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Ecologia espacial associada a personalidade em sapos

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Ilhéus, Bahia Junho de 2022





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Tese apresentada à Universidade Estadual de Santa Cruz, como parte das exigências para obter o título de doutor em Ecologia e Conservação da Biodiversidade.

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Le dedico esto Al universo Y a su infinito poder Y a los terraplanistas y su infinita ignorancia.

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RESUMO GERAL

Esta pesquisa estudo o a ausência de informação e a pouca padronização em dados existentes para pesquisas relacionadas com movimentação em anfíbios arredor do mundo, também foi estudada a personalidade dos sapos da espécie (Rhinella ornata) em áreas agrícolas, florestais e urbanas no município Cachoeiras de Macacu estado do Rio de Janeiro. O primeiro capítulo contém uma descrição das irregularidades nas pesquisas desenvolvidas na área da ecologia espacial em anfíbios, das espécies de anfíbios descritas no mundo 0.5 % possuem dados relacionados à área de vida ou movimento, essas espécies apresentara uma correlação negativa entre o tamanho do corpo (SVL) com e movimento médio diário e uma correlação negativa com a SVL e a área de ocorrência (especificamente para anura). Em caudados os dados foram muito escassos não permitindo realizar análises estadísticos. Nossa pesquisa destacou a necessidade de padronização dos métodos de análise e coleta de dados, uma vez que abordagens diferentes dificultavam ou até impossibilitavam a coleta de dados para todos os grupos. A falta de padronização da coleta de dados e da priorização de certas variáveis não nos permitiu encontrar padrões dentro do deslocamento diário e da área de vida dos anfíbios, e foram sugeridas certas coletas de dados para diminuir essa variação de metodologias. No capítulo 2 procuramos entender as diferenças comportamentais entre os habitantes de Rhinella ornata nos habitats urbanos, agrícolas e florestais. Estudando um grupo de indivíduos in-situ e ex-situ realizamos análises comportamentais para definir como mudam esses traços comportamentais ao longo dos habitats. Foram observadas mudanças comportamentais ao longo dos habitats e estas diferencas podem ter implicações na função trófica dos habitantes de cada tipo de ambiente. Com este estudo pretendemos preencher a lacuna na compreensão de como a história de vida a nível individual pode modificar os padrões de comportamento em diferentes tipos de habitats, e as consequências ecológicas e evolutivas a longo prazo.

Palavras chave: *Rhinella ornata*, Personality traits, Amphibians, Daily movements, Home range, Telemetry.

ABSTRACT

This research studies the lack of information and the little standardization in existing data for research related to the movement of amphibians around the world, it was also studied the personality of *Rhinella ornata* in agricultural, forest and urban areas in the municipality of Cachoeiras de Macacu, Rio de Janeiro State. The first chapter contains a description of the irregularities in the research developed in the area of spatial ecology in amphibians, of the amphibian species described in the world 0.5% have data related to home range or movement, these species had a negative correlation between body size (SVL) and average daily movement and a negative correlation with SVL and home range (specifically for anura). In caudates the data were too sparse to allow statistical analysis. Our research highlighted the need for standardization of data collection and analysis methods, as different approaches made data collection difficult or even impossible for all groups. The lack of standardization of data collection and prioritization of certain variables did not allow us to find patterns within the daily commuting and home range of amphibians, and certain data collections were suggested to decrease this variation in methodologies. In chapter 2 we sought to

understand the behavioral differences between *Rhinella ornata* inhabitants in urban, agricultural, and forest habitats. By studying a group of individuals in-situ and ex-situ we performed behavioral analyses to define how these behavioral traits change across habitats. Behavioral changes were observed across habitats and these differences may have implications for the trophic function of the inhabitants of each environment type. With this study we aim to fill the gap in understanding how individual-level life history can modify behavioral patterns in different habitat types, and the long-term ecological and evolutionary consequences.

Palavras chave: *Rhinella ornata*, Personality traits, Amphibians, Daily movements, Home range, Telemetry.

INTRODUÇÃO GERAL

Há amplas evidências demonstrando variações comportamentais interespecíficas, e durante décadas o objetivo de entender as características comportamentais tem sido uma prioridade para determinar o efeito de vários comportamentos individuais nas populações (Carter *et al.*, 2013; Garamszegi e Herczeg, 2012; Réale *et al.*, 2010). A variação comportamental entre os indivíduos sustentada ao longo do tempo é denominada "personalidade" (Carter *et al.*, 2013; Kelleher *et al.*, 2017). Somente nos últimos 10 anos de pesquisa na área de etologia foi possível desvendar causas relacionadas a variações de personalidade e síndromes comportamentais, especificamente no campo da ecologia comportamental houve uma explosão na pesquisa nesta linha, o que permitiu classificar vários traços de personalidade, quantificá-los e padronizar protocolos para coleta de informações relacionadas à personalidade animal (Bell *et al.*, 2009; Crews, 2013). Sih *et al.*, (2004; 2008) explicam a correlação entre síndromes comportamentais e teoria da personalidade animal, indicando que os vários traços comportamentais podem ter uma relação inata (comportamentos ligados a genes específicos) ligados diretamente à pressão ambiental e, por sua vez, tornando-se um traço hereditário (Sih *et al.*, 2015).

Desta forma, a personalidade e os síndromes comportamentais podem ter fortes efeitos sobre a aptidão física de indivíduos com plasticidade comportamental limitada e podem enfrentar situações que afetam sua sobrevivência e/ou desempenho reprodutivo por não apresentarem comportamentos que promovam uma resposta positiva em tais ambientes (Sih *et al.*, 2004, Sih *et al.*, 2003). Olhando-a de uma perspectiva evolutiva e ecológica, a personalidade pode ter um efeito dramático na aptidão individual e ter consequências que se fixam nas

populações e consequentemente modificam nichos ecológicos, hábitos alimentares e relações tróficas (Réale *et al.*, 2010; Sih e Del Giudice, 2012; Wolf e Weissing, 2012).

É por isso que a inclusão da personalidade animal na ecologia nos permite compreender como o comportamento pode fazer parte de síndromes adaptativas que nos permitem compreender algumas restrições e problemas ecológicos em diversas espécies (Bell *et al.*, 2009; Réale *et al.*, 2010; Sih and Giudice, 2012). Estudos de personalidade mostraram repetitividade em traços como agressão, ousadia, atividade e exploração (Carlson e Langkilde, 2013; Delnat *et al.*, 2017; Kelleher *et al.*, 2018), mas o poder de certos conceitos como síndromes comportamentais ou personalidade animal é melhor apreciado quando ligado a outros traços fenotípicos e seu valor adaptativo é reconhecido (Crews, 2013). Ultimamente, tem havido um grande interesse em relacionar estes traços de personalidade bem documentados à variação individual da cognição (Carere e Locurto, 2011; Niemelä & Dingemanse, 2014; A. Sih & Del Giudice, 2012).

Isso sugere que a personalidade e a cognição devem ser covariáveis no contexto "individual" (Guenther e Brust, 2017). As diferenças individuais no desempenho de aprendizagem e correlações fenotípicas com a personalidade estão bem estabelecidas em algumas espécies (Brust e Guenther, 2015, 2017). Guillette e Griffin, (2017) observam que alguns traços de personalidade podem afetar as taxas de exposição em situações naturais e experimentais, que ao não levar em conta possíveis riscos podem levar a um encontro com predadores, ou em outros casos, a exposição a alguns riscos, tais como a morte na estrada (Skelly 1992; Arbuckle 2015, Sillero *et al.*, 2019; Wang *et al.*, 2019).

