-rank test, p = .051). Cox Regression (p = .031) explained survival by initial weight (p = .039) but not by treatment (p = .352).

Planarians of the Luab group ingested significantly more food than planarians of the Mixed group after 10 days, with the Dela group showing intermdiate values (ANOVA, $F_{(2,30)}$ = 4.110, p = .026). After 20 days, the Luab group continued to have the highest ingestion rate, but the lowest value was that of the Dela group (ANOVA, $F_{(2,26)}$ = 3.405, p = .049). After 31 days, no significant difference was found between groups (ANOVA, $F_{(2,20)}$ = 2.335, p = 0.123), although a tendency similar to that of 20 days is still seen (Fig. 2). Planarians of the Mixed group ingested a larger proportion of specimens of the flatworm *L. abundans* than of the slug *D. laeve* (t-test, t = 3.494, df = 11, p = .005) (Fig. 3).

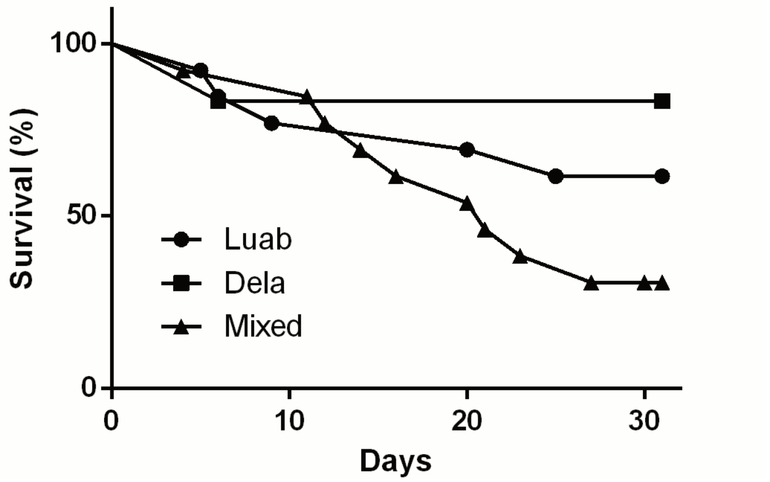

Fig 1. Meier-Kaplan survival curves for specimens of the flatworm *Obama anthropophila* that received three different diets for one month (31 days). Luab: only the flatworm *Luteostriata abundans*; Dela: only the slug *Deroceras laeve*; Mixed: *L. abundans* + *D. laeve.*

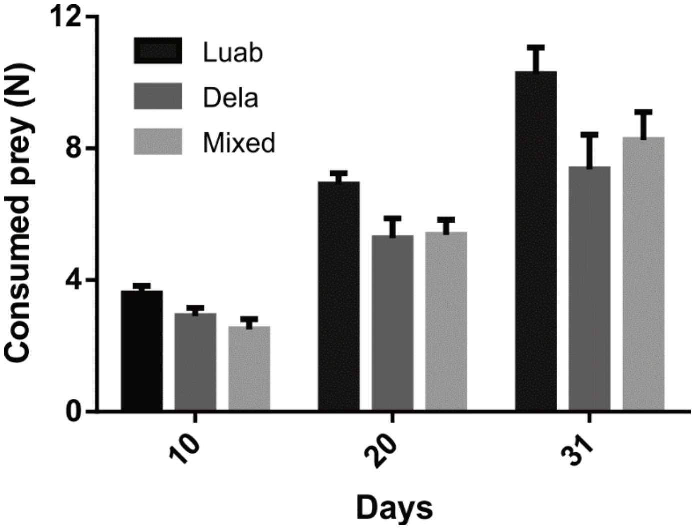

Fig 2. Mean number of prey items consumed after 10, 20 and 31 days by specimens of the flatworm *Obama anthropophila* that received three different diets. Luab: only the flatworm *Luteostriata abundans*; Dela: only the slug *Deroceras laeve*; Mixed: *L. abundans* + *D. laeve*. Bars indicate standard error.

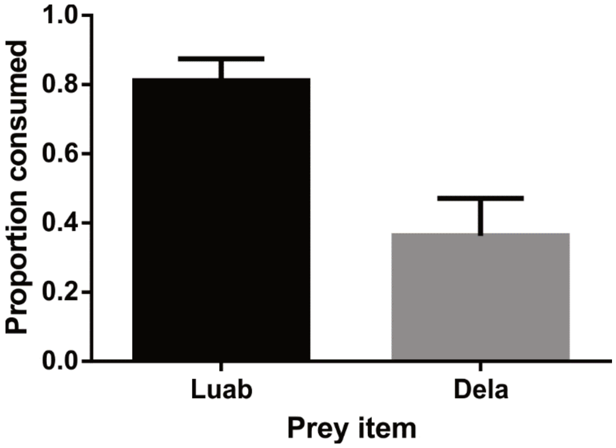

Fig 3. Mean proportion of prey items of each type consumed by specimens of the flatworm *Obama anthropophila* that received a mixed diet for a month (31 days). Luab: *Luteostriata abundans*; Dela: *Deroceras laeve*. Bars indicate standard error.

Mean weight gain was not significantly different for specimens in all groups after 10 (ANOVA, $F_{(2,29)} = 1.115$, $p = .342$), 20 (ANOVA, $F_{(2,25)} = .542$, $p = .588$) or 31 days (ANOVA, $F_{(2,19)} = .828$, $p = .452$). Weight gain was negatively related to initial weight, with smaller planarians gaining more weight than larger ones after 20 days ($R^2 = .163$, $F = 5.061$, $p = 0.033$) and after 31 days ($R^2 = .292$, $F = 8.240$, $p = 0.009$) but no significant difference was found during the first 10 days ($R^2 = .299$, $F = 2.941$, $p = .097$) (Fig. 5).

