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FERNANDA GUIMARÃES FAVA

ECOLOGIA DO MOVIMENTO DE ANUROS NEOTROPICAIS: CIENTOMETRIA, ANÁLISE METODOLÓGICA E USO DO PÓ FLUORESCENTE

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RESUMO

A ecologia do movimento diz respeito ao deslocamento de um organismo no espaço e sua interação com fatores internos e externos. Esta é uma área em crescimento e com muito a ser descoberto. Os anfíbios se movimentam de diferentes maneiras, dependendo da fase de vida em que se encontram. A maioria possui reprodução aquática e após a metamorfose se deslocam para um ambiente terrestre. Enquanto juvenis, eles estabelecem uma área de vida e voltam a se deslocar para o local de reprodução quando atingem a maturidade sexual. Alguns indivíduos também fazem movimentos para fora da população de origem. Para se medir o movimento dos organismos existem várias técnicas, mas poucas são adequadas para se medir o deslocamento em uma escala de micro habitat. O uso do pó fluorescente permite que se conheça todo o caminho percorrido pelos indivíduos, além de poder ser usado em espécies ou indivíduos pequenos, possibilitando a identificação de padrões do movimento. Levando em consideração os poucos estudos existentes na região neotropical em comparação com regiões temperadas e rápida perda de áreas naturais, essa tese foi estruturada em três capítulos e teve como objetivos: 1) sumarizar o conhecimento sobre a ecologia do movimento para os anuros neotropicais; 2) avaliar a técnica de translocação durante o período reprodutivo e o uso do pó fluorescente para rastrear pequenas espécies; 3) avaliar os padrões do movimento de juvenis de *Rhinella hoogmoedi* em área de Mata Atlântica. Nossos resultados evidenciaram algumas lacunas do conhecimento em relação ao movimento, como a falta de estudos em áreas antropizadas, de movimentos na fase juvenil e de dispersão. Encontramos que o pó fluorescente pode ser utilizado em áreas com alta umidade e que as cores podem ter diferentes efetividades. Os juvenis de R. hoogmoedi se movimentam diariamente em uma área menor que 1m², com passos curtos e caminhos sinuosos.

Palavras-chave: cientometria; deslocamento; Hylidae; Bufonidae.

ABSTRACT

The movement ecology concerns the displacement of an organism in space and its interaction with internal and external factors. This is a growing area with a lot to be discovered. Amphibians move in different ways, depending on the life stage to which they are. Most have aquatic reproduction and after metamorphosis, they move to a terrestrial environment. As juveniles, they establish a home range and move back to the breeding site when they reach sexual maturity. Some also make movements outwards of the original population. There are several techniques for measuring the movement of organisms, but few are suitable for measuring displacement on a microhabitat scale. The use of fluorescent powder allows knowing the entire path taken by individuals and being able to be used in species or small individuals, allowing the identification of movement patterns. Considering the few studies in the Neotropics compared with temperate regions and its rapid loss of natural areas, this thesis has been structured into three chapters and aimed to: 1) summarize the knowledge about the movement ecology for the Neotropical anurans; 2) evaluate the translocation technique during the reproductive phase and use fluorescent powder to track small species; and 3) evaluate the movement patterns of Rhinella hoogmoedi juveniles of an Atlantic Forest. Our results highlighted some knowledge gaps in relation to the movement, such as the lacking studies in anthropized areas, movements in the juvenile and dispersion phase. We found that fluorescent powder can be used in high humidity areas and that colors can have different effectiveness. The R. hoogmoedi juveniles move daily in an area smaller than 1m², with short steps and winding paths.

Key-words: scientometry; displacement; Hylidae; Bufonidae.

INTRODUÇÃO GERAL

Você já ouviu falar na ecologia do movimento? Sabe do que se trata? Há quem possa imaginar, por exemplo, aves em uma dança de acasalamento, ou algo do tipo, mas não é nada disso! Quando falamos de ecologia do movimento é em relação ao deslocamento de um indivíduo de um lugar para outro no espaço e sua interação com fatores internos e externos (Nathan *et al.* 2008). Este é um ramo da ecologia em crescimento e que ainda tem muito o que ser descoberto. E como estudar a ecologia do movimento? Quais variáveis medir? O que podemos descobrir a partir desses dados? Para exemplificar vou usar o grupo dos anfíbios. Primeiro é importante saber em que fase da vida os indivíduos estão. Isso porque, com o passar do tempo, eles mudam suas atividades e a forma como usam o ambiente (Pittman *et al.* 2014). Para os anfíbios com reprodução aquática, que são a maioria no grupo, após a metamorfose, os indivíduos saem do local onde nasceram e se refugiam nas imediações (Pittman *et al.* 2014; Sinsch 2014). Neste período fazem movimentos de forrageamento, procurando por locais adequados, que contenham refúgios e alimento (Fahrig 2007), já que após um período sem a boca formada e consumindo recursos da cauda (McDiarmid and Altig 1999), os jovens vão se alimentar o quanto puderem para crescer e alcançar a vida adulta.

E assim como nós, seres humanos, os outros animais, como os anfíbios, também têm “personalidade” (Joly 2019)! Alguns indivíduos são mais “aventureiros” e buscam por outros ambientes, longe da população de origem, o que ajuda na diversificação da espécie, devido à interação genética entre populações. Depois de escolher um lugar adequado, os indivíduos tendem a estabelecer uma área de vida (Pittman *et al.* 2014), sendo este um espaço onde realizam as atividades diárias rotineiras, principalmente de forrageamento (Duellman and Trueb 1994). Quando se tornam adultos, tendem a voltar para a poça de origem (ou vão para outras poças, no caso dos aventureiros) na época reprodutiva para procura de parceiros (Pittman *et al.* 2014). Nesse momento eles tendem a deixar de fazer movimentos curtos na escala do microhabitat e passam a fazer movimentos mais longos e direcionais para as poças, sendo este evento conhecido como migração, pois após a reprodução eles retornam para os ambientes de refúgio e forrageio (Pittman *et al.* 2014). E quando os indivíduos vão à procura de novos ambientes reprodutivos, sejam jovens ou adultos que têm seus ambientes perturbados, este evento é conhecido como dispersão, que tem importante contribuição para o fluxo gênico (Pittman *et al.* 2014). Algumas espécies de anfíbios possuem hábitos terrícolas e a reprodução ocorre dentro da área de vida, sem a necessidade de fazerem migrações para se reproduzirem em corpos d'água (Duellman and Trueb 1994).

Vimos que os anfíbios se deslocam basicamente em três escalas: de forrageamento, migração e dispersão. O forrageamento ocorre diariamente, em um nível local, onde os indivíduos buscam basicamente por recursos, como refúgios e alimentos. A migração dos anfíbios ocorre em um nível intrapopulacional, em movimentos de ida e volta dos locais reprodutivos. E a dispersão ocorre em um nível interpopulacional, em um movimento só de ida (Pittman *et al.* 2014). Mas os anfíbios vão se mover somente quando necessário, já que se deslocar no ambiente, apesar de inevitável, envolve muitos riscos, como predação e dessecação, além do gasto energético (Fahrig 2007; Wells 2007).

E para estudar todo esse movimento é necessário "seguir" os indivíduos. Mas como seguir um sapo? Podemos fazer o uso de tecnologias como rastreamento por rádio (Pitt *et al.* 2017), capazes de monitorar a localidade do indivíduo de tempo em tempo, mas o custo é muito alto, ainda mais se pretendemos monitorar uma boa quantidade de indivíduos. Além disso, o peso de qualquer dispositivo acoplado ao corpo do indivíduo não pode ultrapassar 10% do peso total do animal (Heyer *et al*. 1994), o que limita o uso em pequenas espécies. Uma técnica bem conhecida para reconhecer os indivíduos é o corte de artelhos, que consiste no corte combinado das falanges distais das patas dos anfíbios. Possíveis impactos desta técnica podem ser infecção devido ao corte e/ou interferência no movimento dos indivíduos (Funk *et al.* 2005). Assim como em outras metodologias de captura, marcação e recaptura, seja utilizando o reconhecimento de padrões de manchas na pele (Beck *et al.* 2017), uso de anéis (Neckel-Oliveira and Gascon 2006), ou pigmentos fluorescentes (Moser *et al.* 2019), é necessário que se reencontre os indivíduos, após a liberação no ambiente, o que pode ser um desafio, sendo que muitos não são reencontrados. Existem duas técnicas interessantes que marcam todo o caminho percorrido pelos indivíduos, sendo possível até reencontrá-los no final da trilha, mas só podem ser utilizadas para estudar o movimento a curto prazo, pois têm suas limitações. Uma delas é o uso de carretéis de linha (Tozetti and Toledo 2005), que são acoplados no corpo do animal e por onde passa a linha fica no ambiente, funcionando até para espécies arborícolas (Mejía *et al.* 2021). Mas um problema nessa técnica é a limitação em relação ao tamanho do corpo do animal. A outra é o uso do pó fluorescente (Rittenhouse *et al.* 2006), que é passado no corpo do indivíduo, geralmente em uma parte ventral, e quando solto no ambiente, deixa marcas no substrato por onde passa. Uma vantagem dessa metodologia é que além de relativamente barata e poder marcar muitos indivíduos, pode ser usada em espécies ou indivíduos pequenos, que não seriam capazes de carregar um equipamento.

A partir do uso dos métodos apresentados podemos calcular as direções e distâncias percorridas em um intervalo de tempo especificado e saber o ambiente utilizado pelos indivíduos monitorados. Dessa forma, é possível identificar o tipo de atividade que estão desenvolvendo a partir do padrão do movimento. Por exemplo, indivíduos em atividade de forrageio andam caminhos curtos e sinuosos, explorando o ambiente, diferente dos indivíduos que estão na fase de reprodução, que percorrem caminhos mais longos e retilíneos (Dingle and Drake 2007). Também é possível calcular o tamanho da área de vida para a espécie, sendo que algumas passam quase toda a vida em poucos metros quadrados e outras utilizam um espaço maior para suas atividades diárias (Wells 2007), sendo essa uma informação valiosa para gestores de conservação, pois para proteger o ambiente de uma espécie é importante saber o tamanho da área que ela requer.

Em relação à ecologia do movimento, assim como em outras áreas do conhecimento, existem mais artigos publicados nos países da zona temperada e as espécies desses lugares são mais bem conhecidas. Nas zonas tropicais, onde existe uma maior diversidade de espécies, menos tempo de pesquisas e menos investimentos, existe uma carência de informações, e estas se fazem urgente, vista a rápida destruição dos ambientes naturais (Stuart *et al.* 2004). Neste contexto, com o objetivo de sintetizar informações, realizar testes metodológicos e a aplicação da técnica avaliada para revelar padrões do movimento, a presente tese foi baseada no estudo da ecologia do movimento de anuros da região neotropical, sendo organizada em três capítulos:

O **capítulo 1**, intitulado "Movement ecology of neotropical anurans – a scientometric approach" trata de uma avaliação de estudos realizados na região neotropical, sumarizando informações que podem auxiliar pesquisadores e gestores de áreas de conservação;

O **capítulo 2**, intitulado "Fluorescent powder combined with mineral oil: testing a technique to track small frogs in translocation experiments" busca avaliar uma técnica de rastreamento para pequenos anuros;

E o **capítulo 3**, intitulado "Movement patterns of *Rhinella hoogmoedi* juveniles in a forest area of Northeast Brazil" mede e avalia os padrões do movimento de uma espécie de anuro na fase juvenil.

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Capítulo 1

Movement ecology of neotropical anurans – a scientometric approach

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Movement ecology of neotropical anurans – a scientometric approach

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Abstract

Movement ecology evaluates the organisms, which change their position in time and space, interacting with others and the environment. Many factors affect anuran movement, from individual physiological aspects to environmental factors. There are several techniques for tagging or recognizing individuals, as well as for tracking them. We performed a scientometric analysis to assess when, where and how studies about movement ecology of Neotropical anurans have been carried out. We found 67 articles, published in 39 journals, and there was a clear upward trend in the article's numbers in the area. Studies covered 23 ecoregions, distributed in 15 countries. Most studies were carried out in preserved environments, at the local level, with few populations evaluated. There is information about movement for 50 Neotropical species belonging to 11 families. Most studies have evaluated the movement of males, adults, and species with terrestrial habit. The main movement variables analyzed were "home range size", "movement pattern", "homing", "locomotor performance" and "territory size". Most studies identified individuals through individual patterns and the toe-clipping technique, and to track them, the main technique was mark-recapture. Our synthesis on the anuran movement can provide insights for management and conservation actions. Research efforts in the future should focus on aspects that still represent gaps in the movement ecology of Neotropical anurans, such as studies in anthropized areas, with tree frogs, juveniles and females, focusing on dispersion and using of new technologies.