A partir destas observações, é possível que animais mais exploradores ou ousados encontrem informações a serem aprendidas mais rapidamente em comparação com animais menos exploradores ou tímidos (Dugatkin & Alfieri, 2003; Sneddon, 2003). Neste contexto, a ecologia comportamental nos permite compreender em profundidade alguns detalhes específicos relacionados a como os indivíduos podem variar no comportamento, como os sexos podem se comportar e como as populações também podem variar (Start e De Lisle, 2018; Harrison *et al.*, 2022). Enquanto podemos medir a personalidade nos indivíduos, olhando para a tomada de decisões em uma escala relativamente pequena (arenas, atividade neural em resposta a estímulos etc.) (Kelleher *et al.*, 2018), quando vamos ao contexto mais elevado de como podemos ver a influência dos traços de personalidade na forma como um indivíduo se move em sua área de residência, precisamos recorrer à ecologia espacial. Este ramo da ecologia nos permite ver quanto e como as espécies se movem em habitats

ocupados. Neste contexto, as ferramentas existentes nos permitem ter uma sequência das diversas relocalizações dos animais dentro da área estudada. Nosso modelo biológico sempre foi idealmente de sapos do gênero *Rhinella*, pois além de ter uma estrutura corporal que facilita o estudo do movimento, também apresentam uma plasticidade no tipo de habitat que ocupam, podendo estar distribuídos de áreas com intervenção antrópica zero para áreas urbanas (DeVore *et al.*, 2021).

O gênero *Rhinella* tem sido amplamente estudado desde a ecologia comportamental até a ecologia espacial, por situações relacionadas à sua invasividade em algumas partes do mundo, mostrando variações de personalidade como esperado e ainda trazendo questões quanto ao tipo de estrutura ambiental onde os animais estudados estão vivendo (Gonzalez-Bernal *et al.*, 2014; Gruber *et al.*, 2017, Shine *et al.*, 2021; Shaykevich *et al.*, 2022). Portanto, criamos esta abordagem para estudar *R. ornata* no município de Cachoerias de Macacu, no estado do Rio de Janeiro. No contexto inicial, a metodologia ia nos permitir trabalhar mais tempo estudando indivíduos usando telemetria e ter padrões ao longo de vários dias, mas o lockdown e todas as situações relacionadas com a pandemia COVID- 19 impossibilitou a aquisição de dispositivos de telemetria VHF, e nos forçou a usar ferramentas mais simples como o carretel e o dispositivo de linha usando uma adaptação da metodologia criada por Mejía *et al.*, (2021). Estas variações de deslocamento baseadas na personalidade são a porta de entrada para entender como as espécies se dividem funcionalmente em personalidades e como o grau de intervenção do ecossistema pode modificar e assim modular o comportamento na escala individual.

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CAPITULO 1

GAPS IN AMPHIBIAN MOVEMENTS AND HOME RANGE STUDIES: THE NECESSITY TO UNDERSTAND THOSE PATTERNS.

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ABSTRACT

Spatial distribution within habitats is part of virtually absent knowledge related to the natural history and ecology of amphibians understanding patterns of movement and home range is crucial for conservation management in human-modified landscape. For 60 decades telemetry as tool allowed to elucidate and fill the gap that exists on this research area. Using Web of Science, we reviewed studies related with amphibians, telemetry, home range and movement. Of the 8090 species of amphibians there are 42 species with data related to area of life or movement, distributed in 18 types of habitats with emphasis on temperate forest and agricultural lands. Based on these results we identified for anurans Kernel density 95% (n: 5) = mean 106.85 m², SD= 268.76 m²; MCP95% (n: 19) mean 395.401.3 m² SD= 1991618 m²; daily mov (n: 19) = mean 33 m SD= 65.28 a relatively large area for small animal species, we observe a negative correlation between body size of anuran snout-vent length (SVL) with and average daily movement and a negative correlation with the SVL and the home range. Caudata had an MCP95% (n: 3) Mean 16770.5 m^2 SD = 26315.31 m^2 ; Daily mov (n: 3) = mean 31.62m. SD = 44.49. Our research highlighted the need for standardization of analysis and data collection methods, as different approaches made data collection difficult or even impossible for some groups. The lack of standardization of data collection and prioritization of certain variables did not allow us to find patterns within the displacement and home range of amphibians globally and understand also how human pressure such as deforestation and climate change impacts movement and home range.

Keywords:

Amphibians, Daily movements, Home range, Telemetry, informational gaps

INTRODUCTION

Amphibians are a diverse group of vertebrates, distributed in almost every ecosystem in the world (Duellman, 1999) and with very different biology which allows them to adapt to a large number of habitats and microhabitats (McDiarmid and Altig, 1999; Trimble and Van Aarde, 2014; Cortés-Gomez, et al., 2015; Villacampa, et al 2019). They are also a group threatened worldwide and their populations have been declining for the last decades. Among the causes are habitat loss and fragmentation, which affects amphibians just as it affects any other organisms: through population isolation, inbreeding and edge effects (Pounds et al., 2006). However, changing climate has been suggested as one of the most important drivers of global amphibian declines, either directly (via a direct response to changing abiotic conditions) or indirectly by modifying species interactions and on average, 3.4 percent of amphibian species are disappearing from local amphibian habitats each year (Miller et al., 2018). In a general way, population growth and environmental modifications (Carey and Alexander, 2003; Lips et al., 2008, Blaustein et al., 2010) have led to the decline of amphibian populations in a wide variety of environments. That is why during the last years, this group has been the centre of attention around the world in the context of conservation (Stuart et al., 2008; IUCN, 2013). Amphibians are therefore among the most endangered groups of animals in the world and represent 51% of the threatened species that inhabit the sites that host species in imminent danger of extinction (including mammals, birds, reptiles and conifers) (Ricketts et al., 2005).

The most frequently used method to study the ecology and habitat preferences of tropical species has been the collection of descriptive data from simple field surveys (Heyer *et al.*, 1994; Duellman 2005; McDiarmid *et al.*, 2012; Beirne *et al.*, 2013). However, while this method still provides important ecological knowledge, it is generally limited to providing single data points for individuals (Waddel *et al*, 2016). Research involving amphibian monitoring began decades ago (Madison and Chopp, 1970) and has become more frequent in recent years due to the application of diverse methods of animal tracking such as Spool and Line "Thread Bobbin" (Dole, 1965; Tozetti and Toledo, 2005; Silvester *et al.*, 2019), capture-recapture (Sutherland *et al.*, 2016), Gps (Kays *et al.*, 2015) and VHF radio transmitters (Van Nuland and Claus,1981; Blomquist and Hunter, 2007; Indermaur *et al.*, 2008; Madison *et al.*, 2010). Tracking methods can generate large amounts of detailed ecological data over several days (Heyer *et al.*, 1994) and can be used to understand home ranges, dispersal, activity patterns, habitat preferences, and microhabitat use. They also

support the research line related with how amphibians perform *in-situ* providing information about natural behavior (Indermaur *et al.*, 2008; Madison *et al.*, 2010).

Radio telemetry is the most used technique which allows free movement without the hindrance of the spool and line device (Van Nuland and Claus, 1981; Tozetti and Toledo, 2005; Blomquist and Hunter, 2007; Indermaur et al., 2008; Madison et al., 2010; Silvester et al., 2019). This is also a helpful tool when studying animals in their habitat, it helps to identify the position of individuals providing the possibility to find the animal easier than other techniques. Radio telemetry of amphibians has historically been limited by the size and mass of the transmitter. Whether implanted or attached on the surface of the animal, the concern has been that the subject's mobility would be influenced by the volume of the transmitter (Fitch 1987; Richards et al., 1994). But recent advances in radio telemetry technology, such as decreasing transmitter's size and increasing battery life, facilitate the tracking of smaller individuals (Berg et al., 2010). Furthermore, it seems that the fact that an animal is equipped with a transmitter does not alter the movement patterns revealed by the location of amphibians in the field. Observations support this idea: in all species studied, frogs with transmitters appeared to be uninhibited in their movement, were commonly observed in association with frogs without transmitters, and were frequently observed calling and in amplexus (Rowley and Alford, 2007).