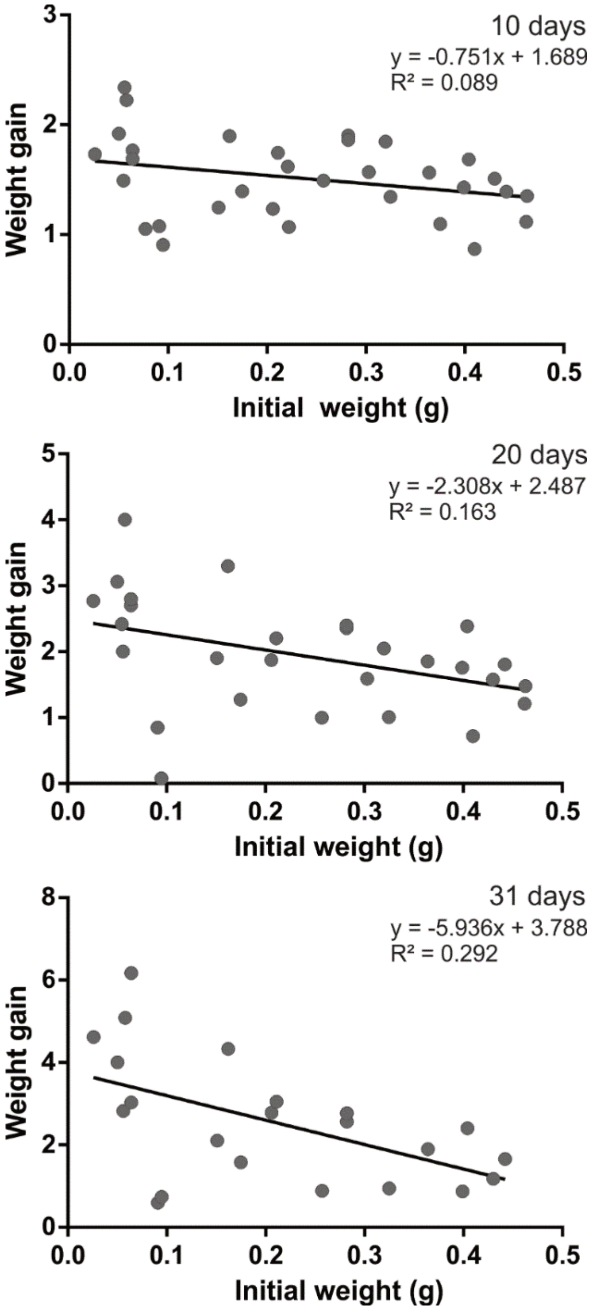

Fig. 5. Relation between initial weight of the flatworm *Obama anthropophila* and weight gain after 10, 20 and 31 days.

The total number of egg capsules laid during the experiment was 28, the mean weight being 0.044±0.015 g. Of those, 16 were laid by the Dela group, 7 by the Mixed group and 5 by the Luab group. The mean weight of the egg capsules was significantly different between groups (ANOVA, $F_{(2,25)} = 4.375$, $p = 0.023$). Egg capsules laid by planarians in the Mixed group were significantly heavier (0.054±0.017 g) than those in the Dela group (0.037±0.013 g), with the Luab group having an intermediate value (0.049±0.006 g).

Discussion

Studies on the nutritional value of different food items of predators and omnivores often reveal that different food types are significantly different in quality (Kaneda et al. 1992, Hauge et al. 1998, Oelbermann & Scheu 2002) and sometimes mixed diets are more beneficial than diets consisting of a single prey type (Eubanks & Denno 1999, Toft & Wise 1999). Regarding *Obama anthropophila*, there was no significant difference in growth between the three different diets. Survival was not strongly different either, but specimens in the group receiving a mixed diet survived less than those feeding only on *D. laeve.* This is usually not seen in other species, except if one of the prey items is unsuitable or less nutritious to the predator (Eubanks & Denno 1999, Bilde & Toft 2001, Oelbermann & Scheu 2002, Allard & Yeargan 2005). However, since the survival was not lower in any of the single-diet groups, it seems unlikely that any of the prey items has a low nutritional value or has a deleterious effect on *O. anthropophila.* A possible explanation is that both food items, although equally nutritious, and therefore substitutable, have antagonistic effects when mixed (Tilman 1980) because of the necessity to undergo physiological changes to allow the proper digestion of each one (Whelan et al. 2000). If this is the case, selection for one food item over another may eventually lead to diet specialization and sympatric speciation (Whelan et al. 2007).

In our study, smaller planarians, regardless of diet, gained weight faster than larger ones. It is well known that a higher growth rate in younger individuals is common in many organisms (von Bertalanffy 1957, Blake & Kudenov 1981, Forsythe 1984, Avsar 2001). Planarians, however, have an ability to decrease in size during starvation and regrow when food is again available (González-Estévez 2009), so that smaller individuals are not necessarily younger, although they could be physiologically equivalent to younger ones. As we captured the planarians in the field, their age could not be determined. Studies on the invasive flatworm *Arthurdendyus triangulatus* (Dendy, 1984) indicated that it grows from autumn to winter and degrows during summer (Blackshaw 1997). Such information is currently unavailable for *O. anthropophila.* Considering a likely similar pattern, despite the smaller variation in temperature and rainfall along the year in southern Brazil, all specimens used in this study would be in the period of growth, as they were all captured during winter and spring. Therefore, although age and annual cycle could have affected our results, this information was not available to include in our analysis.

The amount of food ingested was related to both treatment and time. Specimens feeding only on *L. abundans* ate more than the other groups during the whole month. Planarians receiving the mixed diet ate less but in an apparently continuous rate, while those feeding only on *D. laeve* reduced the number of food ingested as time passed. As the weight gain was not different between groups despite the different consumption rates, one could conclude that those differences occur due to compensatory feeding. It is known that many species compensate the low quality of some food items by eating more (Cruz-Rivera & Hay 2000, Nicolson & Fleming 2003, Berner et al. 2005) and by observing only the result of the single-diet treatments one could conclude that the higher consumption rate of *L. abundans* indicates that this prey item is less nutritious than *D. laeve*. However, the fact that planarians receiving a mixed diet fed more on *L. abundans* than on the apparently more nutritious *D. laeve* goes against the idea of compensatory feeding.