Introduction

Animals can move without necessarily leaving the place, stimulated by some behavior, such as the interaction between frogs of the same species, which through limbs movements, produce visual displays to signal and communicate [1]. But if an individual moves, changing its location in space and time, we are talking about the main issue studied in the movement ecology, which evaluates the organismal movement, at individual, population or community level, and its interactions with others and with the environment [2]. An animal may change its location in space for several reasons, related to survival and reproduction, such as search for resources (shelter, food, sexual partners), to avoid intraspecific competition, to escape from predators and due to unfavorable environmental conditions [3,4]. However, moving can be expensive as it increases exposure and death risk from desiccation and predation [4,5]. Movement is a key process in ecology and evolution, which allows connection between populations [3] and is commonly divided into three phases: foraging, dispersion, and migration. The main differences between them are related to the spatial and temporal scales [3,6,7].

For aquatic-breeding amphibians, Pittman et al. [8] proposed and characterized the movement phases, dividing them into five moments in the individual's life (pre-departure, initial movements, home range, foraring and adult migration). Starting with the juvenile, recently metamorphosed, which remains in the place where it was born and evaluates the terrestrial environment conditions, this is the pre-departure phase. The next phase covers the juvenile's initial movements, in which they search for suitable places to take refuge and complete development. They look for environments that provide ideal conditions, such as low density of conspecifics and predators, in addition to having a breeding site close by, such as ponds [8]. After finding a suitable place, the third phase begins, establishing the homing range area, which must contain shelters against desiccation and predators, in addition to food resources. Within the homing range, the individual performs foraging movements, beginning the fourth phase [8]. Upon completing sexual maturation, the individual begins to perform migratory movements, in which it leaves the homing range, moves to an aquatic breeding place, and returns after the reproductive period [8]. This is the fifth and last stage. Some species may also exhibit movements and territorial behavior at this stage [9–11]. It has been reported that during the second movement phase, the juveniles, who still don't have spatial experiences and memories, search for new places, often performing dispersion process, in which the individual leaves the area where it was born and reaches a new location, being able to form or compose a new population, after reaching reproductive age [12,13]. Adults can also perform dispersion movements, in cases for example, in which the environment becomes unfavorable and the individual is forced to look for a new location, and often ends up finding an environment distant from his native range [12].

Many factors can influence the movement ecology of anuran amphibians, being variable between species and within and between populations [14]. Species and individual characteristics, such as habit (terrestrial, arboreal) [15] age [8,16,17] and sex [18,19] can affect the individual's movement capacity and trajectory, which hampers generalizations in relation to the movement's capacities for the amphibian group as a whole [15]. Furthermore, heterogeneous landscape features can influence the movement [4], as the risks and benefits offered by different coverage types and land use are quite variable [5,20–24]. In this way, it is necessary to evaluate the species movement in natural and anthropized environments, especially in regions that are undergoing rapid landscape transformation, where the species have been suffering various threats and which still harbors great biodiversity. The Neotropical region [25], contains the greatest amphibian's richness in the world, with practically half of the world species, of which around 40% are threatened globally [26].

To be able to remotely monitor frog movement, individual recognition is sometimes necessary. Several techniques are available (tag, polymers and pigments, transponders, toe clipping and branding) for marking or recognizing anurans. They have improved over time, and allow individuals identification through registered coloring patterns, photographs, or drawings [27,28]. There are also some technical options to track anurans. The anuran movement can be observed directly or through video recordings, in the field or laboratory, usually in short studies [29–31]. One of the most used technique is mark-recapture, which is ideal for monitoring many individuals and for long-term studies [27]. However, the researcher needs to count on luck to be able to recapture the individuals marked. Other options are the use of fluorescent powders [32,33], thread bobbins [34,35], harmonic direction finding [36] and radio transmitters [37,38]. Each of these marking/identification and tracking techniques has advantages and limitations and should be chosen considering the study scale (local or landscape), researcher available time and resources, in addition to the chosen species characteristics (habits and body size) [27].

There are different parameters for measuring animal movement, such as the distance moved over a time, the probability of leaving the current location, the probability of crossing boundaries between different habitat types and the movement path tortuosity [4]. These variables can be plastic and vary according to the individual's physiological state [4]. Through these data we can get information about movement patterns, home range, territory size, tendency to return to the home range when displaced (homing), locomotor performance, navigation ability, spatial learning, daily movements, tadpole transport by adults, among others. This information helps to understand how organisms use their environment and can provide insights for management and conservation actions [7,39,40]. Scientometric studies can help synthesize the existing data for a specific group or subject with a broader view of the analyzed subject, in addition to providing good article sources, which can assist researchers in the field and decision makers [41,42].

To understand how research is being done and what are the main features and methods covered in the studies about the movement ecology of anuran amphibian species in the Neotropical region, we carried out a scientometric study looking to bring information about: 1) Temporal trend and characteristics of publications (time trend in research, journals and impact factor, countries and ecoregions); 2) Characteristics representativeness of the studies sites, species and individuals (area status, extent, number of population studied, habit, species, family, sex, and age); and 3) Methodologies used to study movement and temporal trends (marking/individual identification and tracking techniques). We also tried to find out if there is a relationship between the species' habit and the tracking method used. Using this information, we intend to show when, where and how research is being done on the movement ecology of Neotropical anurans.

Material and methods

We used the Google Scholar search site to search for peer-reviewed articles, which proved to be as inclusive or even more than other sites such as Web of Science and Scopus [43]. We performed our search from January 5th to 15th, 2020 using six keyword combinations: “Neotropical anurans” AND “movement ecology” AND “tracking” OR “orientation”; OR “homing”; OR “navigation”; OR “migration”; OR “dispersion”. We conditioned the search for articles to the first 20 pages of the search for each combination of words, 10 per page [44,45]. The eligibility criteria used to choose the relevant articles were: 1) study conducted in the Neotropical region and with native anuran species, and 2) article measuring and analyzing aspects of the individual’s movement, as movement patterns, home range size, territory size, homing, locomotory performance, mobility, spatial memory, navigation capacity, among others. We also added articles from our personal library that dealt with the frog’s movement and that fall within the scope of our study. We discarded research that did not directly measure the movement of individuals, such as studies that used genetic approaches or community studies. These are based on the gene flow and species distribution across the landscape to describe movement patterns, but do not report important mechanisms of movement or physiological consequences organisms’ conditions for movement [8] See the flowchart in figure 1.

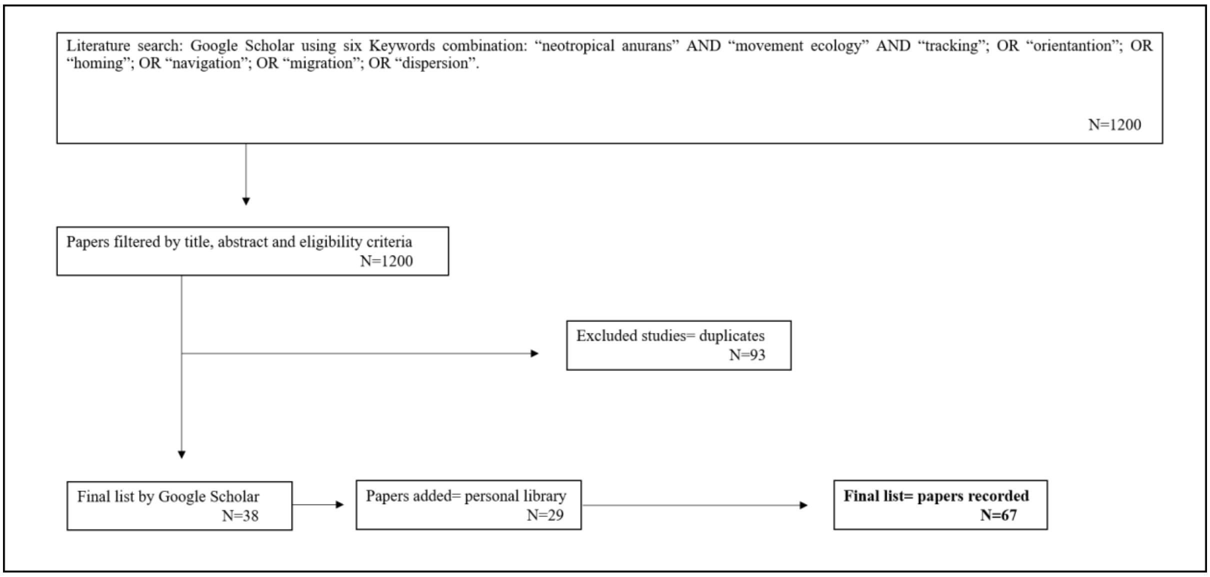

Fig 1. Flowchart with article selection processes and number of studies for each step of the review.

For each study we extracted the following information: 1) publication year, journal name, impact factor, country, ecoregion, geographical coordinates, family, species; 2) studies sites, species and individuals characteristics: habit, conservation status, stage (adult/juvenile/both), sex (male/female/both), study area extension (local/landscape), area status (preserved/anthropized/both), movement variables, movement analysis (how movement was measured), marking/identification and tracking techniques (S1 Table). Studies undertaken in more than one country or ecoregion, with more than one analyzed species and which used more than one mark/identification or tracking method and analyzed more than one movement variable, had such information extracted separately and independently for each variable.

Regarding status, areas were classified as preserved if the study was carried out in reserves or natural areas; as anthropized if the study was carried out on plantations or roads. For the following variables taken from the articles (age, gender, population, tagging, ecoregion and status area) NA means that the information was not available. For the other variables (conservation status, impact factor and extent) NA means that the information does not apply to the studies.

Methods used to estimate some movement variables, such as the home range or territory size, may vary, and a methodological pattern is absent. Different approaches exist to estimate these variables [54]. Among the methodological options there are variations, such as the Kernel method: fixed-kernel method [55], fixed kernel estimates [56], fixed kernels [57]; and the minimum convex polygon method (MCP) 100% [56] e MCP 90% [58]. These were grouped as the same, Kernel method (KM) and minimum convex polygon (MCP). Some marking methods were also grouped, such as: fluorescent pigments (fluorescent pigments, fluorescent subcutaneous labels, nontoxic pigment powder, thermoplastic fluorescent pigments) and individual patterns (dorsal, ventral, lateral patterns; color of legs and flanks).

The journal's impact factor was taken from 2019 Journal Citation Reports (JCR). We updated the taxonomic nomenclature following Frost [59]. Information about the species' habit was retrieved from *AmphibiaWeb* [60] and the *IUCN* database [61]. Species conservation status follows the *IUCN* database [61]. When the number of population studied was evident in the article, this information was also recorded. Ecoregions were defined according to Olson et al. [62], *WWF* [63] and *Ecoregions* [64]. When coordinates were not available in the article, we extracted an approximate coordinate for the indicated region through *Google maps* [65].

Data analysis

We used a generalized linear model (GLM) to analyze whether there are temporal trends in the studies number. To analyze if there is a relationship between the species habit (arboreal or terrestrial) and the used tracking method, we performed a g test of independence. All analyzes were performed in the R environment [66].

Results

Articles search and study characteristics

The Google Scholar search returned 1200 articles and our database comprised 38 valid ones that measured some aspect of movement ecology in Neotropical anurans and adding 29 from personal library, we had a total of 67 valid studies (Figure 1). The oldest article we found in our search dates to 1974 and the article numbers increased over the last 45 years (R^2= 0.5883, p-value < 0.001). Since 2005, long intervals between the publications were no longer observed and the article numbers still tend to increase, since the curve shows no tendency to stabilize (Figure 2). Studies were published in 37 different journals, of which 11 (28.2%) belonged specifically to the herpetological area. About 38% journals published more than one article on the topic, but the majority (about 61% of the journals), published only one article on the subject. The Journal of Herpetology had the largest publications number, with six articles, followed by Amphibia-Reptilia and Phyllomedusa with four articles each. The impact factor of 4 journals (about 10%) was greater than 3, twenty-six journals (about 70%) ranged between 1,032 and 2,869, and 6 (about 16%) had an impact factor below one and only one journal had no classification (Figure 3).

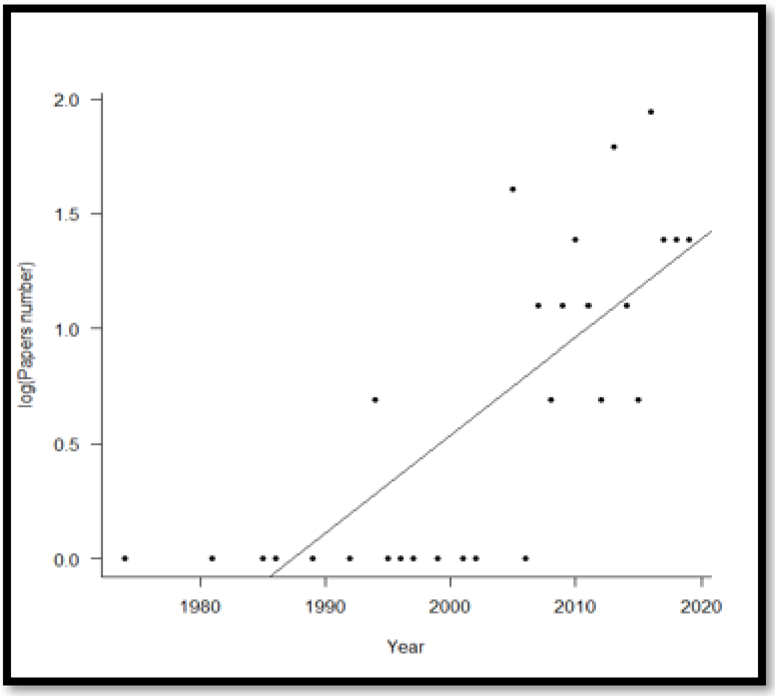

Fig 2. Time trends of research with neotropical anuran movement ecology.