Some of the studies that can be developed with radio telemetry are related with movement patterns and home range of individuals. These research line supports the understanding on how animals are moving through their habitat and how big is the territory needed by an individual/population to survive (Rechetelo *et al.*, 2018). As a tool, radio tracking allows us to understand and fill up the lack of basic ecological information which needs to be considered as one of the main factors hindering the planning of conservation actions for amphibians (Rowley and Alford, 2007). In this sense, data on movement patterns and home range can provide important information from individuals and extend them to a population scale (Lima and Zollner, 1996; Gibbs, 1998; Haddad, 1999).

Even with innovations on this technique there is still a large gap of information for some groups of animals, and mostly amphibians, since we still don't know for certain the general patterns of movements as well as the size of home range for all groups. For this reason, the aim of this study was to gather data related to telemetry in amphibians, in order to analyse the gaps of information concerning this area, especially regarding home range.

METHODS

We searched for peer-reviewed journal articles from March to July 2020, using the Institute for Scientific Information (ISI) Web of Science. We used the keywords "Home range + Movements + Amphibians + Frogs + Salamander + Newts + Telemetry + Radio Tracking" and we filtered 75 scientific articles from a total of 160. The articles needed to be related to telemetry as the method of data collection, and have information of daily movement, mean time of monitoring for each individual, home range using Kernel 95% and 50% and Minimum Convex Polygon (MCP) (Worton, 1987). We reviewed only articles published in peer-reviewed journals in English and yielded by our ISI search (above).

We calculated means of all values available within all studied species to understand the movement patterns and home range of each one of them. We also described which kind of procedure was used to attach the transmitter to the animal. We checked how many studies were made per country and per kind of habitat using a modification of habitat type based on Ramsar Convention Secretariat, (2016).

We made a linear regression between the snout-vent length and the home range and another one between the snout-vent length and daily movement for Anura and Urodela separately. All the statistical analyses were made using R software (R Core Team, 2019), all values were log-transformed. To describe the gaps on information we summarized the information provided in all articles and identified the patterns on the missing information.

RESULTS

We found 160 publications corresponding to the keywords that were used in Web of Science for. From these, 75 had specific data on telemetry and information of at least one variable of interest.

The countries that had the highest number of telemetry research projects are led by the United States with 48 studies, Canada with six and Spain with five (Fig.1). Most of the information generated in this type of research began in 1999 with a systematic increase in the number of investigations, with its highest point in 2017 with a total of 11 scientific articles followed by 2012 (eight).



Figure 1: Ranking of home range and movement studier per country

Of 42 studied species, 33 were Anurans (16 genera, seven families) and nine Caudata (five genera, four families). These species are distributed in 18 types of habitats (Ramsar Convention Secretariat, 2016) with most research done in temperate forests, agricultural crops, followed by alpine habitats and temperate grassland.

Habitats	N# studies	N# speci es
Grassland – Temperate	4	3
Alpine habitat	7	6
Cold Lava/ Early S. vegetation	1	1
Desert – Temperate	1	1
Plantations	9	6
Forest – Boreal	2	2
Forest - Subarctic	1	1
Forest – Subtropical/tropical moist lowland	3	4
Forest – Subtropical/tropical moist montane	1	1
Forest – Temperate	33	22
Sandhill habitat	1	1
Shrubland – Subtropical/tropical dry	1	1
Urban Areas	1	1
Timber harvesting	1	1
Wetlands (inland) – Permanent freshwater marshes/pools	1	1
Wetlands (inland) – Permanent rivers/streams/creeks (includes waterfalls)	4	2
Wetlands (inland) – Šhrub dominated wetlands	3	2
Wetlands (inland) – Seasonal/intermittent saline, brackish or alkaline lakes and flats	1	1

Table 1. Assessed habitat, with number of studies develop per each habitat type and species studied

The most studied species are *Anaxyrus boreas* (five studies), *Epidalea calamita, Bufo bufo, Lithobates pipiens* and *Lithobates capito* (four studies each).

The relationship between the snout vent length and the mean daily distance which the individuals moved gave a small negative correlation R^2 : 0,049; MSE: 9,35 (Fig.2.a). We

only had information of daily movement for 19 species and this result showed a mean of 33 m., SD=65,28 (Fig.2.a).



Figure 2: a) Correlation between average snout-vent length and daily movement Minimum Complex Polygon-MCP. b) Correlation between average snout-vent length and home range area using Minimum Complex Polygon-MCP.

With data from five species, we found a home range based on Kernel density 95% with a mean of 106,85 m², SD=268,76 m². For the data available of MCP we found 19 species with home range of 395401,3 m², SD= 1991618 m².

The linear regression of the mean body size of the individuals, present a negative correlation showing that smaller animals had a larger home range than larger species R^2 : 0.1852 (Fig.2.b).

Aspects related to Urodeles are very scarce and most species are analysed just a descriptive way, without any relevant relationship between the size or weight of these species and the space that they use or the distance they move. The studies which described home ranges give information by the MCP technique of three species, with a mean of 16770,5 M^2 SD=26315,31 M^2 . The mean of daily movement was also analysed for a total of three species with a mean of 31,62m. SD=44,49.



Figure 2: Proportional distribution of data regarding home range with Kernel density of 95%, Kernel 50%, MCP, Daily movement and total distance (P: present information /A: absent information).

When we analysed the number of articles containing data related to the variables of interest, we found that from the 75 publications, 11 included analysis using Kernel 95% (14%), three Kernel 50% (4%), 28 MCP (37.3 %), 34 daily movement data (46.3) and 45 total distance data (60%) (Fig.2).

DISCUSSION

There is not a homogeneous distribution in the amount of scientific information generated by country in the area of interest. The dominance of the extent of research made in the United States is explicitly related to the high investment in research made by this country (\$476,452.0M), and a large part of these funds is headed to the research made by universities (\$62,346.0M) (Unesco, 2019). This allows for a greater number of herpetologists to carry out their research in U.S. universities and therefore this could be the reason why we found that the species studied were mostly North American. Also, regions that lie exclusively in the temperate zone include continental U.S. and most of Canada and this corresponds to the zones where most research was conducted (Ramsar Convention Secretariat, 2016). For the

other countries that follow the US on the list (Canada and Spain) an obvious decrease in production is due to the amount of economic resources used by each one of them regarding research (\$27,793.5M and \$19,356.2M respectively) (Unesco, 2019).

On the other hand, in recent years, the number of companies involved in the manufacture of VHF devices around the world has increased. In general, "Holohil Systems Ltd" has become the most used brand in research studies concerning telemetry. They not only produce the smallest and lightest transmitters currently available for a variety of species, but also have an accessible price. This is important concerning their competitiveness because in some countries, like Brazil for example, the high value of import taxes added to the fluctuating rates in the increase of the currency makes the acquisition of these devices as well as radio receivers very difficult (DECRETO-LEI N° 2.472, DE 1° DE SETEMBRO DE 1988), and even if Brazil has an important number of herpetologists, research in the area of telemetry in amphibians is still unexplored. Also, the decrease of the transmitters size allows their use in most species, thus increasing the number of studies worldwide. This will contribute to answer more questions concerning the natural history and other important biological aspects of amphibians *in-situ*. Used appropriately, telemetry has the potential to provide information that is often unachievable using other techniques and can reduce uncertainty in the assignment of conservation status (Cooke, 2008).