Most studies investigating the food preference of carnivores suggest that preference is related to either availability (Landenberger 1968, Redford & Dorea 1984) or nutritional value (Gutiérrez & Gallardo 1999). Conversely, our results suggest the opposite, i.e., that *O. anthropophila* prefers to feed on *L. abundans*, an apparently less nutritious food, than on *D. laeve*, a more nutritious one. It is worth noticing that *L. abundans* is a native prey of *O. anthropophila*, while *D. laeve* is an introduced species. Could *O. anthropophila*, therefore, prefer native over exotic prey? Previous observations did not find a significant preference of *O. anthropophila* for either native or exotic prey, whether they were land planarians or slugs (Boll & Leal-Zanchet 2016). Those observations, however, were based on several single feeding instances and did not include specimens monitored for several weeks as the current study. It is interesting to notice that, in studies on other predators of different taxonomic groups, preference for exotic prey over native prey is common, possibly because exotic species require less effort to be captured (MacNeil et al. 1999, Dudas et al. 2005, Savini & Occhipinti-Ambrogi 2006).

Although most planarians did not lay egg capsules in the present study, more than half of the capsules were laid by planarians feeding only on *D. laeve*, which could indicate an increased fecundity under this diet. If one considers *D. laeve* to be more nutritious than *L. abundans*, this result is expected, as more nutritious food is usually able to increase the reproductive output of an organism (Kaneda et al. 1992, Allard & Yeargan 2005). Interestingly, egg capsules laid by planarians that received the mixed diet were heavier than those from the single-species diets, even though survival under this diet was lower. Although such an outcome may seem surprising at first, it may be related to the terminal investment hypothesis, the idea that reproductive effort should increase when future events of reproduction are not expected, i.e., when the organism approaches the end of its life (Clutton-Brock 1984). Evidence for the terminal investment hypothesis has been found in several animal groups (Thompson 1983, Velando et al. 2006, Creighton et al. 2009), but this seems to be the first evidence that this mechanism may also apply to planarians.

Although apparently preferring to feed on other land planarians, *O. anthropophila* is able to use slugs as a suitable alternative prey without compromising its survival or fecundity. Its acceptance of slugs as food seems to be greater when the preferred prey is not available. When survival becomes compromised, *O. anthropophila* may increase its investment in reproduction. These factors, together with its proximity to humans, could help its survival and establishment in new areas during accidental transportations, therefore making it a potentially invasive species.

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CAPÍTULO 3

Lazy to prey and eager to run: behaviour of a Neotropical land planarian (Platyhelminthes: Geoplanidae) in the presence of its prey and predators[1]

PITER KEHOMA BOLL* and ANA MARIA LEAL-ZANCHET

Instituto de Pesquisa de Planárias and Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos – UNISINOS, Av. Unisinos, 950, Cristo Rei, São Leopoldo, RS 93022-750, Brazil

*Corresponding author. E-mail: piterkeo@gmail.com

ABSTRACT

We analyzed the behavior of a woodlouse-eating Neotropical planarian, *Luteostriata abundans*, as a predator and as prey and investigated the planarian's ability to detect prey and predator by environmental cues. The results indicate that the planarian detects but does not follow woodlice chemical trails and cannot track the source of remote chemical and mechanical stimuli. We conclude that this woodlouse-eating planarian is likely to be an ambush predator, patrolling the environment and waiting for prey to come near it. As a prey, the planarian escaped during direct contact with two predators and ignored most non-predators. Contact with secretions of a predator and encounters with its slime tracks rarely triggered anti-predator behaviors. However, a non-predatory species that is phylogenetically closely related to a predator also triggered an escape response after contact with anterior and posterior body ends. It has a similar predator-detection ability along the body and shows antipredator behaviors toward species that are phylogenetically closely related to its predators. The unexpected lack of anti-predator behavior during encounters with chemical cues may indicate the need for a tactile stimulus for predator identification.

ADDITIONAL KEYWORDS: antipredator mechanisms – investigative behavior – predation – predator avoidance – prey tracking

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INTRODUCTION

A crucial part in the life of organisms is the ability to detect resources and threats and respond effectively. As a result, animals have evolved a variety of sensory adaptations specific to their ecological context (Stevens, 2013). In environments such as leaf litter, where visual information is of limited use, predators are often detected through chemical cues (Kats & Dill, 1998; Moore & Crimaldi, 2004).

The use of sensory information has been extensively studied in wolf spiders, an important group of predators in leaf litter (Persons & Uetz, 1999; Gordon & Uetz, 2011). However, another group of predators in this environment, land planarians, is still understudied both taxonomically and ecologically (Sluys, 1998; Winsor, Johns, & Barker, 2004; Carbayo & Froehlich, 2008), even though several species have been introduced outside their native range and have become invasive, posing a threat to ecosystems (Sugiura, 2010; Murchie & Gordon, 2013; Sluys, 2016).

Having only a series of very simple eyes (Ball & Reynoldson, 1981), land planarians likely rely on chemical information to explore the environment (Ogren, 1995; Fiore *et al.*, 2004). Recent studies (Fiore *et al.*, 2004; Iwai, Sugiura, & Chiba, 2010; Boll & Leal-Zanchet, 2015) have revealed that species that feed on earthworms and gastropods can track a chemical trail left by the prey, probably by using sensory structures at the anterior region (Fernandes *et al.*, 2001). However, to our knowledge prey detection by arthropod-eating land planarians has not been studied.

For some time, land planarians were thought to be top predators because few species were known to feed on them (Sluys, 1999). However, some predators have now been identified, including predatory beetles (Gibson, Cosens, & Buchanan, 1997), snails (Lemos, Canello, & Leal-Zanchet, 2012) and other land planarians (Boll *et al.*, 2015; Boll & Leal-Zanchet, 2016). Thus we predict that at least some prey species of land planarian should be able to detect predators and respond to their presence to avoid predation while simultaneously minimizing the negative effects on fitness that result from predator avoidance (Lima & Dill, 1990; Creel & Christianson, 2008; Sih *et al.*, 2010).