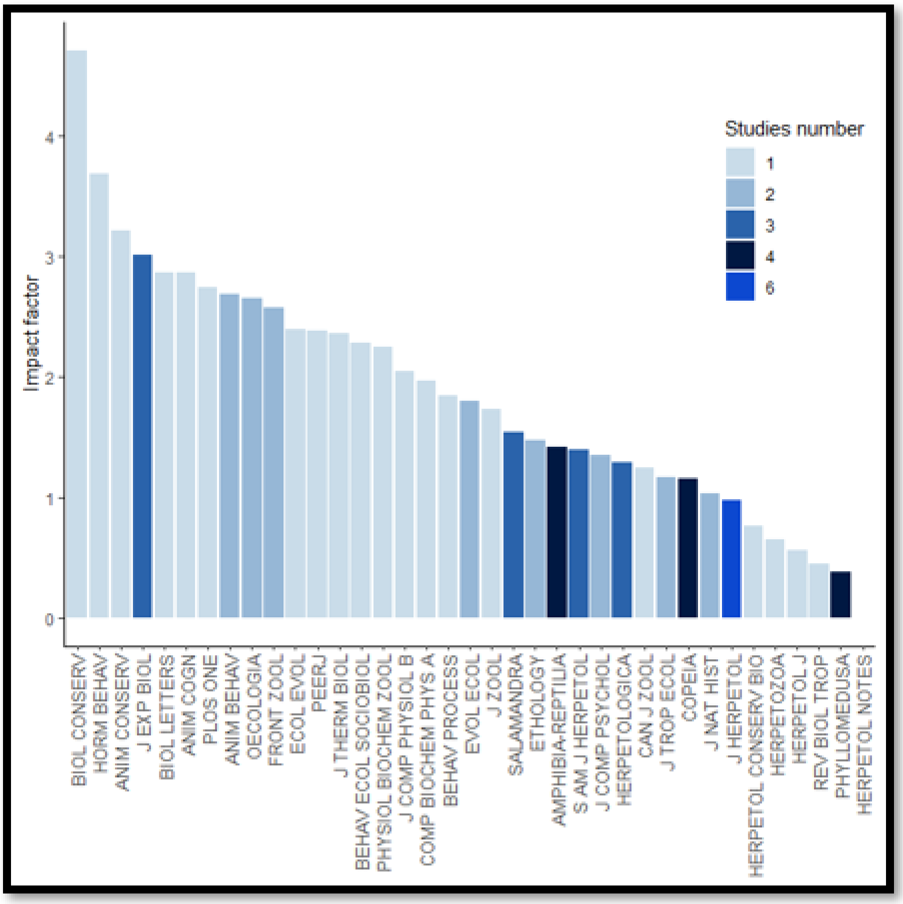

Fig 3. Journal name, impact factor value and studies number found in each journal.

Studies were carried out in the Neotropical region and cover 23 ecoregions in 15 countries (S2 Table). Brazil was the country with the largest number of studies (15), encompassing eight different ecoregions, followed by French Guiana, with 13 studies and Costa Rica with 11. The ecoregion with most studies was Guianan Lowland Moist Forests with 14 studies, the Isthmian-Atlantic moist forest with eight and Cerrado with seven (Fig 4). Most studies (51) were undertaken in preserved environments, with only three in anthropized environments and two studies covering preserved as well as anthropized environments. A large part of the studies (55) was done at the local level and only four at the landscape level. Studies carried out in the laboratory (8) did not fall into this classification. Most studies (31) did not show the evaluated number of population studied, 29 evaluated one population, 5 evaluated two, and a single study evaluated three populations and another four.

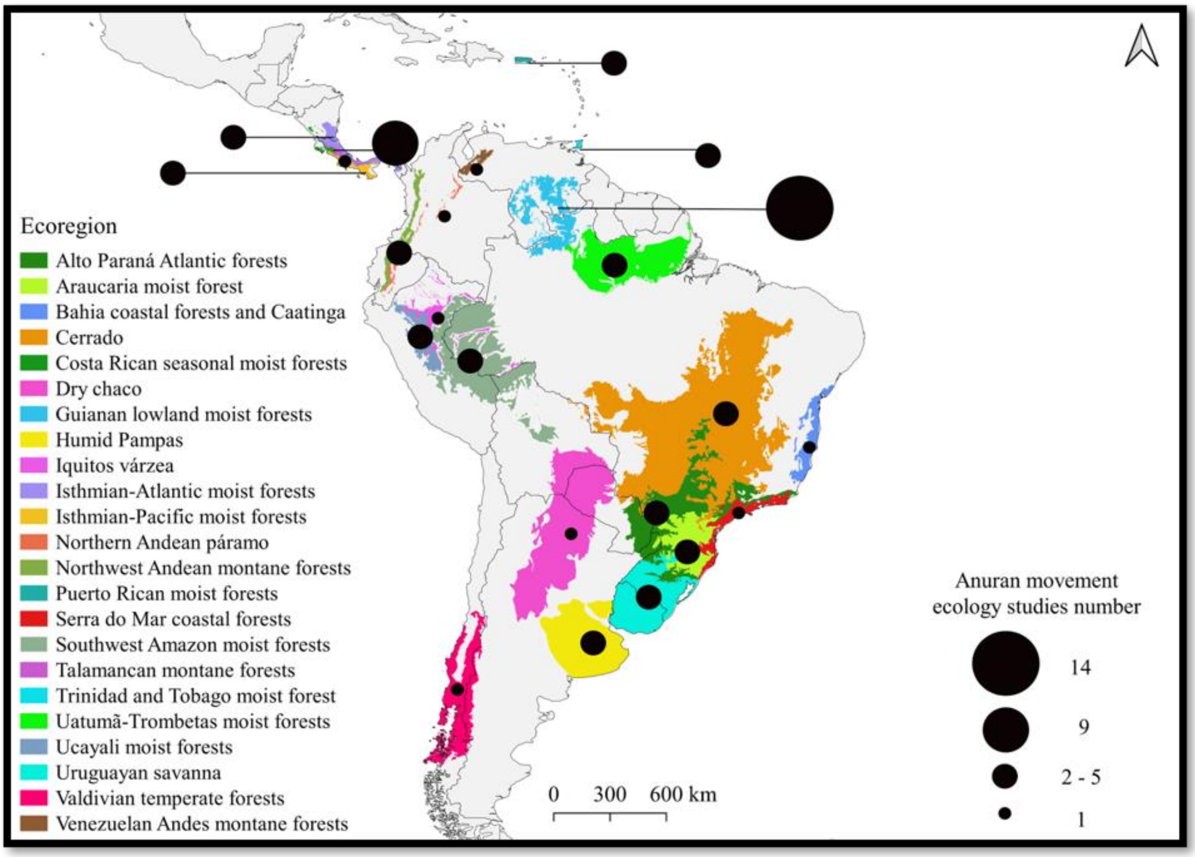

Fig 4. Map showing the studies number carried out by ecoregion.

Species characteristics

Fifty species belonging to 11 families were studied. *Allobates femoralis* (Boulenger 1884) and *Oophaga pumilio* (Schmidt 1857) were present in 12 and seven articles, respectively (Fig 4). In two cases, individuals were only classified at the genus level and thus they were not added to the species number. According to *IUCN* red list [61], four species were classified as critically endangered (CR), one as endangered (EM), four as vulnerable (VU), three were part of the data deficient (DD) category and 35 were classified as least concerned (LC). Three species have not yet been assessed by the IUCN (Fig 5). Among the 67 analyzed studies, only eight addressed more than one species. The Dendrobatidae family was the most represented with 21 studies and 11 species, followed by Bufonidae with 20 studies and 15 different species.

Representatives of the Aromobatidae family were studied in 17 articles but were represented by only three species.

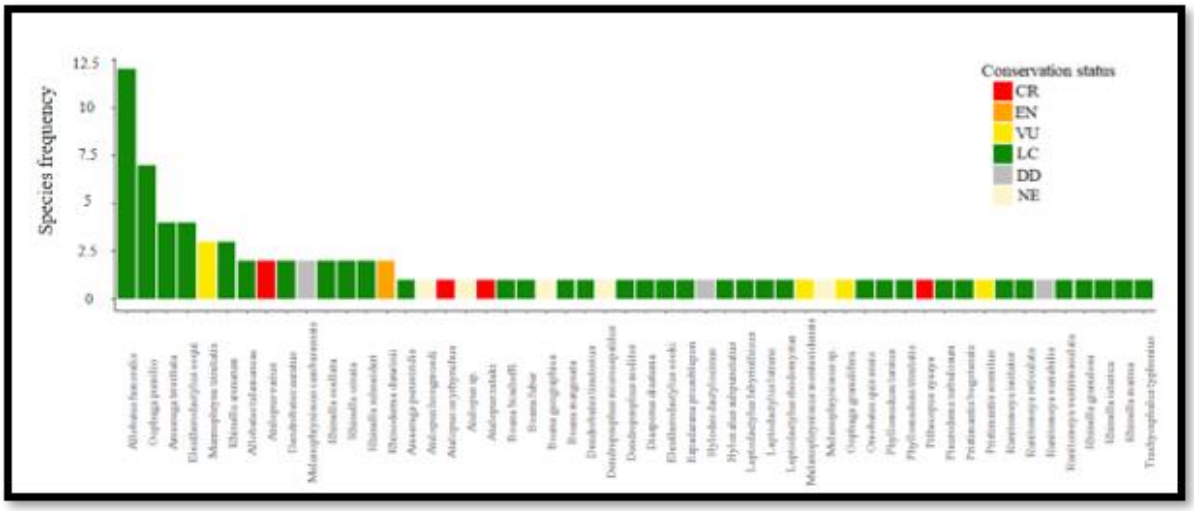

Fig 5. Studied species and their respective conservation status.

Most of the studied species have a terrestrial habit (35), while 15 are arboreal. Of the 67 analyzed articles, 36 studied females and males together, 24 studied only males and one article evaluated only females. The studied anurans sex was not reported in six studies. 58 articles studied only adults while one article analyzed juveniles separately. In six articles adults and juveniles were studied simultaneously while in two articles the authors did not make the development stage evident.

Movement, marking/identification, and tracking variables

The main movement variables analyzed were “home range size”, movement pattern", "homing", "locomotor performance" and "territory size", with 15, 12, 10, seven and six studies, respectively. Other variables were used twice or only once. The most common marking or individual identification modes were the individual patterns, used in 27 studies and the toe-clipping method, used in 18. Other techniques such as fluorescent pigments and rings were seldom used. In 13 studies, no marking method was used and in seven studies this information was not provided (Fig 6a). Most studies (41) used the mark-recapture technique to track individuals, nine studies were done through direct observation and six used video recordings to monitor movement. Radio transmitters, harmonic direction-finding and thread bobbins were used in four studies each (Fig 6b). Both, the marking, and tracking methods have undergone changes in the use frequency over the years (Figs 7 and 8). We found no relationship between the tracking methods and the species' habit (G test= 6.3823, df= 5, P- value= 0.2708), although the radio transmitters and harmonic direction-finding methods were used exclusively in species with terrestrial habits (Fig 9).

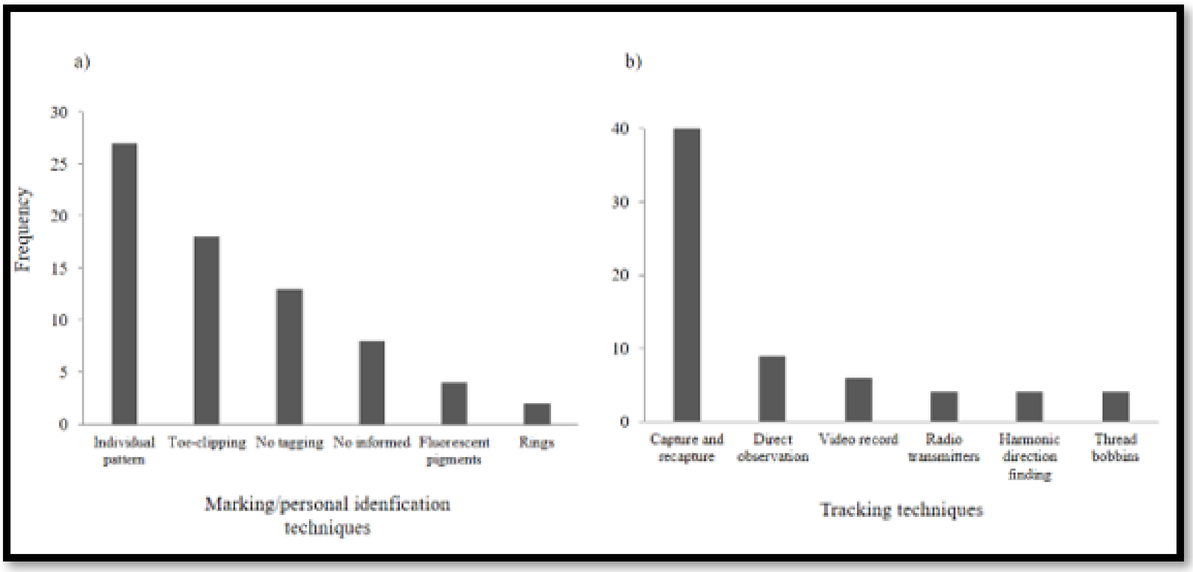

Fig 6. Use frequency of marking/personal identification (a) and tracking techniques (b).