IMPLICATIONS FOR CONSERVATION

At present, the IUCN assessed a total of 6771 species of amphibians, from this number 31,8 % of species are at risk (2157 sp) (IUCN, 2019). There are three species that were used in different studies (*Anaxyrus boreas, Epidalea calamita, Lithobates pipiens*) and that are categorized as Least Concern (LC) with decreasing populations by IUCN, and only one was assessed as LC and stable (*Bufo bufo*). All these species correspond to common Batrachofauna in their geographic range (Beja *et al.*, 2016; Hammerson *et al.*, 2004; Muths *et al.*, 2011), and this can be the main factor of why they are the most studied organisms. *Lithobates capito* is considered Near Threatened (NT), and even with a decreasing population it is possible that the facility to encounter this species in the field motivates researchers to choose this species as a biological model.

In spite of the scarce number of directed research for different species there are 11 that are the objective of study patterns within the home range and movement of endangered amphibians; four are categorized as Vulnerable (VU), three as Endangered (EN), three as Critically Endangered (CR) and one as Extinct in the Wild (EW) (*Anaxyrus baxteri*). This number represents only 0.5 percent of all species that are in actual risk (2157 sp.) (IUCN, 2019).

To categorize species, IUCN uses rational and defensible criteria to objectively assess endangerment status. At the present time with the generalized environmental crisis, a necessity in the taking of actions regarding conservation is needed in order to understand the requirements and biological aspects of species (Cooke, 2008). It is logical that the great number of species does not allow many aspects to be studied in their totality, but to understand the patterns required by these organisms is necessary and that is where telemetry plays an important role and will allow to answer many questions, and therefore could offer important information on the assessment of species and the placement in the correct endangerment category (Cooke, 2008).

Radio-telemetry is a helpful tool to understand the size of the conservation area of a species in order to maintain its population; it also provides an idea on the specific characteristics of the vegetation structure to support the biological requirements in order to maintain and preserve the species. Species with large home ranges normally have long distance movement, and for that reason it is important to understand the migration patterns in the planning of conservation strategies (Longepierre *et al.*, 2001; Himes, 2006; Cooke, 2008; Hamer *et al.*, 2008). Also, studies related to species movement can help conservation organizations and specialists' groups to understand how certain strategic decisions, such as focusing on the management of a threatened species and its habitat benefit the accomplishment of conservation goals in a more effective way. Further, to have an idea of the home range of a species has important implications for conservation not only of a species but for biodiversity in general.

In view of the above, studies on the behavior and spatial movement patterns of individuals are necessary for a thorough understanding of what is really happening to species in their home ranges, and if they are able to reach the habitats they need. For most amphibian species, movement patterns and habitat use are not very well known, especially for locations used away from breeding sites (Rowley and Alford, 2007). Along the years, the study of wildlife behavior has provided important information for wildlife management and conservation (Sillero-Zubiri *et al.*, 2004) but for many years, the only way to trace wildlife was simply to follow and observe the movement and habits of an animal. Radio telemetry

has improved the ability of ecologists to monitor wild animals (Mekonnen, 2015), increasing the chances of examining detailed ecological and management issues related to movement (Ramazin *et al.*, 2007), animal behavior (Lodé, 2011), habitat use and activity (Martin *et al.*, 2010). So the use of transmitters is a more precise methodology to understand the spatial ecology of species all along the habitats they use.

IMPLICATIONS FOR A BETTER ECOLOGICAL INSIGHT OF ANURA AND URODELA

If we analyze the average displacement per day for the 19 species (33 meters), there is an evident difference in daily displacement rates within amphibian species in all groups when they are in different habitats. The environmental structure may represent a barrier to locomotion and the differential use of diverse microenvironments may condition the individual to maintain or not patterns of displacement between microhabitats for refuge, hunting or reproduction, which may be fundamental to understand where, how and when these organisms have to move (Douglas and Monroe, 1981; Sinsch 1990; Rittenhouse and Semlitsch, 2006; Marsh et al., 2005; Sinsch, 2014). With other methodologies such as passive radars these species would be outside the range of perception of the antenna (Alford and Rowley, 2007; Pašukonis et al., 2014). All of this shows that at the time of studying these patterns in a more precise way and without restricting the animal's activity, radio telemetry would be a more effective detection technique. Some techniques such as monitoring by GPS transmitters would allow remote detection with programmed data collection, but the high cost of this equipment would limit large-scale development of research using GPS devices. Experts in the field of study still criticize the devices, mainly because of their size, which is a limitation for the study of smaller species.

When studying the average daily displacement distance of anurans in relation to their size we observed a small relationship with negative trend. This can be explained by the fact that smaller species correspond to species of higher standard metabolic rates which can consequently generate states of higher activity, more frequent search for prey and displacement at shorter intervals and greater distances (Davison, 1955; Seymour, 1973). The cost of energy expenditure is very dependent on body mass. A large animal expends less energy to move a unit of its mass than does a smaller animal (Bennett, 1978). For example, dendrobatid frogs show that high rates of metabolism lead frog move actively through the understory looking for invertebrates (Born *et al.*, 2010; Lima and Magnusson, 1998).

On the other hand, small displacements of large species may be related to low metabolic rates associated with large body size (Davison, 1955; Seymour, 1973; Bennett, 1978) and also to hunting strategies as "sit-and-wait" of Ranidae (Hayes and Tennant, 1985), Bufonidae (Strüssmann *et al.*, 1984; De Mattos *et al.*, 2013) and Leptodactylidae (Strüssmann *et al.*, 1984). In the case of Bufonidae, there are variations in hunting strategies depending on maturity, where juveniles are active hunters and adults show sit-and-wait strategies (Hinckley, 1963; Flowers and Graves, B. M. (1995); Evans and Lampo, 1996; Crnobrnja-Isailović, 2012).

In the case of urodeles, there are limited studies, which makes it impossible to use them for the analysis related to SVL and daily movement. However, we know that for *Ambystoma* there are observable patterns of migration where some individuals moved from 600 to 2200 meters in their reproductive period (Jenkins *et al.*, 2006; Bar-David *et al.*, 2007; Orloff, 2011). Studies showed that there is not an estimate of the average daily movement of individuals. When we multiply the number of days of the study by our results of general average displacement in salamanders, we can infer that it corresponds with the values between the generalized expected intervals (1896 m, in 60 days). As with anurans, we believe that there is a need for more studies in these groups in order to better understand these patterns as well as to answer more questions concerning not only movement ecology but also natural history.

There was a great variation in the number of species that were studied using both estimation methods. MCP analysis was the most used proxy for home range. Regarding the Kernel density analysis, the number of studied species was low and thus we were not able to make a non-descriptive analysis. In the MCP analysis there is a slightly negative correlation between the snout-vent length and the size of the home range, these trends as well as the daily movement could be related to the activity of the organisms (Davison, 1955; Seymour, 1973; Bennett, 1978).

It is possible that environmental heterogeneity is one of the causes influencing the heterogeneity of the size of the home range in organisms of similar sizes (Rabinowitz and Jr, 1986; Crawshaw, 1997; de Azevedo and Murray, 2007; Cavalcanti and Gese, 2009; Harmsen *et al.*, 2009). Anurans are highly dependable on environmental quality, and studies have demonstrated that in some areas abiotic factors (rainfall, temperature, and vegetation) have greater effects on the structure of anuran communities than biotic factors (competition and predation) (Parris 2004; Werner *et al.*, 2007). The first studies on the area of telemetry

are mainly descriptive and revealed that environmental heterogeneity is important for structure of anuran assemblages, however they did not test this hypothesis (Cardoso *et al.*, 1989; Pombal 1997; Arzabe *et al.*, 1998; Bernarder and Kokubum 1999). Today, with the development of statistical software, several studies have tested the prediction that environmental heterogeneity affects the structure of anuran assemblages. These studies also mention which environmental factors are most important for the assemblage (e.g., Parris and McCarthy 1999; Ricklefs and Lovette1999; Parris, 2004; Afonso and Eterovick 2007; Bastazini *et al.*, 2007; Keller *et al.*, 2009). Future studies with sympatric species of different sizes and similar biology will allow to determine the influence of environmental heterogeneity on their home range. There's not enough information from habitats that are not from temperate forests and there is also emptiness of data about home range of tropical species, and this needs to be filled.