Studies on *Luteostriata abundans* (Graff, 1899), a land planarian that feeds exclusively on woodlice (Boll & Leal-Zanchet, 2016), showed that it captures prey very quickly (Prasniski & Leal-Zanchet, 2009; Boll & Leal-Zanchet, 2018). However, all observations have been made based on predator and prey moving freely in a Petri dish and most encounters occurred when the woodlouse accidentally touched the planarian (Prasniski & Leal-Zanchet, 2009). Therefore, how *L. abundans* locates its prey in the wild has not been investigated.

This species is also interesting because it seems to be one of the main prey of other land planarians, especially *Obama anthropophila* Amaral, Leal-Zanchet & Carbayo, 2015 (Hauser & Maurmann, 1959; Boll & Leal-Zanchet, 2016) and shows an immediate escape response when contacted by the predator (Boll & Leal-Zanchet, 2018). It is unclear whether *L. abundans* needs physical contact with the predator to recognize it as such or whether indirect cues, such as a slime trail left by *O. anthropophila*, may induce a change in behavior that results in predator avoidance.

To understand how *L. abundans* behaves both as predator and as prey, we conducted a series of laboratory experiments to test its ability to detect and locate prey, and to recognize predators based on direct or indirect clues.

MATERIAL AND METHODS

We captured adult specimens of *L. abundans* and woodlice (*Benthana* spp.) in urban gardens and groves in the Metropolitan Region of Porto Alegre, Rio Grande do Sul, Brazil. In the laboratory, the planarians were kept individually in closed plastic containers, measuring 9 × 5.5 × 2.6 cm, with a cotton ball moistened with mineral water to retain humidity and a piece of wood bark for refuge. The cotton balls were changed and the containers washed with tap water weekly. Woodlice were kept together in a closed plastic container, measuring 17.8 × 12.8 × 11.5 cm, half-filled with soil, leaf litter and pieces of wood bark that were periodically sprayed with mineral water to retain humidity. All containers were maintained in the dark in an incubator at 18–20°C.

We conducted two groups of experiments, one for prey detection and one for predator detection. All experiments were conducted under low diffuse light provided by a door.

PREY DETECTION BY *L. ABUNDANS*

We used 39 specimens of *L. abundans* (about 5–7 cm long) to conduct three different experiments. All planarians were deprived of food for at least a week prior to the experiments and were used at most in one trial every 48 h. The specimens for each treatment were randomly chosen from the available animals, without using the same specimen twice in the same treatment.

Detection of chemical trails of uninjured woodlice (1.1)

We covered the bottom of a 14-cm-diameter Petri dish with a layer of filter paper moistened with mineral water and then created a corridor by placing two plastic plates measuring 14 × 5 cm (length × height) across the diameter of the dish, with a space of 1 cm between them. A line was drawn with a pencil along the margins of the plates that touched the paper to outline the area between the two plates. We placed three uninjured woodlice (about 0.8 cm long) over the paper in the 1 × 14 cm area of the corridor and let them move freely for 5 min, touching them gently with a soft brush to keep them moving. Afterwards, we first removed the woodlice and then the plastic plates and placed a planarian at a random point on the filter paper and left it free to move until contacting the outlined area (Fig. 1A). Two different controls were used, one where we created a 1-cm-wide track of moistened soil across the diameter of the plate and another where a 1-cm-wide track was outlined with a pencil but not otherwise differentiated from the surrounding area. We performed 16 repetitions for each treatment (woodlouse, soil and empty tracks). The reaction of the planarian after finding the tracks and the time that it spent exploring each track before leaving it were recorded. We considered that the planarian was exploring the track when its anterior exploratory region was over the track. The exploratory region was defined as the part of the body that is constantly lifted from the substrate and moves from side to side during exploratory behavior (Boll & Leal-Zanchet, 2018).

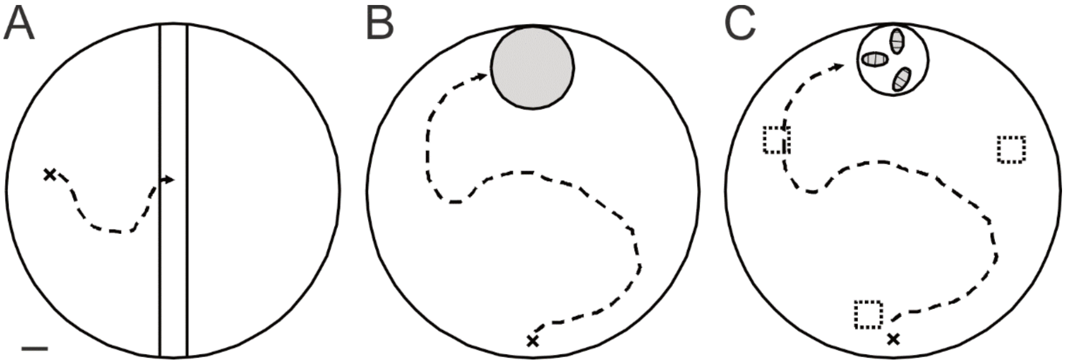

Figure 1. Experimental designs for the three experiments of prey detection in Luteostriata abundans in a Petri dish: A, chemical trail of woodlice occupying a 1-cm-wide area across the dish; B, 3.5-cm-diameter circle containing crushed woodlice; C, 3-cm diameter lid containing live prey. Dashed lines indicate the possible path taken by the planarian, 'x' gives the planarian's initial position and dotted squares indicate plastic pieces used to lift the filter paperslightly. Scale bar = 10 mm.

Remote chemical detection of woodlice (1.2)

As in the previous experiment, we covered the bottom of the Petri dish with moistened filter paper. We outlined a 3.5-cm-diameter circle with a pencil at the border of the plate and crushed several woodlice inside the circle so that the fragments occupied the whole area outlined. Afterwards, we placed a planarian at the point diametrically opposite this area and allowed it to move freely until contacting the circle with its anterior tip (Fig. 1B). As a control, we covered the area with moistened soil instead of crushed woodlice. We performed 15 repetitions for each treatment (woodlice and soil) and recorded the time taken by the planarian to reach the area.