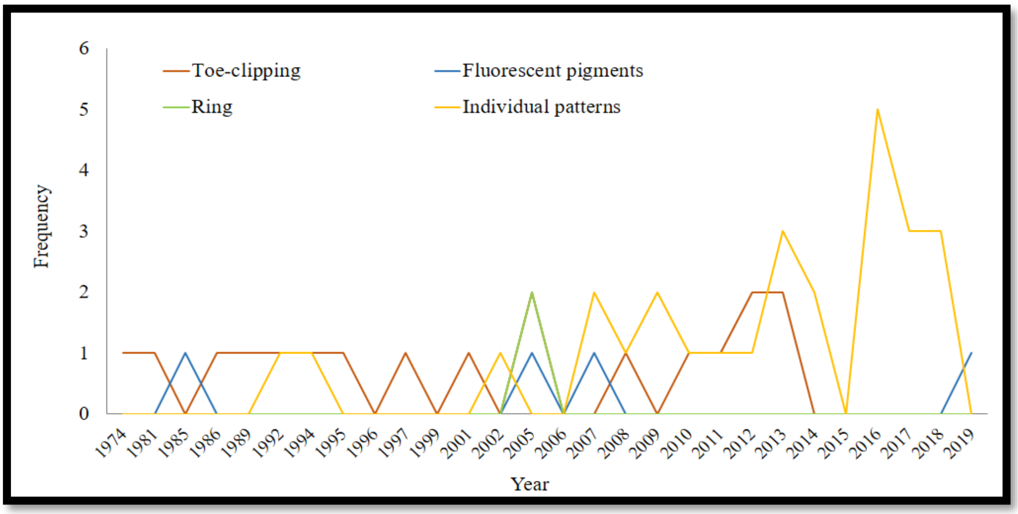

Fig 7. Temporal trends in the marking/personal identification techniques use.

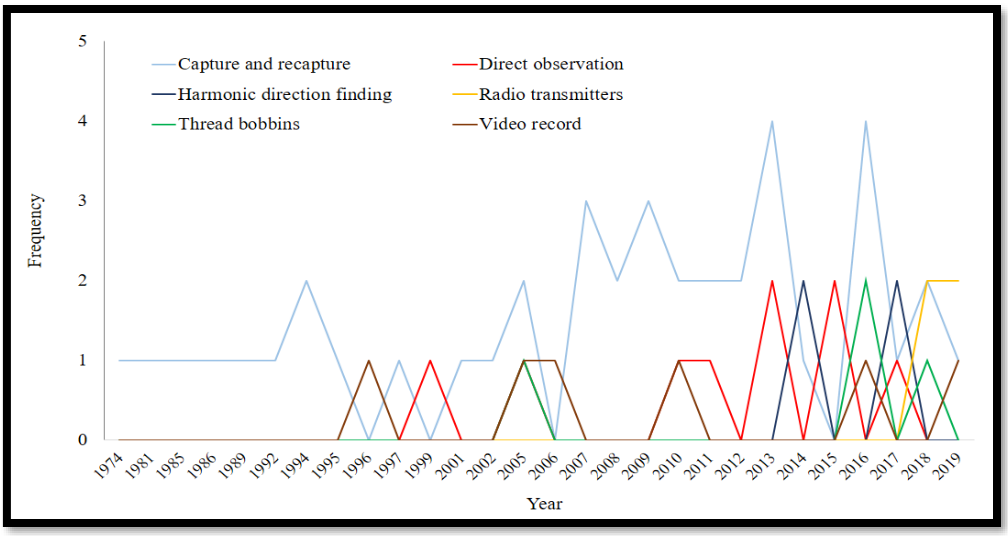

Fig 8. Temporal trends in the tracking techniques used in anuran movement ecology studies.

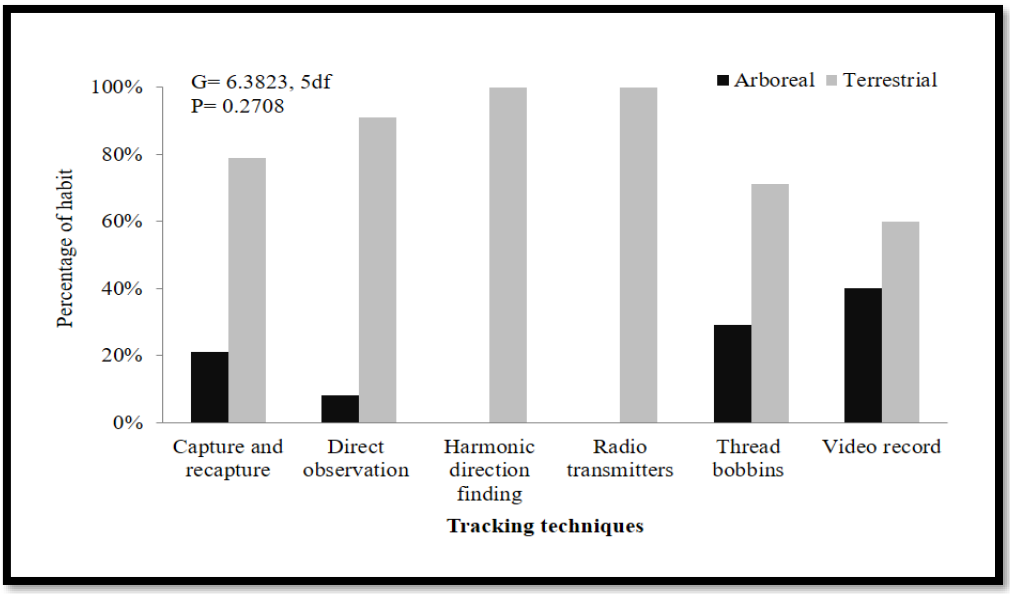

Fig 9. G independence test showing the relationship between the species' habit and the registered tracking method.

Discussion

In this review, we present the main trends and study characteristics, species analyzed and the used methods to study movement ecology of Neotropical anurans. We registered a low number of studies over the 45 years with an increase from 2005 and a clear tendency to continue in the coming years, being a potential field on the rise. Studies were carried out in a small number of ecoregions (23) which are well distributed throughout the region. Few species were analyzed (50) in view of the large number of amphibian species in the Neotropics. We identified the movement variables used in the studies for each species, in addition to trends in the marking/identification and tracking techniques.

Temporal tendencies and studies publication

There is a global trend in the increase of publication of articles in the biological area in the last two decades [67–72]. In Latin America, this increase is probably related to the increase in investments and the research valorization by governments, as well as the increase in the creation of graduate research grants [73]. Journals that most published on the movement ecology of Neotropical anurans are specialized in the herpetological field (e.g., Journal of Herpetology, South American Journal of Herpetology, Salamandra, Herpetological Conservation and Biology and Phyllomedusa), however most journals are not specialized in the herpetological area, showing that there is a scientific community interest in the theme and that frogs are a good model to study movement ecology.

Study characteristics

The Neotropical region currently is formed by 178 ecoregions [62,63], which harbor almost half of the world's amphibian species, about 2.916 [74], with 2.623 anuran species only in South America [72]. Despite studies having been conducted only in a few ecoregions, they are well distributed across South America, Central America, the Caribbean and French Guiana. Eleven of the 23 ecoregions where the studies were undertaken are considered strategic areas for the terrestrial vertebrate's conservation, according to data of species richness, endemism, and threat [75]. The large number of studies in the Guianan Lowland Moist Forests ecoregion can be explained by the high number of articles published by the same author or the same research group that developed many of his projects in this region, as are the cases of the authors Pašukonis, with seven articles and Ringler, with three (Table 2).

Most research has been done in preserved areas but considering the rapid trend of conversion of natural habitats into fragmented and anthropized environments in the Neotropics [76–79], it is expected that future studies also focus on the anuran's movement in altered landscapes. Further, movement between these two contrasting environments should also receive attention. Most studies were carried out at local scale, which may also explain the large number of studies carried out with one population, which reduces the generalizability of the result for the species in general. Movement can vary between populations or even among individuals of the same population [14]. However, these large study numbers carried out with one population and at a local level can be justified by the difficulty of tracking and monitoring many individuals at the same time and across a wide territory. Acquiring detailed knowledge about the frog's movement may not be easy, in addition to requiring large amounts of time and resources [15].

Movement studies generally evaluate one species [80]. Our results show that *Allobates femoralis* (Boulenger 1884) was the most studied species, present in twelve articles. This high number of studies, almost twice as many as the second most studied species, is related to works carried out by authors who deepened their research efforts on the same species. For example, Pasukonis [81–85] and Ringler [11,86,87], who published five and three articles, respectively, about this same species, in addition to other four authors. Most of the species evaluated are not in any threat category, but some studies have addressed species movement aspects that are critically endangered, endangered, and vulnerable, as well as for species with insufficient data, which can assist in filling gaps about such species. About 40% of global amphibian species are in some threat category [26] and the rapid advance of emerging diseases, pollution, climate change and habitat loss and fragmentation further accelerate population declines [74,88,89]. In this context, a deeper evaluation of the species movement seems necessary, especially of threatened ones since this knowledge can provide a basis for more realistic and efficient conservation policies [39,40]. The advance in knowledge about the biology and ecology of species helps in decision-making and its correct use can optimize the use of time and resources by decision makers.

The high representativeness of the Dendrobatidae, Bufonidae and Aromobatidae families is reflected in the high terrestrial habit species number in relation to arboreal ones, probably because tracking in the horizontal stratum is much easier than in the vertical one. Data on the movement of arboreal anuran species from temperate regions is also scarce [15]. Despite the lack of information, these data are important because species with different habits may also differ in relation to how they use their habitat and how they move [15]. In addition, arboreal species may be more affected by sudden changes in the landscape than terrestrial species, which do not use the arboreal layer to move around.

In general, little is known about juvenile's movement ecology [8,15,90], most likely due to the difficulty of marking and tracking these small and still growing individuals [90,91]. For Neotropical species, we also found few studies addressing this life stage. However, we have information on the juvenile movement of seven species, *Allobates femoralis* (Boulenger 1884); *Hylodes dactylocinus* Pavan, Narvaes, and Rodrigues, 2001; *Atelopus zeteki* Dunn 1933; *Trachycephalus typhonius* (Laurenti 1768); *Boana geographica* (Spix 1824); *Pleurodema nebulosum* (Burmeister 1861) and *Rhinoderma darwinii* Duméril and Bibron, 1841 [92–97]. Information on the juvenile's movement is important as they are believed to be long dispersion agents [12,98,99], playing a crucial role in maintaining the populations dynamic persistence [8].

Although many studies have evaluated male and female movement, a larger number of studies with males exists, probably because they are easier to find because of their conspicuousness during calling activity through their reproductive season [100]. Many studies that compared the individual's movement of different sexes found no significant differences. Some authors found no differences between the male and female home range area of *Atelopus oxyrhynchus*, *Hylodes dactylocinus*, *Ranitomeya reticulata* and *Ameerega trivittata* [92,101–103]. Wooldbright [104] showed that there are no differences in the movement number and the distance moved between males and females of *Eleutherodactylus coqui*. Crump [105] analyzed whether there was a difference in the homing frequency in males and females of *Atelopus varius* but noted that both sexes had similar frequencies, for the dry and rainy seasons. On the other hand, there are also results that show differences between sexes, as observed by Pröhl [10] and Pröhl & Berke [106], in relation to the *Dendrobates pumilio* home range area. In both cases the home range area of the females was larger than that of males. Therefore, further empirical studies are needed, comparing different populations, using different estimation methods, to identify general patterns in movement in both sexes for Neotropical species.

Movement, marking/identification, and tracking variables

Most anurans go through different life stages, in aquatic and terrestrial environments, from the larval/juvenile stage to the adult [100]. Throughout animal development, individuals also go through different movement phases, which vary and are characterized by different patterns. So, it is important to consider the movement phase in which the analyzed individuals are when evaluating some movement aspect [2,8]. Studies on the movement ecology of Neotropical anurans focused on assessing the home range size, movement patterns, homing, movement performance and territory size, thus, the variables measured in the studies in this review covered practically all the amphibian movement phases: initial juvenile movement, home range, foraging and adult migrations [8]. The gap to be filled in relation to the Neotropical anuran movement phases refers to long distance movements, which can measure the individual's dispersion capacity. Only one article recovered in our review evaluated dispersion movements. Robertson et al. [107] and Pašukonis et al. [108] measured the adults' influence on tadpole dispersion of two dendrobatid species. Juvenile dispersal events are difficult to find, in addition to the difficulty of tracking small individuals. Adults can also make dispersal movements [8], but these are also hard to identify and track. To carry out dispersion studies, long-term research is required, using good marking and tracking techniques. Unfortunately, these are factors that limit the development of this area since most of the research probably is carried out by graduate students and they have limited time and resources to carry out field activities.