There is very little research concerning home range and much less done with urodeles, thus limiting even more the scope of the estimates. Studies with telemetry were done for only three species and this is the only data available to analyze daily movement and home range in these species. This data shows that the average movement is similar to that observed in anurans. Some species of salamanders migrate to breeding sites and many times these migrations are done in territories that are cut by highways and urbanized areas. Some of these factors reduced up to 51% of the movement of individuals studied (Marsh *et al.*, 2005), and these patterns must possibly be repeated in multiple species. Scientific basis with movement patterns studies can provide necessary information that can be used to establish protected areas which include important resources for urodeles.

Used resources by salamanders (shelter, food and water) as well as their distribution is not homogeneous in the environment, and their availability determines the size of their home range (Jaeger, 1980; Collins, 1981; Holomuzki, 1986; Loredo *et al*,1996; Madison, 1997; Semlitsch, 1998; Rittenhouse and Semlitsch, 2007; Crawford and Semlitsch, 2008), in some cases needing to travel great distances to make the differential use of them (Mohr, 1944; Semlitsch, 1985; Phillips and Sexton, 1989; Homan *et al.*, 2003; Searcy *et al.*, 2013). The dispersal capacity looking for resources is small proportionally with the age, juvenile of some salamander species has low fidelity with home range and are also considerate as unsteady animals, looking for needed resources until they become adults (Jaeger *et al.*, 1995; Marsh *et al.*, 1995; Sinsch, 2014).

With the low standardization of the obtained data related to daily movement we observed the necessity to standardize the data collection related to amphibians' movement. Many of these studies could generally provide such information, but possibly by the nature of the objectives this information was not collected. Daily activity patterns along with descriptions of activity intervals as well as other factors such as directionality (Pašukonis *et al.*, 2014) and tortuosity would allow for a deeper understanding of the patterns of displacement and thus modelling of those patterns. In this way we could use them in conservation actions where species with long displacements are a priority (Whittington *et al.*, 2004; Pittman *et al* 2014; Sinsch, 2014; Cline and Hunter, 2016). For all these reasons, this study helps to understand the need to standardize the collection of home range and movement data in amphibians, since in this way these patterns can be understood.

CONCLUDING CONSIDERATIONS AND RECOMMENDATIONS

It is essential to understand the relationship between populations and their habitats to facilitate wildlife conservation. There is currently the possibility of estimating approximately the population size by knowing the area of distribution of the studied species along with the probable number of home ranges that fit within the area of occurrence. Unfortunately, we observed a lack of data for salamanders with few studies that make it impossible to analyze patterns. For anurans there were more studies, but these lacked a pattern in the collection of data and the types of analysis were different. In this work, we suggest the use of the home range estimators Kernel 95 and 50% together with MCP, as well as independently of the scientific questions to take data of daily movement and total movement during the period of the investigation. In some of the studies we revised it was not possible to make estimates of variables which normally are statistically solid. 85,33 % of the articles did not carry out Kernel 95% analysis, 96% Kernel 50%, 62,67% MCP. Mean estimates of daily displacement were exempted in 53.6% of the investigations and finally 40% of the articles did not consider including total movement data throughout the investigation. For all the above, we highly recommend to carry out research including species from Urodela which is a totally unknown group in terms of its spatial ecology and mobilization patterns. But above all to carry out standardized protocols for investigations of movement ecology in amphibians.

Minimum recommended information.

-Home range analyses with kernel should have 50% and 95%

-Average daily movement (if the study includes movements)

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CAPITULO 2

Tell me what neighbourhood you're from and I'll tell you how you act: Personality and movements of *Rhinella ornata* in forestal, agricultural and urban ecosystems

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ABSTRACT

Animal personality can have dramatic effects on individual fitness, and in turn great ecological consequences. As new scientific evidence on animal personality emerges, new questions about how ecosystems can modulate behavior appear. In this research we seek to understand the behavioral differences between *Rhinella ornata* inhabitants of urban, agricultural and forest habitats. For this we collected 70 individuals distributed among the different habitats and first studied the total daily movement and the size of the area used. Once the in-situ data was collected, we carried out personality analysis in an OFA (open field arena), to obtain the personality traits (boldness-shyness, exploration and coefficient of straightness). We didn't found statistical differences (p=0.0658) in the boldness presented by the toads for all areas, but the toads from forest areas were the boldest individuals leaving the refuge before 2983.3 sec sd= 7399.5, followed by the toads from forest areas 5417, 19 sec sd= 7399.59 and finally, the shyest toads were those from agricultural areas, taking an average of 8007.4 sec sd= 13705.95 to leave the shelter. We found statistically significant differences in the exploration levels presented by individuals in this study (p=0.0052) being the toads of urban areas the most exploring individuals. The exploration as well as the straightness coefficient served as predictive models to determine the size of the used area. Our results show notorious behavioral differences for toads distributed in anthropized and natural habitats. These differences may have implications for the trophic function of the inhabitants of each type of environment. With this study we hope to help fill the gap on understanding how life history at the individual level can modify behavioral patterns in different types of habitats, and the long-term ecological and evolutionary consequences.

Keywords:

Rhinella ornata, Personality traits, Behavior, Bufonidae, Boldness, Shyness, Exploration

INTRODUCTION

Amphibia is a scarcely studied vertebrate class within animal behavior, even though there is convincing evidence that amphibians show personality and behavioral syndromes (Kelleher *et al.*, 2018; Baxter-Gilbert *et al.*, 2021). It is also known that their personality can be just as essential from an ecological point of view as any other vertebrate group (Gruber *et al.*, 2017; de Azevedo and Young, 2021), with a strong influence by recognizing variations of these phenomena that can assist with animal conservation (Merrick & Koprovski, 2017).

According to Réale *et al.*, (2007), the major personality trait categories are activity, sociability, aggression, boldness/shyness and exploration. Amphibians display most of these behavioral axes (Brodin *et al.*, 2013; Carlson and Langkilde, 2013; Kelleher *et al.*, 2018) and therefore, they are an ideal model to further examine personality traits and see how they correlate to one another (Kelleher *et al.*, 2018;). In the last decade, research about amphibian personality has demonstrated that it can impact relevant behaviors. For example, it can influence dispersal (Brodin *et al.*, 2013; Gruber *et al.*, 2017), foraging and hunting sites selection (González-Bernal *et al.*, 2014), and even the possibility of getting a disease or and parasitosis (Koprivnikar *et al.*, 2012).

As one of the main personality characteristics, boldness can be defined as the tendency of the studied organisms to take risks in a new area or under unusual situations (Wilson *et al.*, 1994). According to Kelleher *et al.*, (2018), boldness is present in juvenile amphibians and remains to adult life stages as a consistent behavioral characteristic, which tends to be hormonally influenced as an innate or learned response (Koolhaas *et al.*, 1999; Wilczynski *et al.*, 2005; Haun, 2007; Van Oers *et al.*, 2011). Boldness levels in toads vary based on environmental characteristics, differing according to the type of habitat, its structure and its biota (Gruber *et al.*, 2017; Hegde *et al.*, 2019; Shine *et al.*, 2021; Zamora-Camacho, 2022). As well as other changes in personality the relationship of boldness to other behavioral changes is not necessarily going to happen in the same direction (Putman *et al.*, 2020) being present in an inversely proportional variation (e.g.: increase of exploration and decrease of boldness) for one habitat continuum or for different habitats (Baxter-Gilbert, *et al.*, 2021).