Remote mechanical detection of woodlice (1.3)

In this experiment, three live woodlice were placed inside a 3-cm-diameter circle outlined on the filter paper and were covered by a 3-cm-diameter plastic lid. Two different controls were used, one in which the three woodlice were replaced by a nymph of the cockroach *Pycnoscelus surinamensis* (Linnaeus, 1758), about 1 cm long, and another without any animal under the lid (empty). We performed 15 repetitions for each treatment (woodlouse, cockroach and empty). We placed the planarian at the point diametrically opposite the lid and recorded the time it took to reach the lid with its anterior tip. Three 1 × 1 cm plastic plates were placed under the filter paper in a triangular arrangement to keep the paper slightly elevated and facilitate the spread of vibration (Fig. 1C).

We compared the treatments in experiments 1.1 and 1.3 using a one-way analysis of variance (ANOVA) followed by a Tukey test, and in experiment 1.2 using a *t*-test. All statistical analyses were performed by means of the program IBM SPSS Statistics 19.

PREDATOR DETECTION BY *L. ABUNDANS*

We used 45 specimens of *L. abundans* to conduct the four experiments, some of which had been used in the previous set of experiments. Each planarian received three woodlice per week as food and the specimens for each treatment were randomly chosen from the available animals, without using the same specimen twice in the same treatment.

Predator recognition by contact through the posterior end (2.1)

To test the ability of *L. abundans* to differentiate predators from non-predators upon contact with the posterior end, we placed a specimen of *L. abundans* in a 14-cm-diameter Petri dish and let it move freely for 5 min to reduce the influence of any alarm state caused by handling the animal. Afterwards, we touched the dorsal side of the posterior end of the planarian with one of the following species: *Obama anthropophila* (a predator), *Obama ficki* (Amaral & Leal-Zanchet, 2012) (a non-predator phylogenetically closely related to the above predator), *Paraba multicolor* (Graff, 1899) (also a predator), *Imbira guaiana* (Leal-Zanchet & Carbayo, 2001) (a non-predator land planarian phylogenetically distant from the predators), *L. abundans* (a conspecific), *Luteostriata ernesti* (Leal-Zanchet & Froelich, 2006) (a phylogenetically close species) and *Deroceras laeve* (O. F. Müller, 1774) (a non-predator gastropod). We performed

15 repetitions for each of the seven treatments and the reaction of *L. abundans* after being touched was recorded and classified as either escape or no escape. The escape reaction was defined as the escape response of *L. abundans* described in Boll & Leal-Zanchet (2018). Other responses were considered as no escape.

Accuracy of predator recognition by anterior and posterior ends (2.2)

To test whether the accuracy of recognizing a predator was different between the anterior and posterior ends of *L. abundans*, we placed a specimen of *O. anthropophila* or a specimen of *O. ficki* in front of a specimen of *L. abundans* moving freely through a 14-cm-diameter Petri dish and recorded its reaction (as escape or no escape) after intercepting the predator. Both treatments were repeated 15 times and compared with the 15 instances from the previous experiment in which *L. abundans* was touched by those two species at the posterior end.

Predator recognition by contacting predator secretions through the posterior end (2.3)

This experiment was similar to experiment 2.1. However, instead of touching the posterior end of *L. abundans* with a specimen of the predator, we touched it with a soft brush containing secretions of the predator. The secretions were collected by passing the brush repeatedly over the dorsal surface of *O. anthropophila* until a visible amount had adhered to the brush. As a control, we used secretions of the slug *D. laeve*, which were collected in the same manner. Both treatments were repeated 15 times and the reaction of *L. abundans* was recorded and classified as either escape or no escape.

Predator detection by a chemical cue in the environment (2.4)

In this experiment, we covered the bottom of a 14-cm-diameter Petri dish with a layer of filter paper and deposited a specimen of *O. anthropophila* over it, letting it move freely until it created a slime trail. We then removed the predator and immediately deposited a specimen of *L. abundans* on a random point on the filter paper outside the slime trail and let it move freely until contacting the slime trail, recording its behavior as escape or no escape after finding the trail. As a control, we used a slime trail of the slug *D. laeve*. Both treatments were repeated 15 times.

All results were statistically analysed with the program IBM SPSS Statistics 20. We used a Generalized Linear Mixed Model (GLMM) assuming a binomial error distribution and logit link function. Stimulus was used as a fixed factor and planarian individual as a random factor.

RESULTS

PREY DETECTION BY *L. ABUNDANS*

The planarians spent more time exploring woodlouse tracks than soil and empty tracks (ANOVA, $F_{(2,45)} = 4.736$, $p = .014$; Fig. 2). After finding a woodlouse track, the planarian investigated the surroundings of the point at which it touched the track but did not follow it further, continuing to move across the dish a few seconds later. This investigative behavior was not observed during encounters with soil or empty tracks.

There was no significant difference in the time spent by the planarians in reaching either the woodlouse and soil areas of experiment 1.2 (t-test, $F = .006$, $df = 28$, $p = .864$) or the woodlouse, cockroach and empty lids of experiment 1.3 (ANOVA, $F_{(2,42)} = 1.017$, $p = .371$). However, after reaching the crushed woodlice, the planarians identified the remains as food and everted their pharynx, starting to ingest the remains.

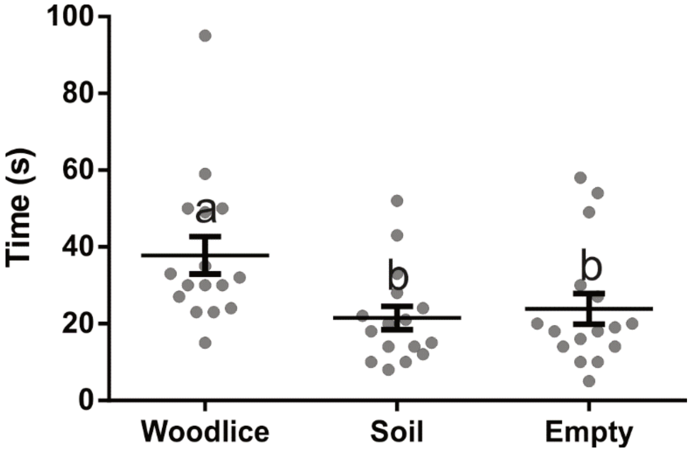

Figure 2. Time spent by *Luteostriata abundans* exploring woodlice, soil and empty tracks (N = 16 for each treatment). Circles indicate individual values; horizontal lines and bars indicate mean and standard error, respectively; different letters indicate significantly different means.