We found a temporal trend in the marking/identifying individuals' techniques (Fig 7). From 1974 to 2005 most studies used toe-clipping. The procedure was first described by Bogert [109] and has been widely used in both short and long term studies. Toe-clipping became popular due to its low cost [91], the possibility of marking several individuals at the same time and of all sizes [110]. Despite widespread use, possible impacts on anurans have sparked some debates over the last two decades [47,110–114]. Due to the knowledge of the negative impact it can cause in some species, ethical guidelines were created to regulate its use, which may explain the technique's use reduction. From 2007 to 2013, more studies began to use individual patterns for identification; however, some works still used toe-clipping. Color pattern use is considered a non-invasive marking method [47,91], being the technique that causes less stress in individuals, since manipulation is minimal. Its use has some limitations because not all species have distinguishable patterns between individuals [115] and when the patterns exist, they can change over time [91]. Despite this, since 2014 practically all studies on frog movement carried out in the Neotropical region have used individual patterns, with no further use of the toe-clipping technique. This result may be only an effect of the chosen species, with most of the recent studies done with species where individuals were discernible by individual color patterns.

We also identified a temporal trend in the tracking techniques used (Fig 8). From 1974 to 2005, most tracking studies were done through mark-recapture. While Tozetti et al. [34] used spools, most of the tracking studies continued to rely on mark-recapture. However, in 2014 Pasukonis et al. [82,83] started using harmonic direction finding and mark-recapture stopped being the most used method. It was only in 2018 that radio transmitters were used to study movement ecology of Neotropical amphibians [116], although it has been tested and used in studies with anurans already in the 80s, in Europe [117]. Despite many technological advances in the animal tracking area, such as the development of miniaturized tracking devices [40,118], most of the methods used in studies in the Neotropical region are still old and, generally, difficult to apply to small individuals.

Conclusions

The movement ecology study of the Neotropical anurans has been gaining more space, especially during the last 15 years, and shows a strong tendency to rise in the coming years. The construction of knowledge in a field that has not yet been studied can seem at first to be an enormous challenge, but it can represent new opportunities and hypotheses to be tested [119]. Research efforts for the future should focus on aspects that still represent gaps for the ecology movement of Neotropical anurans, such as studying movement in anthropized areas; being able to cover the landscape and different populations; increasing studies with arboreal species and juveniles; identifying differences in movement between males and females; studying dispersion movements and considering new technologies that allow to track small individuals. Often there is little information about a species movement, which is a start. Some information, such as home range size, can help decision makers decide on conservation measures.

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Supporting information

S1 Table. All data.

S2 Table. Studies carried out by ecoregion.

article	Studies by ecoregion	article	Studies by ecoregion
	Guianan Piedmont and Lowland Moist Forests		**Alto Paraná Atlantic forests**
[1]	Beck et al. 2017	[2]	Oliveira et al. 2016
[3]	Luger et al. 2009	[4]	Santos & Grant 2011
[5]	Munteanu et al. 2016	[6]	Santos et al. 2010
[7]	Pašukonis et al. 2013		**Humid Pampas**
[8]	Pašukonis et al. 2014a	[9]	Cairo & Zalba 2007
[10]	Pašukonis et al. 2014b	[11]	Daneri et al. 2015
[12]	Pašukonis et al. 2016	[13]	Sotelo et al. 2015

[14]	Pašukonis et al. 2017		**Isthmian-Pacific moist forests**
[15]	Pašukonis et al. 2018	[16]	Navas 1996
[17]	Pašukonis et al. 2019	[18]	Pichler et al. 2017
[19]	Poelman & Dicke 2008	[20]	Wijngaarden & Gool 1994
[21]	Ringler et al. 2009		**Southwest Amazon moist forests**
[22]	Ringler et al. 2011	[23]	Neu et al. 2016
[24]	Ringler et al. 2013	[25]	Nothacker et al. 2018
	Isthmian-Atlantic moist forests	[26]	Waddell et al. 2016
[27]	Cove & Spínola 2013		**Costa Rican seasonal moist forests**
[28]	Donnelly 1989	[29]	Crump 1986
[30]	McVey et al. 1981	[31]	Haase & Pröhl 2002
[32]	Nowakowski et al. 2012		**Araucaria moist forest**
[33]	Pröhl & Berke 2001	[34]	Moser et al. 2019
[35]	Pröhl 1997		**Bahia coastal forests e Caatinga**
[36]	Robertson et al. 2008	[37]	Prates et al. 2013
[38]	Robinson et al. 2013		**Dry chaco**
	Cerrado	[39]	Sanabria et al. 2013
[40]	Anderson & Andrade 2017		**Iquitos várzea**
[41]	Borges et al. 2018	[42]	Werner et al. 2011
[43]	Caldwell & Shepard 2007		**Northern Andean páramo**
[44]	Titon Jr. Et al. 2010	[16]	Navas 1996
[45]	Tozetti & Toledo 2005		**Northwest Andean montane forests**
	Puerto Rican moist forests	[46]	Hutter et al. 2016
[47]	Ten Eyck 2005		**Serra do Mar coastal forests**
[48]	Gonser & Woolbright 1995	[49]	Narvaes & Rodrigues 2005
[50]	Rogowitz et al. 1999		**Talamancan montane forests**
[51]	Woolbright 1985	[52]	Lindquist et al. 2007

	Trinidad and Tobago moist forest		**Uatumã-Trombetas moist forests**
[53]	Downie et al. 2005	[54]	Kaefer et al. 2012
[55]	Gourevitch & Downie 2018	[56]	Neckel-Oliveira & Gascon 2006
[57]	Royan et al. 2010		**Uruguayan savana**
[58]	Smith et al. 2006	[59]	Henrique & Grant 2019
	Ucayali moist forests	[60]	Pereira & Maneyro 2016
[61]	Brown et al. 2009		**Valdivian temperate forests**
[17]	Pašukonis et al. 2019	[62]	Valenzuela-Sánchez et al. 2014
[63]	Roithmair 1992		**Venezuelan Andes montane forests**
[64]	Roithmair 1994	[65]	Dole & Durant 1974
			NA
		[66]	Daneri et al. 2011
		[67]	Liu et al. 2019

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Capítulo 2

Fluorescent powder combined with mineral oil: testing a technique to track small tree frogs (*Dendropsophus elegans*) in translocation experiments

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Fluorescent powder combined with mineral oil: testing a technique to track small tree frogs (*Dendropsophus elegans*) in translocation experiments

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Abstract

In the study of movement ecology, different tracking techniques allow obtaining different pieces of information. Fluorescent powder has been used in different taxa and, like many techniques, it has its positive and negative points. In assessing animal movement, it is important to consider the movement motivation standardization and translocation experiments can be useful for this purpose. In this work, the objective was to test the effectiveness of the fluorescent powder and the combination with mineral oil, comparing two colors. The experiments were carried out in two ponds located in pastures with the species *Dendropsophus elegans*. Male individuals in calling activity were captured and moved a standardized distance, received the treatments, and were released shortly thereafter. The tracks were followed with the support of a UV light lantern. 86 individuals were monitored, 38 with yellow and 48 with orange colors.

A Wilcoxon-Mann-Whitney nonparametric test was performed to assess whether there is a significant difference between the distance marked by the powder and the powder + oil combination and between the two colors used. After translocation individuals did not return immediately to the pond. The yellow color leaves a longer trail length, is more visible under UV light, and has longer durability in the rain than the orange color. The fluorescent powder or combination with mineral oil lasts in the environment but does not last for long on the individual's body. This technique has already been tested in other amphibians, but this is the first study to use fluorescent powder to analyze tree frog movement.

Key-words: Atlantic Forest, tree frog, durability in the rain

Introduction

Movement ecology, which analyzes the displacement of individuals in space at a given time, deals with processes that determine the individual's destiny, connect populations, communities and regulate an entire ecosystem (Nathan *et al.* 2008). Different tracking techniques have been developed and used in the study of animal movement (Kays *et al.* 2015), through direct observations (Sotelo *et al.* 2015), capture and recapture (Pérez-Lustre and Santos-Moreno 2010), use of GPS (Burdett *et al.* 2007), spool-and-line device (Tozetti and Toledo 2005), harmonic direction finding (O'Neal *et al.* 2004) and fluorescent powder (Furman *et al.* 2011). The use of these techniques has allowed obtaining information about the individual's movement such as habitat use, home range size, locomotor performance, homing, navigation capacity, habitat fidelity, dispersion capacity, territory size, and movement patterns.

The fluorescent powder tracking technique has been used for a long time and in different animal groups, such as small mammals (Lemen and Freeman 1985; The *et al.* 2011), reptiles (Stark and Fox 2000; Furman *et al.* 2011), and amphibians (Rittenhouse *et al.* 2006; Ramírez *et al.* 2017). The advantages of using the fluorescent powder are the low cost, the short time needed to handle the animal for the product application, the low interference of the technique in the individual's behavior, besides allowing detailed information on the path taken. The disadvantage is the durability of the fluorescent powder on the animal's body and in the environment, which decreases with increasing humidity (Eggert 2002; Graeter and Rothermel 2007). The fluorescent powder mixed with mineral oil can be a way to solve this problem (Eggert 2002; Williams *et al.* 2014).

An important aspect to be considered in the movement analysis is the movement motivation standardization between individuals, which facilitates comparison, despite being a great challenge (Bélisle 2005). One of the techniques used to achieve standardization is translocation experiments (Betts *et al.* 2015), in which individuals are away from the capture place, with monitored paths. When individuals are captured and translocated in the reproductive period, it is expected that individuals will behave in a homing manner (Betts *et al.* 2015), as they have a motivation to return to their initial location (Bélisle *et al.* 2001). Translocation experiments have been widely used in studies with several taxa (Betts *et al.* 2015) and their relevance has been recognized for the study of movement related to the landscape structure (Mazerolle and Desrochers 2005; Betts *et al.* 2015). That is, they can help to understand how biotic and abiotic characteristics influence movement behavior.

Few studies with amphibians used the mixture of fluorescent powder with mineral oil to mark individuals (Eggert 2002; Williams *et al.* 2014; Okamiya and Kusano 2018). Of these, only William et al. 2014 tested the effectiveness of mixture use but using carrots to mark the trails, which can change the way the product is left in the environment, with species moving in different ways on the substrate. Given the personal observation made by Eggert 2002, on the effect of using mineral oil associated with fluorescent powder to increase the durability of the trail under dry and wind conditions, our objective was: 1) to test the influence of the mineral oil and color addition on the marking of trajectories, measured by the marked distance using a species of neotropical anuran, *Dendropsophus elegans* in the reproductive period.

Material and methods

Study sites

The experiments were conducted in the municipality of Ilhéus, Bahia, Brazil, in two pasture areas (Fig 1). The first is located in the Sambaituba district (Location 1: -14.667553, -39.082881 decimal degrees), and the second on the Jorge Amado highway (Location 2: -14.804235, -39.140527 decimal degrees). These were chosen because 1) they have permanent ponds (cattle tank), which provide a good source of reproductive area for frogs and usually harbor many individuals of common species, such as *Dendropsophus elegans*, and 2) they have pasture around the pond, standardizing the vegetation type in open areas and exposure to climatic conditions.

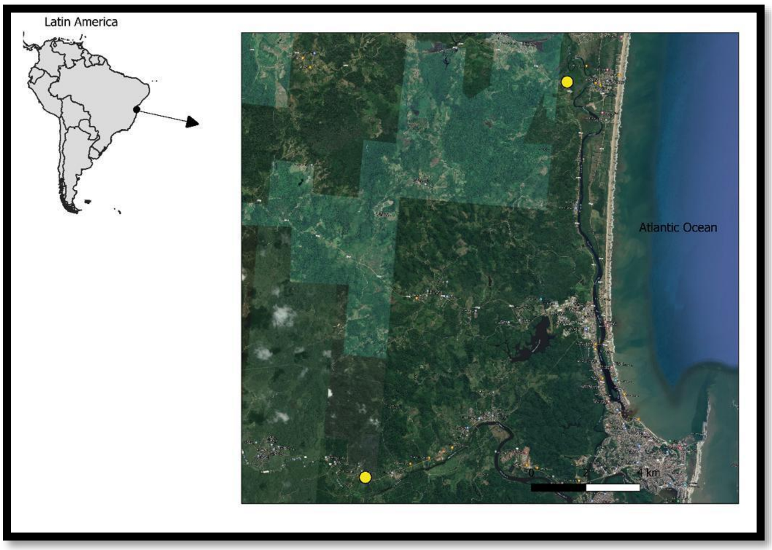

Figure 1. Studied locations in map. The point above right represents location 1, in Sambaituba. The dot below on the left represents location 2, on the Jorge Amado highway, in Ilhéus, Bahia, Brazil.