Another personality trait that has been widely studied is exploration, as this personality trait has been considered essential for dispersal and resource acquisition. It can be a decisive trait in the later stages of amphibians' life, as their fitness changes over time. Therefore, their movements between and within their habitats have bigger consequences. Larval amphibians are naturally highly inactive, but adult amphibians are wired for dispersal (Wilson and Krause 2012). Many amphibian species travel long distances (even tens of kilometres) to find food, shelter, or to breed (Wells, 2007). iIn a 2017 study the exploration behaviour in invasive cane toads was investigated and revealed that it significantly correlated with dispersal patterns. Furthermore, the results showed that exploratory toads are dispersing further than less exploratory toads and that this personality trait cannot be neglected anymore (Gruber *et al.*, 2017). Therefore, it is likely, that the exploration/avoidance axis can be a strong indicator of the movement patterns of amphibians, and even an determinant of home range areas size, both within and between habitats.

Amphibians' personality is influenced by species-specific, life stage, and ecological factors. Personality could be highly correlate with variables like food, for example, amphibians living in territories with fewer food sources might need to be more exploratory (Kelleher *et al.*, 2018). Moreover, some species need to travel long distances to find shelter or to breed (Wells, 2007). The life stage is also a determining factor in both personality traits, as larvae stage animals are more sedentary, while adult amphibians are geared for dispersal (Wilson and Krause, 2012b). Finally, the ecological factors such as the quality of water or land may also influence the activity and exploration traits; poor conditions can be a key driver for the animals' ventures and movements (Kelleher *et al.*, 2018).

THE CONNECTION BETWEEN PERSONALITY AND MOVEMENT

The differences in activity levels are likely to affect the animals' fitness, as they can affect the chances of acquiring food. The study by Wells (2007) showed that the activity of amphibians positively correlates with food intake (Wells 2007; Pittman *et al.*, 2014). Furthermore, it can also affect the reproductive success of these vertebrates in the adult life stage, for instance, when frogs are active in a chorus at night. Furthermore, this male amphibian's activity positively correlates with mating success during breeding seasons (Russell *et al.*, 2005; Jaquiéry *et al.*, 2010).

Individual differences in activity levels of amphibians could also result in vital compromises (Sih *et al.*, 2004). For example, animals with constantly high activity levels are more prone to be eaten by predators, which means an increased predation risk. However, it also results in a higher resource acquisition rate and food intake (Sih *et al.*, 2015). On the other hand,

higher activity levels may result in higher energy use, an increased calorie intake and a slight decrease in growth rate.

It must be emphasized that the key drivers of these individual differences in activity levels are not well comprehended, and future research is needed to investigate them. According to one hypothesis, the differences in activity levels are caused by variances in the animals' physiological state and metabolic rate (Sinsch, 2014).

MOVEMENT IN ECOSYSTEM HABITATS IN AMPHIBIANS (SPATIAL ECOLOGY OF AMPHIBIANS)

Movement is a central aspect of amphibians' life, both within an individual's home range and at the scale of dispersal. An organism's lifetime movement is arranged around three main purposes: exploitation, exploration, and relocation (Joly, 2019). These are associated with specific behavioral mechanisms and spatial factors. Therefore, examining the movements of amphibians in their ecosystem habitat can be very beneficial, as managing these spatially structured populations requires in-depth knowledge of the behavioral mechanisms that project the animals' movement.

These vertebrates have a complex life cycle regarding exploitation, which includes migrating between different sorts of habitats (Cushman, 2006). This requires them to cross different landscapes that are barely liveable for them. In terms of relocation and exploration, the between-pond movements occur very frequently, making the amphibians' populations very resilient regarding movements. Relocation is an essential type of movement that happens at a large scale, along with wide dispersal to colonize new habitats (Joly, 2019). Each function, at each scale, involves specific interactions between individual motivation (phenotype dependence) and environmental quality (context dependence) that determine decision-making and fitness outputs.

The movement of the dispersal process entails three main stages; departure, transfer, and settlement. Each of these stages involves different movement mechanisms (Ims & Yoccoz, 1997). Today we have substantial insights into the departure choices, which may have a lot of different causes. For example, it can be influenced by phenotype states such as age, sex, physical condition, personality, and hormonal state of the amphibians (Wilczynski *et al.,* 2005; Haun, 2007). Moreover, the quality of the current environment can have a substantial impact on their movements, such as genetic relatedness with other residents, food shortage,

density, the occurrence of predators, parasites, and the deprivation of the environment (Matthysen, 2012).

The dispersion in a natural or artificial habitat can be analyzed by using three main factors: tortuosity, straightness index and path length (Benhamou 2004). The tortuosity of an animal's path is an essential parameter in searching behaviors as well as in orientation. The tortuosity of a path is inversely related to the efficiency of the orientation mechanism. The effectiveness of an oriented path can be estimated by a straightness index calculated as the ratio between the distance (starting point to the goal), and the path length travelled to reach the goal (Benhamou 2004). According to the newest findings, fractal analysis of animals' paths appears as an alternative and promising way to weigh the tortuosity of a random search path as a fractal dimension ranging between 1 being a straight-line movement) and 2 being a Brownian motion (Benhamou 2004).

BUFONIDS FAMILY AS MODEL TO UNDERSTAND MOVEMENTS PATTERNS AND PERSONALITY TRAITS

Rhinella is a widespread genus native to Central and South America, and to the moment it is the most common group of neotropical anurans, inhabiting a great diversity of environments and ecoregions (Pereyra *et al.*, 2021). Specifically, *Rhinella marina* is one of the most studied species at present due to its invasive potential and great impact on biodiversity when introduced in various parts of the world. The greatest focus has been the Australian case (Rollins *et al.*, 2015; Gruber *et al.*, 2017; McCann *et al.*, 2018). This species is well known for its rapid adaptation to new environments and personality traits that promote migration and occupation of new environments. These traits for both *R. marina* and other taxa have been characterized as learned behaviours and even innate traits (Baxter-Gilbert et al 2019). The broad phenotypic plasticity present in this species has possibly allowed for broad tolerance and rapid adaptability to diverse biotic and abiotic factors, not only in invaded habitats but also in habitats where they are native (Candler and Bernal 2015; McCann *et al.*, 2018; Fan *et al.*, 2021).

Some evidence has also been found that personality traits associated with diverse geographic areas can be maintained even in captivity, and at the same time are determinant in understanding the distances they can move in their natural habitats (Gruber *et al.*, 2017). Other traits associated with straightness of movement were recorded by Shaykevich *et al.*,

(2022), comparing navigational patterns with other groups such as Dendrobatidae, breaking the paradigm of amphibian navigation and suggesting that navigational abilities may be widely shared among amphibians.

For the central regions of Rio de Janeiro, the species that occupies the greatest environmental heterogeneity is *R. ornata* Spix 1824, distributed in various types of habitats and having similar body proportions to *R. marina*, which allows us to apply the methodologies used in studies and compare with the same approaches (Maia-Carneiro *et al.*, 2013, Costa and & Nomura, 2014).

Although much is understood about the actuality of *R. marina* in the area of movement ecology and behavioural ecology, many questions remain to be answered, and biological models such as *R. ornata* will help us to answer them, as a homologous species, which occupies similar ecological niches to *R. marina* and because they have a wide plasticity and habitat occupancy we will use a biological model to understand how certain personality traits are distributed in populations associated with non-anthropized, agricultural and urban environments and their effect on daily movement patterns using the spool and line method.