PREDATOR DETECTION BY *L. ABUNDANS*

Predator recognition by contact through the posterior end

Luteostriata abundans showed a high escape response rate during encounters with *O. anthropophila, O. ficki* and *P. multicolor*, while encounters with the other four species triggered few or no escape responses (GLMM, $F_{6,98} = 5.369$, $p < 0.001$) (Figs 3, 4).

Accuracy of predator recognition by anterior and posterior ends

The escape response rate of *L. abundans* was high while touching both *O. anthropophila* and *O. ficki* with either the anterior or posterior ends (GLMM, $F_{3,56} = 0.453$, $p = 0.717$) (Figs 5, 6).

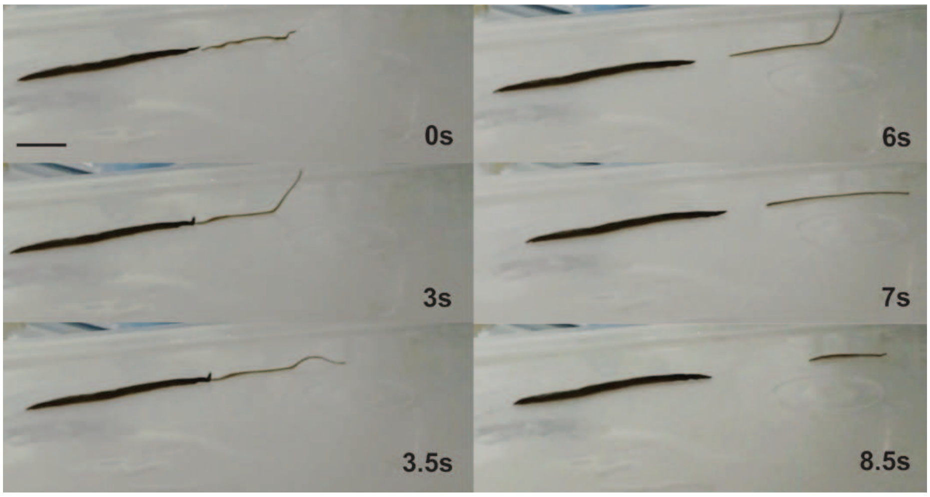

Figure 3. Sequential images of *Luteostriata abundans* (right) escaping from its predator, *Obama anthropophila* (left), after contact at the posterior end of the body. Scale bar = 15 mm.

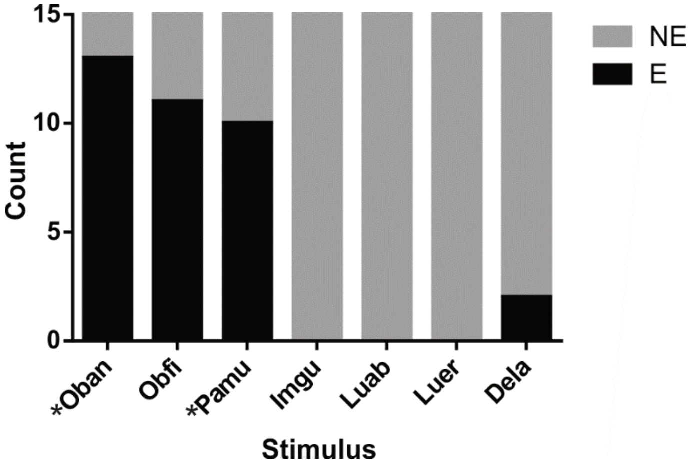

Figure 4. Response of *Luteostriata abundans* after contacting different species through the posterior end. Oban = *Obama anthropophila*; Obfi = *Obama ficki*; Pamu = *Paraba multicolor*; Imgu = *Imbira guaiana*; Luab = *L. abundans*; Luer = *Luteostriata ernesti*; Dela = *Deroceras laeve*; NE = no escape; E = escape. Asterisks indicate predators of L. abundans.

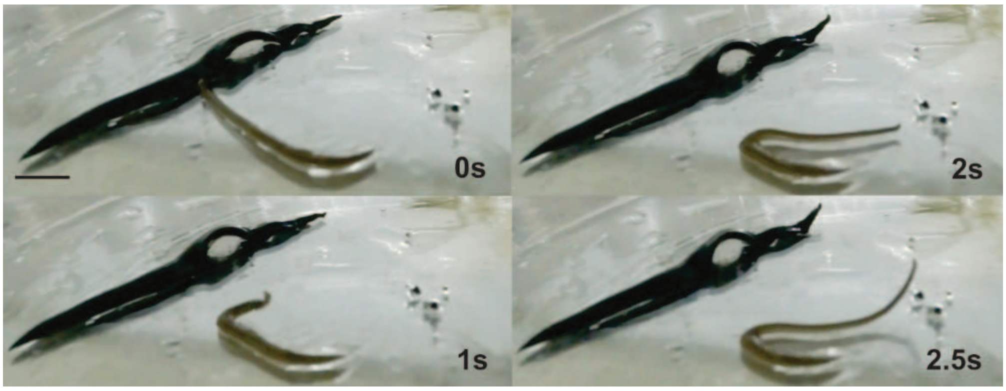

Figure 5. Sequential images of *Luteostriata abundans* (right) escaping from a non-predator, *Obama ficki* (left), after recognizing it as a predator by a touch with the anterior end of the body. Scale bar = 10 mm.