Studied species

Dendropsophus elegans (Wied-Neuwied, 1824), is a species classified as of little concern (LC) by IUCN, with arboreal habit and small size (Haddad *et al.* 2013) with records of the reproductive period from December to February (Abrunhosa *et al.* 2006) in its southern distribution area. It is characterized by having a yellowish-white frame around a dark brown rectangle, which may vary along with its distribution, which ranges from the state of Rio Grande do Norte to the state of Santa Catarina, occurring in areas of the Atlantic Forest and transitions with Caatinga and Cerrado (IUCN 2021).

Experimental design

Male individuals, calling in the pond (Fig 2), were captured and stored in clean plastic bags, without water and vegetation, since they would be released shortly thereafter, thus preventing anything from adhering to the animal's body and thus hampering the application of the fluorescent powder. After being placed in the bags, they were moved at a standardized distance away from the water body, received the application of the fluorescent powder or the combination of the fluorescent powder with mineral oil, and were released shortly thereafter. The fluorescent powder and the powder + oil combination was applied to a part of the belly and the legs with the help of a small soft bristle brush. The proportion of dust and oil was 2: 1, as suggested by William et al. (2014). We used the fluorescent powder (ECO-Series, Dayglo Color Corp, Cleveland, USA DayGlo, free of formaldehyde) in yellow (ECO17 Saturn Yellow) and orange (ECO15 Blaze Orange) colors. The orange color was only tested in the second location. We followed the fluorescent trails left by the individuals the next night using ultraviolet light (Higher UV Radiant Intensity 100UV LED) and measured the displaced distance along the trail with the support of a tape measure.

The trails were monitored daily for two weeks to test the durability of the trail, given the weather conditions at the time, being done only at location 2.

Figure 2. ***Dendropsophus elegans*** **male individuals, calling in the pond, in location 2.**

The first experiment was carried out on September 30, 2020, in location 1 during a non-rainy week, and between the 16th and the 30th in November, in location 2 during a rainy week. For location 1, eighteen individuals were collected, with nine receiving the powder treatment and nine receiving the powder + oil treatment, all in yellow. These were moved about 50 meters from the pond and released after applying the treatments. In location 2 the individuals were displaced and released about 20 meters around the pond. 20 individuals were marked with yellow and 48 individuals with orange treatments. In total, adding up the two locations, 86 individuals were monitored, of which 38 received the yellow treatment, 19 were marked with powder, and 19 with powder + oil. Forty-eight individuals received the orange treatment, 24 were marked with powder, and 24 with powder + oil.

License

The experiment was approved by the Ethics Committee of Animals Use at the State University of Santa Cruz (process n ° 010/20) and the animals were handled under the authorization of SISBIO-ICMBio (n ° 76241).

Statistical analysis

After verifying that the data did not meet the assumptions of normality, absence of outliers, and homogeneity of variances, we conducted a nonparametric Wilcoxon-Mann-Whitney test to assess whether there is a significant difference between the distance marked by the powder and the combination of powder + oil and between the two colors used (yellow and orange).

Results

Only two individuals returned to the pond after displacement. All the others climbed on nearby herbaceous vegetation, perched, and stayed there for a while, most of the time returning to calling activity, even far from the pond.

Evaluating the data in general, there was no difference between the distances marked by the trails between the treatments, powder and powder + oil ($W = 1074$, p-value = 0.198). Evaluating the colors (yellow and orange), there was a significant difference between the distances marked by the trails ($W = 455.5$, p-value <0.001). This color difference was observed both using the fluorescent powder ($W = 91.5$, p-value <0.001) and using the powder + oil combination ($W = 143$, p-value = 0.037). We could see that the distances marked by the yellow color corresponded to longer trails than those marked by the orange color. Evaluating only the yellow color, there was no difference in the distance marked using the powder or using the powder + oil combination ($W = 164$, p-value = 0.6404). Evaluating only the orange color, there was a difference in the marked distance between powder and powder + oil ($W = 409$, p-value = 0.01296) (see fig 3).

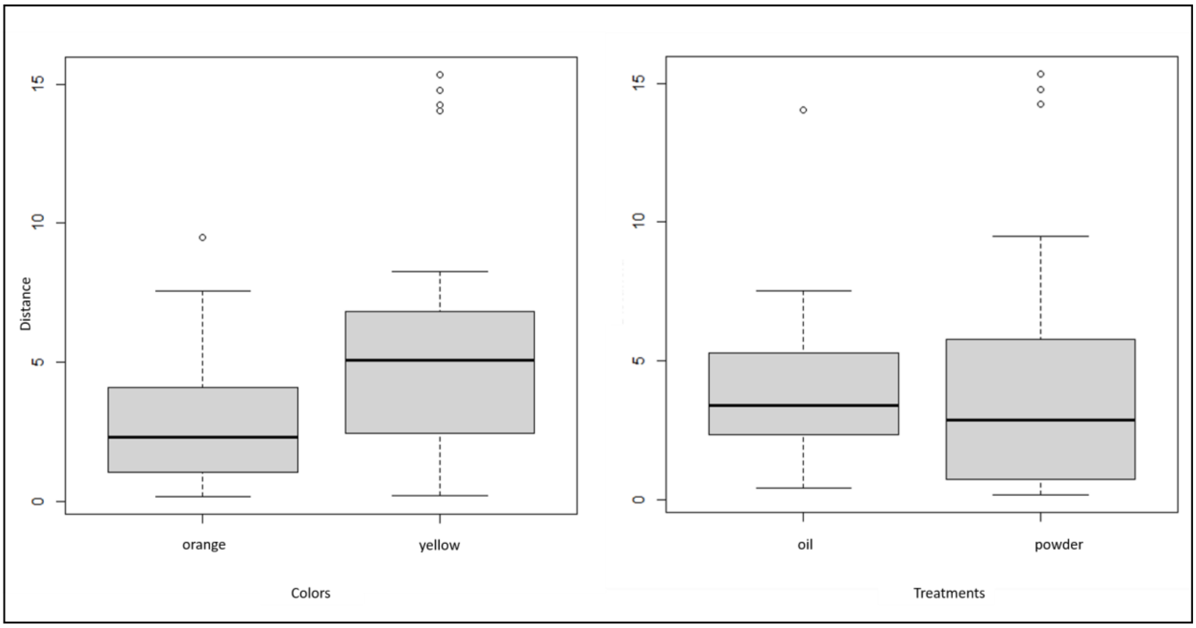

Figure 3. Interaction between colors (orange/yellow) and treatments (powder/powder+mineral oil), showing the minimum and maximum ranges with the median.

We noted differences during the fluorescent powder and the powder + oil combination application. The fluorescent powder is very fine and light and can spread easily in the environment, in the hands, clothes, and accessories. The use of oil makes the application easier, as the combination has greater adherence to the animal's body and does not spread with the action of wind or movements. Also, by personal observation, the yellow color seemed more visible to us under ultraviolet light compared to the orange color (Fig 4).

Figure 4. Visibility difference between yellow and orange colors. While the yellow color is noticeable little orange color appears, being more visible in the branch initial part.

The experiment in location 2 was carried out during an intense rain period, with a daily volume greater than 60mm. The yellow trails remained unchanged for a long time, starting to fade only after two rainy weeks. The orange trails started to fade after a week. Thus, the yellow color has seemed to have greater detectability in the environment than the orange color, even in heavy rains.

Discussion

Our experiment demonstrated that the translocated *Dendropsophus elegans* males did not return to the pond. We also observed that there were differences between the colors, about the distance of the marked trail and a personal observation about the durability in the rain. The results indicate that the fluorescent powder tracking technique is useful to provide information about short paths in time and space, generally marking short initial paths after release. Direction and distance information offset on this scale can be used in analyzes of perceptual ability, homing behavior and short daily movements

Only one study used the translocation method to assess the movement of anurans (*Lithobates clamitans*) using the fluorescent powder and found that the individual's movement was not directed to the original pond (Birchfield and Deters 2005). In most studies done with the fluorescent powder, individuals were captured leaving the water body (Eggert *et al.* 1999; Eggert 2002; Graeter and Rothermel 2007; Graeter *et al.* 2008) or were captured outside the reproductive period (Ramírez *et al.* 2017; Okamiya and Kusano 2018). In the present study, individuals were captured in the pond during the calling activity period. This method was used thinking about the incentive that individuals would have to return to the place where they were captured to reproduce, but they behaved in a different way than expected.

As most individuals perched and restarted calling activity soon after being released, most of the powder or powder + oil remained in the initial perimeter, which shortened the detectable track size. In cases where the tracks were longer, the individuals probably started walking shortly after having been released, instead of perching in the nearby herbaceous vegetation. Considering that the trails varied in length, measuring from a few centimeters to about 15 meters, and because we did not find any individuals after following the trails, we found that both the fluorescent powder and the combination of powder + mineral oil lasts in the environment but doesn't last long on the individual's body to the point of continuing to mark the trail, as also found by Birchfield and Deters 2005.

Using different colors can be useful to avoid confusion between trails marked by individuals when paths are close and cross (Stark and Fox 2000). However, some colors can be more difficult to detect than others (Birchfield and Deters 2005) and can mark different track sizes (Graeter and Rothermel 2007). Interestingly, in the work carried out by Eggert *et al.* 1999, it was observed that the orange color was more visible under ultraviolet light than the yellow color, the opposite of what was observed during our experiments. Graeter and Rothermel 2007 compared different colors of fluorescent powder and concluded that the color chartreuse, which is very similar to saturn yellow, is the best option to use alone, in situations where the trails do not cross, and which has a greater detectability in the trails length about the colors green, orange, and pink. The use of yellow versus orange, indicated that some colors (such as yellow) are more efficient in marking trajectories than others (such as orange), and should be standardized to a single color when the study depends on an analysis using distance covered, but this brings disadvantage because it limits the trajectory individualization. If more than one color is used, this should be considered as a source of variation in the data. The use of oil can improve the colors marking that tend to be less efficient (in the case of orange), although it does not match its efficiency in relation to the color identified here as more efficient (in the case yellow with and without the use of oil).

In addition to the detectability differences and the trail's size about the yellow and orange colors, we also found differences in terms of durability in the presence of heavy rains. Eggert 2002; Graeter and Rothermel 2007; Ramírez *et al.* 2017 found the durability of the trails decrease in the face of light rain (<10mm), and this method is often considered to be of little use in wet areas (Graeter and Rothermel 2007), as is the case of the Atlantic Forest. However, we were able to observe the trails in yellow for two weeks and orange for one week, even during heavy rains (> 60mm). It may not be ideal to run experiments during rainy days or nights, as it can influence the permanence of the powder on the animal's body, however, after the trail has been marked, it persists, even under conditions of high humidity. The yellow color was easier to be visualized and had greater durability in the field under the conditions of the study (personal observation) and could bring extra advantages. The better detection of the yellow color may have influenced the greater efficiency of marking the trajectory by this color.

This study is the first to use fluorescent powder to analyze tree frog movement. Gourevitch and Roger Downie 2018 tried to make use of it in a species of *Phyllomedusa*, but the individuals became lethargic and did not move. It is still unclear why, but it probably occurred because of the species habit of spreading serous secretion over the body using the limbs, which may have waterproofed the entire surface of the body. In a preliminary trial using the fluorescent powder, we observed individuals of *Dendropsophus elegans* in a terrarium and they did not exhibit unusual conditions or behaviors. The other studies that used fluorescent powder in anurans were carried out with bufonids (Eggert *et al.* 1999; Eggert 2002; Graeter and Rothermel 2007; Graeter *et al.* 2008; Okamiya and Kusano 2018) or ranids (Birchfield and Deters 2005; Rittenhouse *et al.* 2006; Graeter and Rothermel 2007; Graeter *et al.* 2008).

Our study brings new information about the fluorescent powder use associated with mineral oil. *Dendropsophus elegans* didn't respond in an expected way to translocation during calling activity, which may or may not occur for other species, since behavior may vary. Mineral oil can be useful to improve the effectiveness of the fluorescent powder use, depending on the used color, in addition to facilitating the product application. Despite research in temperate zones pointing out the method ineffectiveness in very humid environments (Eggert 2002; Graeter and Rothermel 2007; Ramírez *et al.* 2017), our results show that it is quite possible to use this method, even in high humidity environments, like the Atlantic Forest.