MATERIALS AND METHODS

STUDY AREA

The study was conducted between September 2021 and April 2022, in the municipality of Cachoeiras de Macacu in the state of Rio de Janeiro, located at the edge of the slopes of the Serra dos Órgãos (-22° 27' 27.59" S. -42° 39' 5.99" W). This region has a mean annual rainfall of 2600 mm with mean temperatures of 14 to 37 C° Fig.1 (Bernardo *et al.*, 2011).



Figure 1. Study area map.

The studied localities correspond to urban, agricultural, and forest areas peripheral to the city of Papucaia and Cachoeiras de Macacu, an area that is made up of a mosaic of these habitats (Almeida-Gomes & Rocha 2014), ranging from young secondary forests, areas with relatively little intervention, small agricultural communities, and varied organic plantations of coconut, goiaba and lemon (Rocha *et al.*, 2007). Anthropized areas classified as urban were chosen by generating random points in Arcgis within the peripheral zone of each city. Similarly, random points were generated in agricultural areas.

DATA COLLECTION

Adult and sub-adult individuals of *Rhinella ornata* (Spix 1824) were collected by active search during the first hours of the night (lidar 3D image for a studied individual: https://poly.cam/capture/8ADAD2D6-477F-47F4-8B06-1653CB8C8C59). A spool and line device was attached using a strap made from the polypropylene tubing of pericranial

catheters, modifying the model created by Mejía *et al.*, (2021) and adapting it to Bufonids (lidar 3D image for a studied individual with spool and line device attached: https://poly.cam/capture/44F48B6B-13A5-4E71-B48E-E600438CC448) (S.Fig). At the time of collection individuals were placed in plastic bags and weighed to determine the weight and size of the spool and line device that should be assigned to each individual. The spool and line device represented 9% of the weight of the toad (Brown *et al.*, 2006; Mejía *et al.*, 2021). The device didn't influence the mobility of the toad, and the line was 0,04 grams per meter, that added together allowed monitoring of the individual for 24 hours respectively. Once the spool and line device was attached, the toad was released at the same collection point and the release point was marked with a stake and tape.

After 24 hours each toad was collected and transported in plastic containers to an open field arena (OFA) to determine the variables related to personality. The arena used for the personality experiments was made following Gruber *et al.*, (2017) maintaining the 120*120*80 dimensions used for *Rhinella marina* and recording with a Samsung M31 mobile phone with a 64 mp camera. (Fig. 2). Two types of experiments were carried out in the arenas:



Figure 2: Open field arenas, A: Diagram of shyness - boldness (risk taking) experiment with refugia, B : Diagram of exploration and straightness experiment, 1: departure point for all studied individuals, 2: first set of consecutive points 3: Trakctor generalizer map.

EXPERIMENT 1: SHYNESS - BOLDNESS (SC).

This experiment was conducted by filming individuals from above and recording the time it took for them to leave the shelter (fig.2A). This analysis was created by modifying the methodology developed by Brehm *et al.*, 2019 and adapting it to this species. The exit time was determined to be the exact second when the entire body of the individual left the shelter (fig.2A). The time data was measured in seconds. Lower values (exit response of a few seconds) will be translated as greater extroversion of the animal (Wilson *et al.*, 1994; Brehm *et al.*, 2019).

EXPERIMENT 2: EXPLORATION (TDE- TOTAL DISTANCE EX-SITU) AND EX-SITU STRAIGHTNESS COEFFICIENT (SCE).

This experiment was created to determine the exploration degree of the studied animals. Each toad was recorded from above, for 10 minutes (modif. of Brehm *et al.*, 2019) to determine the distance travelled (cm) from the starting point in the centre of the OFA to the final point , and path straightness. We used Python's Tracktor, Scipy and Pandas library (Gomez - Marin et al 2012, Gruber *et al.*, 2017, Brehm *et al.*, 2019, Sridhar *et al.*, 2019) to analyze the videos corresponding to each animal. These data were then analyzed in R using the AdHabitat LT package. The Straightness coefficient was obtained according to the following formula:

SC = D/L

where D is the straight-line distance between the start and end of the trajectory, and L is the length of the trajectory (Benhamou, 2004).

To reduce the stress of the individuals, morphometric data was taken immediately after the open field arena test and a day after the personality test (at morning hours). Used area (UAI=Used area in-situ) and total distance traveled (TDI= total distance in-situ) by the individuals was mapped, using gps, compass and metric tape, the data was analyzed using R software with adHabitat LT (CRAN - Package adehabitatLT r-project.org) and adHabitatHR (CRAN - Package adehabitatHR r-project.org) (Calenge, 2006). Individuals were not fixed or deposited in collections; they were released in their initial habitat after the area was surveyed.

STATISTICAL ANALYSIS

To understand how behavioral traits and which behavior influence daily movement patterns and area use by habitat type, a PCA was performed to simplify the complexity of the multidimensional sample spaces and understand the relationship between the variables. In addition to this, a GLM was performed using the MuMIn package, (https://cran.r-project.org/web/packages/MuMIn/index.html) using a Poisson distribution to assess the influence of all behavioural traits and their relationship with the daily movement pattern and used area (Hammond *et al.*, 2021).

We used a theoretic approach of the data information that averages models to assess both the magnitude and direction of the coefficients of predictor variables. This approach combines information from all candidate models to produce coefficient estimates that result in more precise parameter estimates. Thus, we evaluated all possible combinations of the five variables used in the model, in addition to the null model, using the "dredge" function of the MuMIn package. We used the Akaike information criterion (AIC) and Akaike information weights corrected for small samples (AICc) to rank the models (Burnham and Anderson, 2002).

Finally, to understand the structural composition of habitats used by toads, an NDVI was performed in Arcgis 10.3 to estimate habitat type (Lehmann *et al.*, 2014; Froehly, et al 2020), and vegetation structure present in the study areas and the percentage that it represents in the study area (Wasko & Sasa, 2012; Lehmann *et al.*, 2014; Ramírez-Arce *et al.*, 2021).

RESULTS

Ninety-three individuals were collected and 70 individuals (37 females and 33 males) of *Rhinella ornata* were successfully studied. The loss of 23 individuals was due to spool and line loosening (12), predation (5), and roadkill (4). Individuals were distributed in three habitat types: urban habitats (21 individuals in seven areas), forest habitats (23 individuals in six areas), agricultural habitats (26 individuals in six areas). Males had an averaged of 60g and 7.37cm SVL (urban habitats: 6.68cm; forest habitats 7.63cm; 7.62cm in agricultural habitats) females averaged 111.59 grams and 9.61cm in length (urban habitats 10cm; forest habitats 9.5cm; agricultural habitats 9.2cm).

For the shyness and boldness traits, a greater boldness was observed in males than in females 2717.66 sec sd= 6406.817 vs 8132.08 sec sd= 14671.67 p(0.044). No significant differences were observed between the boldness in individuals between habitat types (p=0,0658). When arranged from highest to lowest we can observe that individuals from forest areas had the highest boldness translated into the lowest response time 12 sec with a mean of 2983, 3 sec sd= 7399.59, followed immediately by individuals from urban areas with 21 sec and a mean of 5417, 19 sec sd= 7399.59, and finally agricultural areas with the boldest individual 54 sec, a mean of 8007.4 sec and sd= 13705.95.

When analysing the distance travelled by individuals in a new environment, sexes didn't show significant differences p=0,071. mean= 275,51cm sd=226,084 for females and 226,9 cm sd=326,84 for males. Comparing between the types of studied habitats, individuals belonging to urban habitats (Mean: 441,32 sd= 399,93) moved more than twice as much as individuals from forest areas (Mean: 193,82 sd= 168,91) followed finally by individuals from agricultural areas (mean: 144,68 sd= 144,135) and when comparing the means of these groups, significant differences were recorded (p=0,0052).