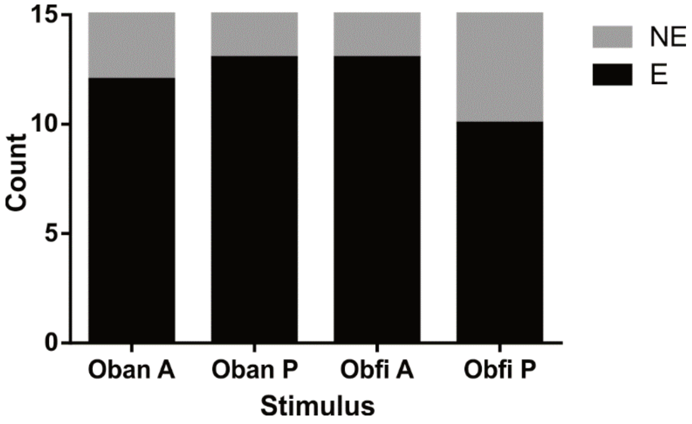

Figure 6. Response of specimens of *Luteostriata abundans* after contacting specimens of the predator *Obama anthropophila* (Oban) and the non-predator *Obama ficki* (Obfi) through the anterior (A) and posterior (P) ends. NE = No escape; E = Escape.

Predator recognition by contacting predator secretions through the posterior end

An escape response was triggered in only three of the 15 contacts with the secretions of *O. anthropophila* and in none of the contacts with the secretions of *D. laeve* (GLMM, $F_{1,28} = 1.626$, $p = 0.213$).

Predator detection by a chemical cue in the environment

Most contacts of *L. abundans* with the trails of either *O. anthropophila* or *D. laeve* did not alter its exploratory behavior (GLMM, $F_{1,28} = 0.820$, $p = 0.346$). Only two encounters with the trail of *O. anthropophila* elicited anti-predator responses, one in which the planarian avoided the trail, changing its direction of movement, and another in which the trail triggered an escape response after the whole body of the planarian was over the trail.

DISCUSSION

Luteostriata abundans increased its exploratory behavior after finding a chemical trail left by woodlice, a behavior that may be a specific form of investigation (Boll & Leal-Zanchet, 2018). This response suggests an ability to detect chemical cues of the prey on the substrate, which triggers an investigation of the surrounding environment. However, differently from land planarian species (Fiore *et al.*, 2004; Iwai *et al.*, 2010; Boll & Leal-Zanchet, 2015), *L. abundans* did not follow the chemical trail, continuing to move randomly after a brief investigation of the area around the point at which it found the trail. Similar behavior has been observed in velvet worms, which feed on arthropods but do not follow trails left by prey that previously passed the area (Read & Hughes, 1987).

The planarians were unable to locate the crushed woodlice from a distance, suggesting that they are unable to track airborne chemical signals. Comparative studies with other groups, such as land snails, have indicated that herbivores are better able to locate distant odor sources compared with carnivores, due to fact that herbivores feed on stationary food, while carnivores rely on mobile prey (Shearer & Atkinson, 2001). However, carnivorous snails, as well as land planarians that feed on snails and earthworms, follow chemical trails of their prey (Clifford *et al.*, 2003; Fiore *et al.*, 2004; Boll & Leal-Zanchet, 2015), which *L. abundans* does not. Likewise, *L. abundans* was unable to locate prey by mechanical stimuli coming through the substrate. In fact, prey detection by mechanical stimuli is more often recorded in aquatic predators (Feigenbaum & Reeve, 1977; Proctor & Pritchard, 1990; Rebora, Piersanti, & Gaino, 2004) than in terrestrial predators (Brownell & van Hemmen, 2001). However, even in an aquatic environment, where vibrations are conducted through the water, many of these species are ambush predators and only react to a stimulus produced very close to their bodies (Feigenbaum & Reeve, 1977).

Carnivorous land snails and land planarians that track their prey by following a chemical trail are slow-moving predators and feed on similarly slow-moving species, including earthworms and other gastropods and land planarians (Shearer & Atkinson, 2001; Clifford *et al.*, 2003; Fiore *et al.*, 2004; Iwai *et al.*, 2010; Boll & Leal-Zanchet, 2015). Arthropods, on the other hand, are fast-moving prey and are unlikely to be overtaken by a slow-moving predator such as *L. abundans*. Therefore, we conclude that *L. abundans* is likely an ambush predator (Curio, 1976), behaving in the same way as velvet worms (Blaxter & Sunnucks, 2011): slowly patrolling the environment, waiting for its prey to come near, and then quickly capturing it (Boll & Leal-Zanchet, 2018).

Regarding *L. abundans* as a prey, the results of the first experiment indicate that contact caused by its predators *O. anthropophila* and *P. multicolor* on its posterior dorsal surface triggers an escape response. Contact with most other harmless organisms is ignored. However, contact by *O. ficki*, a species closely related to one of its predators, but not itself a predator of other land planarians (Boll & Leal-Zanchet, 2016), triggers an escape response as well. The ability to distinguish species that pose a threat from those that do not is important because this reduces instances of unnecessary escape behavior, saving time and energy consumed for other important tasks, such as feeding (Ydenberg & Dill, 1986; Lima, 1998). In fact, most prey species from different animal groups are able to distinguish between predatory and non-predatory species (Bullock, 1953; Coates, 1980; Marko & Palmer, 1991) and even sometimes between more important (i.e., more dangerous) and less important threats (Dias *et al.*, 2016;

Ramesh *et al.*, 2016). The fact that *L. abundans* ignores most non-predatory species suggests that there is a selection pressure in action to develop this discrimination. The escape response triggered by *O. ficki* may be a result of its phylogenetic proximity to one of the main predators, while their chemical signatures may be too similar for *L. abundans* to be able to distinguish the two species, especially considering that other species of *Obama* also feed on *L. abundans* and other land planarians (Boll *et al.*, 2015; Boll & Leal-Zanchet, 2016). Therefore, it is more advantageous for *L. abundans* to flee whenever it encounters an *Obama* individual, whether it is dangerous or not, than to risk of being captured and killed (Lima & Dill, 1990).