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Capítulo 3

Movement patterns of *Rhinella hoogmoedi* juveniles in a forest area of Northeastern Brazil

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Foto: Gabriela Alves

Movement patterns of *Rhinella hoogmoedi* juveniles in a forest area of Northeastern Brazil

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Abstract

Amphibians are organisms that move at different scales, make daily movements on a local scale, and can also move outside the area of the original population, in dispersion events. The individual home range begins to be formed in the juvenile phase of species, as soon as they find an adequate habitat. They move only when necessary since it is an activity that involves many costs. Assessing the frog's movement outside the reproductive period is a challenge, as is monitoring the daily movement of small individuals with appropriate methods. In this study, our aim is to analyze the daily movement patterns of *Rhinella hoogmoedi* juveniles, using a mixture of fluorescent powder with mineral oil. The trails of 57 individuals were monitored. The daily movement is characterized by small steps and many turning angles. The median area sizes of the daily movement range were small, less than $1m^2$. We found no association between body size and distance moved, perhaps because individuals were still developing and establishing a home range. The trails measured considering only the start and endpoints were smaller than the measures considering the entire trajectory, which may corroborate the group's reputation for a sedentary lifestyle. The movement pattern of *R. hoogmoedi* juveniles was characterized as a foraging pattern, with short and sinuous movements. This study provides information about the movement of a little-known life stage for anurans. We consider the use of fluorescent powder to be a good alternative for microhabitat studies of small individuals and it has the potential to be used in future studies that may add detailed information about short movements and may be useful to inform assessments of the threat status of the species.

Key-words: microhabitat, daily movement, step-length, turning angles.

Introduction

Amphibians, in general, move at different scales, from micro to macro (Dingle and Drake 2007; Jeltsch *et al.* 2013; Sinsch 2014), and the study of the ecology and behavior of these organisms can become a challenge, since a single step taken by a human being can cover the entire individual´s home range, with amphibians being the group known to be the most sedentary among terrestrial vertebrates, despite the existence of more active species (Wells 2007). Some individuals also move on larger scales, when dispersing, moving out of the original population (Wells 2007; Pittman *et al.* 2014).

Individual's daily movements generally occur on a microhabitat scale, characterized by short movements to acquire local resources, such as food, escape from predators, and thermal regulation (Fahrig 2007; Sinsch 2014). The home range that an individual occupies is where he performs his normal activities in a given period (Burt 1943), and for amphibians in general, the home range begins to be determined even during the juvenile phase, after the individuals leave the reproductive site, e. g. a puddle and settle in a suitable habitat (Pittman *et al.* 2014). Many remain close to the breeding site while others will be able to disperse and colonize other breeding sites, outside the original population (Pittman *et al.* 2014; Sinsch 2014). Within daily activities, foraging leads to characteristically more sinuous and repetitive movements, in a short time and space interval (Dingle and Drake 2007). However, amphibians move only when necessary, since movement leads to energy expenditure, exposure to predators, and the extreme climate (Fahrig 2007; Wells 2007).

Assessing the amphibian’s movement outside the reproductive period is already a challenge, as the reproductive period is the most conspicuous moment for the group (Wells 2007). Another challenge is to find suitable methods for monitoring small species and assessing daily movements. The most common methods are capture and recapture, with marked individuals (Pröhl and Berke 2001; Nowakowski *et al.* 2012), identified by body patterns

(Poelman and Dicke 2008; Munteanu *et al.* 2016; Pereira and Maneyro 2016), monitored by radio (Henrique and Grant 2019; Pašukonis *et al.* 2019) or harmonic direction finder (Beck *et al.* 2017; Pasukonis *et al.* 2017). The problem with these methods for monitoring daily data is that they report only the start and endpoints over time, measuring the straight-line distance. Other methods, such as thread bobbins (Tozetti and Toledo 2005; Oliveira *et al.* 2016) and the use of fluorescent powder (Rittenhouse *et al.* 2006; Graeter and Rothermel 2007) trace the entire path taken by the individual, making it possible to measure the distance moved, with the difference that the thread bobbins can't be used on very small individuals.

This study aimed to analyze the daily movement patterns of *Rhinella hoogmoedi* juveniles, using a mixture of fluorescent powder and mineral oil. For that, we determine the movement parameters for the species in the juvenile phase: total distance; linear distance; and the daily movement range. We also investigated the individuals' body size influence on the total distance moved and the movement ranges. We hypothesize that individuals with larger body sizes travel greater distances and, consequently, have a greater daily movement range, since bufonids seem to move greater distances as they increase body size as adults (Lemckert 2004).

Material and methods

Species and study area

Rhinella hoogmoedi Caramaschi and Pombal, 2006 is an endemic species in Brazil, distributed in the east of the Atlantic Forest, from Ceará to Paraná. It shows terrestrial habits and inhabits the interior of forests (IUCN 2021). Individuals can be found active or resting under bushes at night, but records of greatest activity have been made during the day. Other species of the same complex (*Rhinella margaritifera*), also exhibit this pattern (Brito *et al.* 2013). Male individuals are medium size, with snout-vent length (SVL) 39.4 to 52.1 mm (Caramaschi and Pombal 2006). We chose this species in the juvenile phase because it was abundant in the study area and period. The study was conducted from March 9 to 11, 2021, in a forest area under restoration, which is located on the campus of the State University of Santa Cruz (UESC), located in the south of Bahia, Brazil (14 ° 47 '37.66 "S, 39 ° 10 '17.46 "O) (Fig 1).

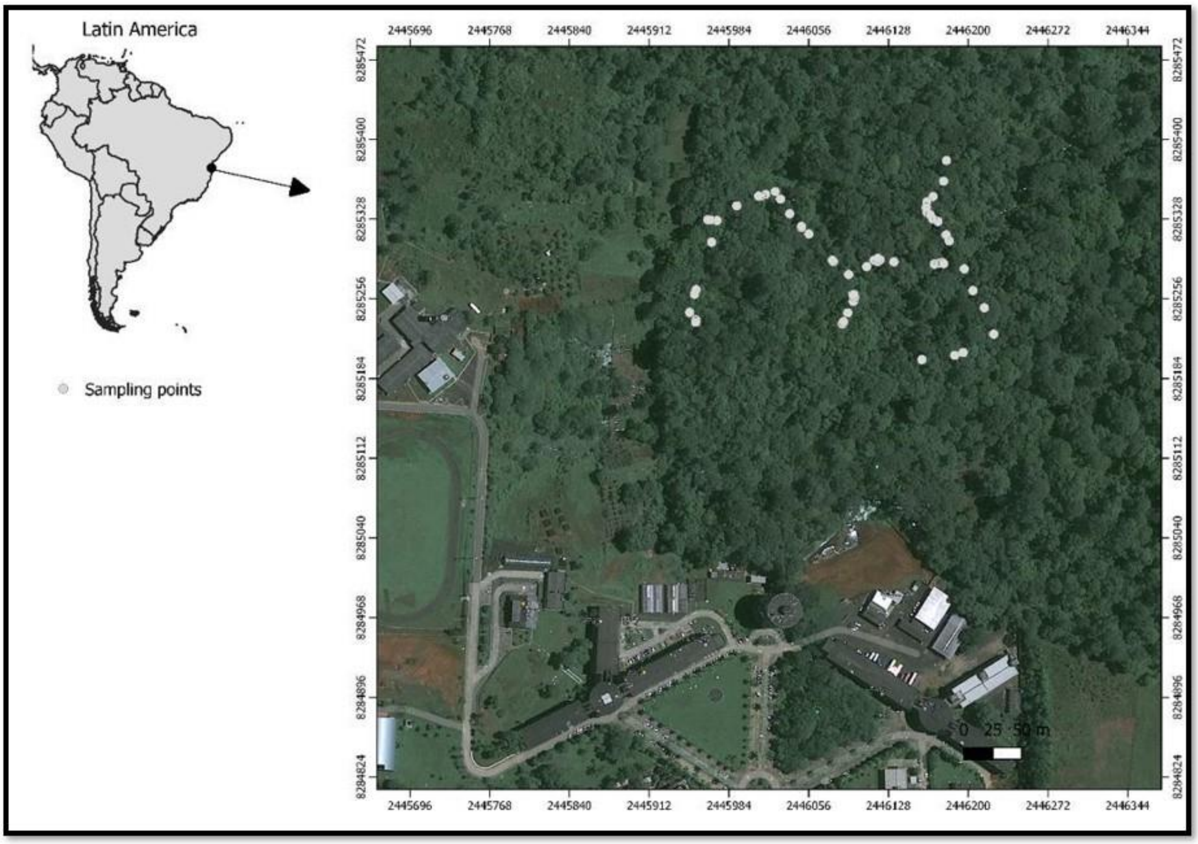

Figure 1. Study area. Campus of the State University of Santa Cruz (UESC), located in the south of Bahia, Brazil. Dots are locations where each individual (57) was captured and subsequently released.

Field tracking

We found individuals through active searches (Heyer *et al.* 1994) carried out during the day. We marked 57 individuals in total, all were found active on the leaf litter. Searches started around 9:00 am. Still during the morning period, when an individual was found, his SVL was measured with a digital caliper with accuracy ± 0.02mm / 0.0008”. Then, the legs and part of the belly were painted with a combination of fluorescent powder (ECO-Series, Dayglo Color Corp, Cleveland, USA DayGlo, free of formaldehyde) and mineral oil (Eggert *et al.* 1999; Eggert 2002; Williams *et al.* 2014), with the help of a brush with soft bristles (Fig. 2) and the toads were released, shortly thereafter, at the same location in which they were found. We used two colors of fluorescent powder, yellow (ECO17-Saturn Yellow) and red (ECO14-Fire Orange), to avoid confusion in the identification between individuals' trails that crossed paths (Fig. 3). We tied a piece of string with identification to make it easier to find the starting point and recorded the coordinates with a Garmin Etrex 10x GPS.

Figure 2. Powder and mineral oil mixture application in *R. hoogmoedi* individuals. UESC, Ilhéus, Bahia, Brazil.

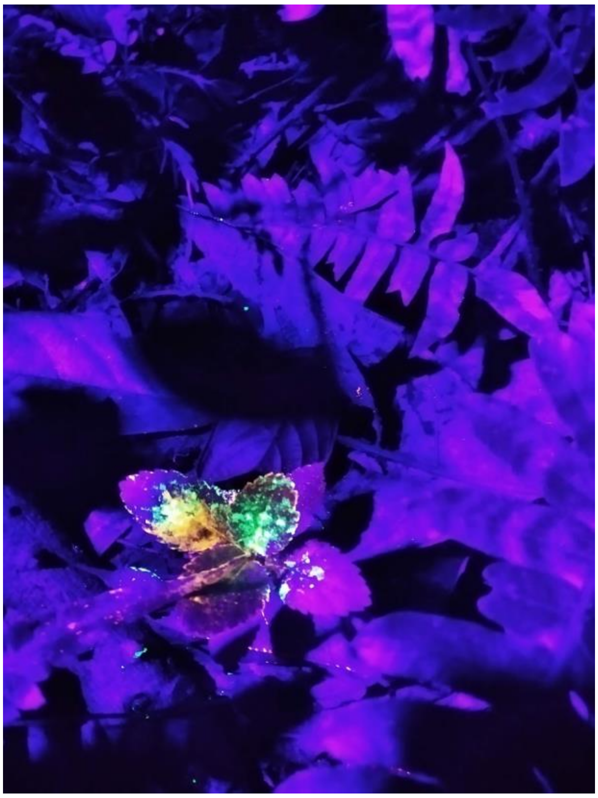

Figure 3. Crossed paths from two *R. hoogmoed* individuals that used the same space. UESC, Ilhéus, Bahia, Brazil.

Trails were followed from the starting point of release to where they ended or to where the individuals were, when found again (Graeter and Rothermel 2007; Graeter *et al.* 2008; Roe and Grayson 2008; Okamiya and Kusano 2018). We monitored trails on the same day, during the night, after about 9 to 12 hours. We considered this time period as the daily movement because this species juveniles are usually active during the day and resting during the night. The trails were monitored with a UV flashlight (Higher UV Radiant Intensity 100UV LED). We measure the following parameters for each trail: the turning angle (a), which measures the angle between the previous and the current step; and the step length (b), which measures the distance between the turning points (Fig. 4). We considered angles greater than 15°. The distances between the turning points were measured with a tape measure and the turning angles were measured with a protractor. As the trails were already marked and the coordinates were not taken in a given time interval (Oliveira *et al.* 2016; Ramírez *et al.* 2017), the step length was not defined by time, but by the turning angles (Okamiya and Kusano 2018).

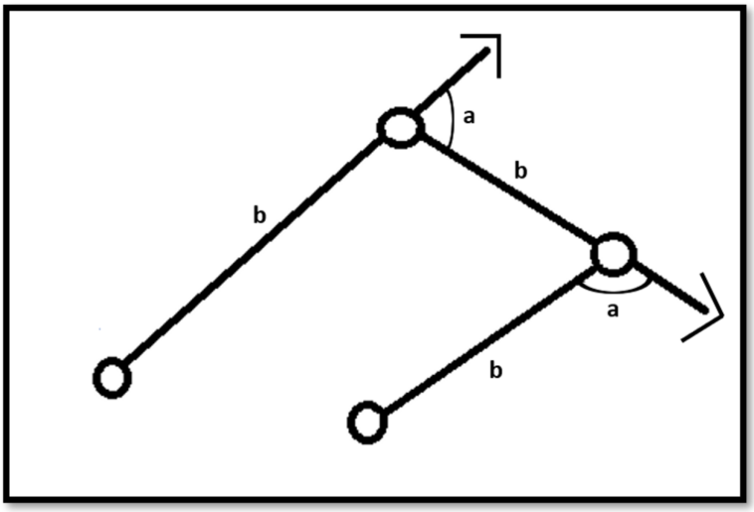

Figure 4. Movement parameters measured in the field. a) turning angles and b) stepsize.