The results of the second experiment do not support possibility that the accuracy of *L. abundans* in discriminating predatory from non-predatory species of *Obama* is related to differences in its sensory abilities along the body, as the escape response during the contact with both *O. anthropophila* and *O. ficki* was similar at both anterior and posterior ends of the body. The sensory organs of land planarians are usually identified as (1) a set of pits or papillae close to the anterior end (Fernandes *et al.*, 2001), which are considered to be chemoreceptors, and (2) the eyes (photoreceptors), which may vary from two at the anterior end to several along the entire body (Ball & Reynoldson, 1981). The nature of the stimulus that allows *L. abundans* to identify species of *Obama* and *Paraba* as a threat is unknown, but a chemical stimulus shows that chemoreceptors are more ubiquitously distributed on the planarian's body than is usually assumed. Experiments with other groups, such as sea snails, indicate a similar ubiquitous chemoreception to detect predators (Dix & Hamilton, 1993). Nevertheless, the results from experiments 2.3 and 2.4, showing that dorsal secretions alone or a slime trail of the predator rarely trigger an antipredator behavior, suggest that a chemical cue alone may not be enough to trigger an escape response and that therefore a tactile element is also necessary. Some receptors in rhabditophoran flatworms are considered as possible mechanoreceptors, although there is little empirical evidence for this (Rieger *et al.*, 1991). Visual detection is unlikely to be of importance, as the eyes of land planarians are simple and the escape response only starts after physical contact, as occurs also in other organisms with poor vision (Bullock, 1953).

Another possible explanation for the apparent lack of response of *L. abundans* to the chemical cues of *O. anthropophila* may be simply a matter of the experimental design. Our experiments 2.3 and 2.4 intended to identify an immediate escape behavior or a similar aversive behavior by *L. abundans* when contacting the secretions, implying a behavior similar to that used when finding the predator itself (Boll & Leal-Zanchet, 2018). However, the response may have been more subtle and thus not recorded, including, for example, a simple displacement in a larger area. This could include a vertical migration, as observed in other species that are attacked by predators with habits similar to those of the prey, such as wolf spiders that are prey of other wolf spiders (Persons, Walker, & Rypstra, 2002) or species that are able to move and be unreachable by the predator (Dalesman *et al.*, 2006). The typical escape behavior of *L. abundans* includes upward stretching of the anterior half of the body (Boll & Leal-Zanchet, 2018), which could indicate an attempt to climb a nearby structure to mislead the predator, thus making it lose the prey's track. A chemical cue of the predator in the environment could, therefore, induce a similar, but not as abrupt, attempt to move upward. Additional observations are needed to address this possibility, including observations in the field, as the anti-predator behavior of organisms may show differences in a natural environment when compared to that in the laboratory (Sullivan, Maerz, & Madison, 2002).

CONCLUSION

Land planarians have high species diversity in tropical regions, and are thus specialized in feeding on several invertebrate groups. As a result, they developed a variety of strategies to capture prey, including pursuit predation of slow-moving prey (Fiore *et al.*, 2004; Iwai *et al.*, 2010; Boll & Leal-Zanchet, 2015), as well as ambush predation of fast-moving prey. The environment occupied by *L. abundans*, which includes human-disturbed areas (Prasniski & Leal-Zanchet, 2009), also hosts other species of land planarians of the genus *Obama*, including species that feed on *L. abundans* and others that do not (Boll & Leal-Zanchet, 2016). The generalized escape response of *L. abundans* toward species of *Obama* may be a result of the contrast between similar chemical signals and different diets, but it also suggests an ongoing process of natural selection reflecting a recent coexistence of these species. Human-disturbed areas may be a new environment occasioning new interactions that were previously much rarer or non-existent. Thus, the possibility that some are new interactions caused by human interference, and the ability to discriminate dangerous from harmless predators increases the overall fitness of a prey (Crawford, Hickman, & Luhring, 2012), suggest that such discrimination is under selection in *L. abundans* and that future generations will be more efficient in their escape response.

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CONSIDERAÇÕES FINAIS

1. A comparação de valores morfométricos de planárias terrestres com dieta e gênero encontrou relações significativas entre algumas variáveis.
2. Ao contrário do esperado, o formato da faringe não se mostrou uma característica eficiente para prever a dieta de planárias da subfamília Geoplaninae, sendo a altura da musculatura cutânea uma característica mais adequada.
3. Na subfamília Geoplaninae, a posição da boca na bolsa faríngea é influenciada pela posição da inserção dorsal da faringe. Da mesma maneira, o formato do corpo em corte transversal (circularidade transversal) é influenciado pela altura da musculatura cutânea.
4. *Obama anthropophila*, uma espécie que se alimenta de gastrópodes e planárias, apresentou crescimento similar quando submetida a dietas de apenas um item ou a uma dieta mista, no entanto a sobrevivência de indivíduos recebendo dieta mista tendeu a ser menor, sugerindo um efeito antagonista dos dois alimentos combinados, talvez pela necessidade de profundas mudanças fisiológicas para digerir cada tipo de presa.
5. Espécimes de *O. anthropophila* se alimentando exclusivamente de lesmas produziram mais cápsulas de ovos que espécimes que receberam uma dieta de planárias ou uma dieta mista, sugerindo que lesmas são mais nutritivas e aumentam a fecundidade. Por outro lado, espécimes que receberam dieta mista, e que apresentaram a menor sobrevivência, produziram cápsulas mais pesadas, indicando investimento terminal para maximizar a reprodução perto do fim da vida.
6. *Luteostriata abundans*, que se alimenta exclusivamente de isópodes terrestres, não seguiu rastros deixados por isópodes e nem foi capaz de detectá-los química e mecanicamente à distância, o que aponta para uma estratégia de emboscada para capturar as presas.
7. Quando em contato físico direto com um predador, *L. abundans* apresenta uma imediata resposta de fuga que não ocorre ao contato com a maioria das espécies não predadoras. No entanto o contato com espécies inofensivas filogeneticamente próximas do predador desencadeou uma resposta de fuga, o que pode ser devido a assinaturas químicas similares e indistinguíveis entre predadores e não predadores próximos destes.

8. O contato com secreções do predador por si só raramente desencadeou respostas de fuga em *L. abundans*, o que supõe uma importância do estímulo tátil ou comportamentos de esquiva mais sutis quando o perigo não é imediato.
9. O conjunto de resultados das investigações dos três capítulos evidencia que, apesar da anatomia simples, planárias terrestres desenvolveram uma grande diversidade de adaptações morfológicas, ecológicas e comportamentais.