To calculate the total distance moved by an individual we added all the step lengths for each trail and also measured the linear distance between the start and the endpoint (Oliveira *et al.* 2016). The initial angles were measured concerning the geographic north indicated in Google Earth, the initial angle was zero. The initial angle determination was limited by angle ranges of 45 °, the first being 0 ° to the north, 45 ° to the northeast, 90 ° to the east, and so on, up to 315 ° to the northwest.

Given the GPS inaccuracy, we calculate the coordinates of each turning point using the initial coordinate, distance from the step, initial direction towards north and turn angles. From the initial coordinate, in UTM, step distance, initial angle, and turning angles, we calculate the remaining coordinates of the individual tracks using a spreadsheet in Microsoft Excel. The latitude of the next point was calculated as follows: new latitude = latitude of the previous point + step size * cos (absolute angle); new longitude = longitude of the previous point + step size * sen (absolute angle). The absolute angle was calculated by adding the angle before the next angle. In this way, we could calculate the coordinates of the turning points and plot each track using a scatter plot.

Data Analysis

All analyzes were performed using software R version 4.0.4 (R Core Team. (2021). R: A language and environment for statistical computing). We calculated the distribution frequency of the following parameters: step size and turning angles. We used the “ggplot” function of the ggplot2 package (Wickham *et al.* 2020) to make a step size distribution histogram and to plot the circular distribution of turning angle frequencies.

To determine the daily movement range of individuals, we calculated the minimum convex polygon MCP (Mohr 1947; White and Garrot 1990). We calculated the MCP 95%, an estimate that excludes 5% of the points, usually excluding outliers; and we calculate the MCP 100%, considering all points. We calculate the daily movement range for each individual using the “mcp.area” function of the AdehabitatHR package (Calenge 2019). We used only the data from the trails that contained at least five coordinates, which is the minimum to estimate the MCP, evaluating a total of 22 individuals.

To analyze the individuals' size effect, measured by SVL, in the total daily distance covered and in the daily movement range, we used a linear model, considering the SVL as a predictor variable, and the distance covered and the daily movement range as response variables. The response variables and the SVL were logarithmized to be used in analyzes. We used the “lm” function and the packages lme4, Matrix, lmerTest, and ggplot2 (Bates *et al.* 2020; Kuznetsova *et al.* 2020; Wickham *et al.* 2020). To find out if there was a difference between the total distance moved and the measured linear distance, from the start point to the endpoint, we performed a non-parametric U test, by Mann Whitney (Zar 1996), using the function wilcox.test.

Results

We monitored the trails (S1 Figures) of 57 *Rhinella hoogmoedi* juveniles (mean SVL = 16.34, range = 8.77 - 25.42mm). Of the 57 marked individuals, 44 were found near or at the end of the trail, 7 were still active and 37 were resting on shrub leaves. During the fieldwork, we noticed that many individuals, already monitored in the past days, remained in the same area.

The step size frequency distribution can be seen in figure 5. The step size; effective moved distance, straight-line distance, and movement range statistics are summarized in Table 1. The individual's movement is characterized by small step sizes, the vast majority less than 0.5 meters, with few steps greater than one and a half meters and rare steps greater than three meters. The turning angles distribution can be seen in figure 6. Turning angles greater than 90 ° were frequent, both to the left and to the right. The daily movement range size was small, less than 1m², even considering the 100% MCP.

Table 1. Step size, total distance moved, linear distance, in meters, and movement range statistics, in square meters.

	min	**max**	**mean**	**median**	**SD**
Step size	0.11	12.4	0.932	0.58	1.005
Total distance	0.54	15.81	3.093	1.97	3.019
Linear distante	0.332	14.746	2.189	1.145	2.05
MCP 95%	0.103	1.469	0.513	0.248	0.432
MCP 100%	0.153	9.578	1.64	0.728	2.653

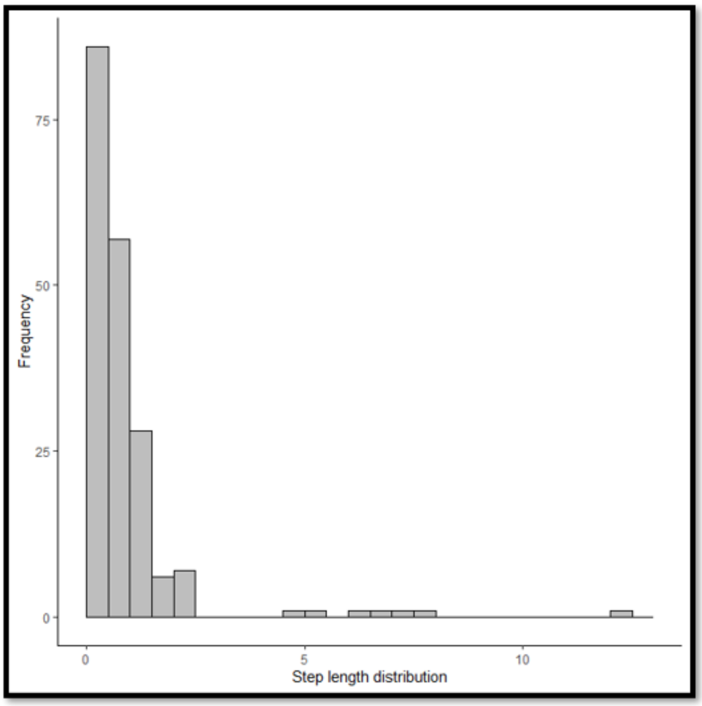

Figure 5. Step size frequency distribution in meters.

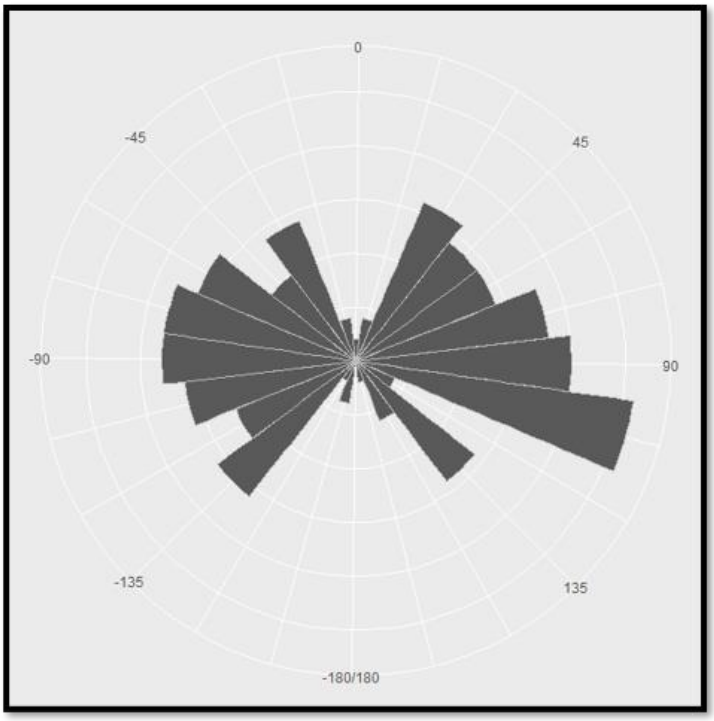

Figure 6. Turning angles distribution in degrees.

We did not find any association between the variables analyzed in the linear models, that is, the SVL did not influence the daily movement or the daily movement range. The median difference in the total distance moved values and the linear distance were different (W = 943, p-value = 0.0001137). Median total distance: 1.97m, median linear distance = 1.145m.

Discussion

Analyzing movement pattern aspects of *R. hoogmoedi* juveniles in a regenerating Atlantic Forest area, we found that most of the steps taken by the individuals were small and most of the turning angles greater than 90 °, showing that many individuals were moving little in a straight line and making many large turns, staying in the same area. The total distance covered and the daily movement's range was not related to the individuals' body size, probably due to the life stage when the individuals are still growing. The values of daily move ranges can change depending on how the displacement of individuals is measured.

In this study, the step size was defined by the distance between turning points. When individuals have the largest number of short steps, it means that the movement does not seem to have a linear pattern, which can be confirmed by the number of turning angles, most of which were greater than 90 °. In this way, the individual can explore the environment, foraging, and return to a place of refuge, remaining within the same area of habitual use, which would be the daily home range of a juvenile individual. But some individuals traveled relatively straight and longer paths, which indicates that they may still be establishing the home range. Another species, from the same family, *Bufo japonicus*, also had the daily movement pattern monitored, using the fluorescent powder technique in adults, during the non-reproductive season. The authors concluded that the individuals had a pattern with many short steps and few long ones, but more straight movements (Okamiya and Kusano 2018). Roe and Grayson 2008 also found a straighter movement in adults than in juveniles in the salamander *Notophthalmus viridescens*.

These results differ from what we found in our study, probably because of the difference in the assessed life stage. Amphibians go through different ecological needs throughout development until they become adults, so naturally, movement patterns also change (Pittman *et al.* 2014). While juvenile individuals devote their time and energy to foraging activity and may even have a more general feeding habit, because of their small body size. As an adult, *Rhinella hoogmoedi* has most of the ant-based diet (Brito *et al.* 2013), but juveniles may preferentially feed on mites until they reach an ideal size.

The daily movement values for *R. hoogmoedi* juveniles were small, as also reported for other species, including adults (Harris 1975; Roble 1979; Caldwell and Shepard 2007; Ramírez *et al.* 2017; Moser *et al.* 2019), which is expected for amphibians (Pittman *et al.* 2014). However, some species show greater daily movements (Tozetti and Toledo 2005; Okamiya and Kusano 2018). The relationship between distances moved and body size is not very clear for amphibians (Wells 2007). Some studies found a positive relationship between displaced distances and/or home range (Valenzuela-Sánchez *et al.* 2014; Okamiya and Kusano 2018), while others could not detect relationships between these variables (Lemckert 2004; Tozetti and Toledo 2005; Oliveira *et al.* 2016). Also, other factors affected the size of the area of use, such as the availability of food and shelters (Wells 2007). We found no relationship between body size and distances moved, perhaps because individuals were still developing and establishing a home range.

The total distance moved was greater than the linear distance values. This shows that the results can be influenced by how the researcher measures the distances moved (Tozetti and Toledo 2005; Oliveira *et al.* 2016). Some widely used techniques, such as capture and recapture marking; radio tracking, and harmonic direction finding, generally evaluate the movement based on the start point and the endpoint of the movement (Roble 1979; Lemckert and Slatyer 2002; Caldwell and Shepard 2007). In this way, the daily movement values can be underestimated, a factor that can corroborate the amphibian group's reputation for a sedentary lifestyle. The technique of tracking with fluorescent powder, as well as the use of thread bobbins, does not necessarily need monitoring based on time intervals, since it has all the detailed path taken by the individual, drawing on the substrate. In this way, it is possible to know the exact value and distances covered by the individual’s range (Graeter *et al.* 2008; Pittman *et al.* 2013; Ramírez *et al.* 2017; Okamiya and Kusano 2018).

Finding frogs outside the reproductive period can be challenging, as this is the most conspicuous period for individuals in the group (Wells 2007). As juveniles are more abundant and are easier to spot in a considerable individual’s number, it may be a good option to study a species movement. The non-reproductive areas are little known, as well as the anuran’s juvenile phase since most studies are done during or after reproduction (Lemckert 2004). Small individuals are difficult to monitor, but using some alternative methods (such as fluorescent powder) it is possible to accurately assess microhabitat movements, but not for long, unless individuals are rescheduled for a few more days (Roe and Grayson 2008). Our results suggest that the daily movement of amphibians may be underestimated, depending on the sampling method, and that the use of fluorescent powder is an efficient way to know more about the species’ daily movement. In this way, this work brings information about an anuran’s little known life stage, mainly about the movement. Detailed information about a species, at the individual level, provides data that can be used to inform status assessments of the species.

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Conclusão geral:

Ainda existem muitas lacunas no conhecimento sobre a ecologia do movimento de anfíbios anuros neotropicais. Faltam informações sobre diferentes espécies, estudos em áreas antropizadas, avaliações sobre a dispersão dos indivíduos, estudos de espécies com hábitos arborícolas, movimento de juvenis, além de estudos que consigam analisar o movimento de espécies em diferentes populações. Também concluímos que o uso do pó fluorescente, associado ou não com o óleo mineral, é uma técnica eficiente para medir o movimento diário de espécies ou indivíduos pequenos de regiões com alta umidade, como é o caso da Floresta Atlântica. Acreditamos que informações detalhadas sobre o uso da área de vida e com maiores períodos de monitoramento possam ser úteis para compor os dados de avaliações das espécies e auxiliar na conservação dos anfíbios.