Regarding the straightness coefficient of movement in a new environment, non-significant differences were observed between sexes (males 0,345 vs. 0,474 p= 0,117). We could observe significant differences between the groups representing each habitat p=0,004, (urban habitat with Mean: 0,29 sd= 0,303, forest habitat Mean: 0,484 sd=0,33 and agricultural habitat Mean: 0,451 sd=0,31).

When studying the distance travelled by individuals within the 24h period, no significant differences were observed between the sexes (males Mean: 40.57 sd=33.59 vs females Mean: 44.15 sd=36.28). And when comparing the distance travelled between the different habitat types, individuals from forest areas moved more than other groups with a mean of 73 m (sd=40.767). The second highest daily movement was by individuals from agricultural areas with 35.61 m per day (sd=13), finally with a mean of 16.67 m (sd=16.41) and these daily movement values presented highly significant differences 6.53E-08. When we analysed the difference between the areas used by the individuals there were significant differences between males (mean= 141,38m2 sd= 265,77) and females (124,495m2 sd=196,495), in a different way when analysing all the individuals between the different habitats the area of the polygon created by the area used showed non-statistically significant differences p(0,08) (urban habitats Mean: 250,26m² sd=360,76, forest habitats Mean: 130,194m² sd=152,803 and agricultural habitats Mean: 39,309m² sd=28,011.

The study areas varied according to the vegetation structure present, presence of exposed soil and buildings-paving. When mapping the agricultural areas there were no significant differences in the vegetation percentage composition and habitat type within these environments (p=0.1206) mean of crops= 50% sd=37.69, shrubs= 6.21% sd=7.04, open bare land=24.25% sd=23.61, water bodies= 0.3 % sd=0.7, Grass=18.5% sd=17.43. Urban areas had significant differences in the means of the proportion of each constituent habitat type (p=0.0002) buildings and paved = 55.57% sd=12.96, shrubs= 38.04% sd=20.37, water bodies= 0.82 % sd= 2.19, Grass=5.52% sd=12.26. Forest areas also had significant differences in the percentage composition of structure types present (p=0.0009), Forest= 93% sd=4.03, shrubs= 5% sd=2.85, open bare land=1.25% sd=1.94 (Fig.3).



Figure 3: Habitat distribution in studied areas, maps represent a sample of the first study area for each habitat type.

The PCA showed most of the variance in components 1 and 2 (Fig. 4). In the same way there was an evident grouping by habitat type indicating an explicit differentiation in the behavioral characteristics and habitat types for component 1. The most significant variables were Urban areas 0.47, TDE (total distance Ex-situ) 0.37 followed by the presence of Shrubs (0.34) and negatively the highest variance was for TDI (total distance IN-Situ) 0.4 (Fig 4.A).



Figure 4: Relationship between behavioral tested traits and tested habitat structure for each kind of habitat. A: Loadings representing the respective values for each variable. B: Scatter-plot showing the relationship between behavioral tested traits and tested habitat structure for each kind of habitat. Purple dots: Forestal habitats, bluish green dots: Urban habitats, yellow dots: Agricultural habitats. SVL: snout vent length, BS: Boldness-Shyness, TDE: Total distance Ex-situ environment (open field arena), SCE: Straightness coefficient Ex-Situ (open field arena), TDI: Total distance In-situ environment (native environment), UAI: Used area In-Situ (native environment), SCE: Straightness coefficient In-Situ (native environment).

The second Component was mostly explained by the presence of open bare land (0.47), Crops (0.41) and Forest areas (0.38). The behavior of the variables evidently grouped the individuals by habitats representing a clear segmentation; this segmentation coincides with the compositional structures of each habitat, and at the same time individuals from agricultural areas are being differentiated from their counterparts from urban and forest habitats in terms of BS values, showing the highest values for this group on this axis. Among the variables that were opposite for the groups, there is the straightness coefficient where the higher the straightness of the travelled path in the native habitats (SCI) the lower the straightness coefficient in the new environment SCE (open field arena), the same happened with the total distance travelled in the new environment TDE (open field arena), the higher this variable the lower the response in the native TDI habitats.

For all explanatory variables in the 70 individuals for the 3 habitat types, 3 model selections were created to understand the used area size (Tab.1), the straightness coefficient (Fig. 5, Tab.1) and the area travelled by the individuals. In Tab.1 the Δ AIC selected two independent variables and these were evaluated separately, these variables accounted for 25 % of the model ω i=0.25 when studying the size of the area used by individuals in the study area.

Table 1. Ranking of Linear Model (LM) selection based on a candidate set of 'best models' $(\Delta AIC \le 2.00)$ predicting the size area *in-situ* recorded in agricultural, urban and forestal habitats for 70 individuals of *Rhinella ornata*. The difference from the best model (ΔAIC), the AIC score (AICc) and Akaike weight (ω i) are shown.

PARSIMONIOUS MODEL	ΔΑΙC	AICc	ω _i
STRAIGHTNESS COEFFICIENT+TOTAL DISTANCE TRAVELED	0.00	261.4	0.25
STRAIGHTNESS COEFFICIENT+TOTAL DISTANCE TRAVELED +EMERGENCE	0.43	261.7	0.20
STRAIGHTNESS COEFFICIENT+HABITAT	0.92	262.3	0.15
HABITAT	1.08	262.4	0.14
STRAIGHTNESS COEFFICIENT+TOTAL DISTANCE TRAVELED +HABITAT	1.10	262.4	0.14



Figure 5. Relationship between (a) size of the area used by the studied animals and Total distance travelled in the open field arena (b) size of the area used by the studied animals and Straightness coefficient recorded in agricultural, urban and forestal habitats for 70 individuals of *Rhinella ornata*.

The second model that aimed to explain the coefficient of straightness of movement in-situ took into consideration as the best model the null model $\omega i=0.43$ (Tab.2),

Table 2. Ranking of Linear Model (LM) selection based on a candidate set of 'best models' ($\Delta AIC \le 2.00$) predicting the coefficiente straightness *in-situ* recorded in agricultural, urban and forestal habitats for 70 individuals of *Rhinella ornata*. The difference from the best model (ΔAIC), the AIC score (AICc) and Akaike weight (ω i) are shown.

PARSIMONIOUS MODEL	ΔΑΙC	AICc	Θi	
NULL	0.00	-94.7	0.43	
STRAIGHTNESS COEFFICIENT	1.38	-93.3	0.21	
EMERGENCE	1.74	-93.0	0.15	
TOTAL DISTANCE TRAVELED	1.89	-92.8	0.16	

Finally, to explain the distance travelled by individuals in-situ the best model was habitat, having the highest weight and explaining 48% $\omega i=0.48$ (Tab.3, Fig.6).

Table 3. Ranking of General Linear Model (GLM) selection based on a candidate set of 'best models' ($\Delta AIC \le 2.00$) predicting the distance travelled *in-situ* in agricultural, urban and forestal habitats for 70 individuals of *Rhinella ornata*. The difference from the best model (ΔAIC), the AIC score (AICc) and Akaike weight (ω i) are shown.

PARSIMONIOUS MODEL	ΔΑΙC	AICc	ωi	
HABITAT	0.00	631	0.48	
HABITAT + EMERGENCE	0.82	632	0.31	
HABITAT + STRAIGHTNESS COEFFICIENT	1.73	633	0.20	



Figure 6. Total distance travelled in-situ through habitat type recorded in agricultural, urban and forestal habitats for 70 individuals of *Rhinella ornata*.

DISCUSSION

Since the beginning of human-animal interaction, the interpretation of variation in behavior with the species we interact with has always been evident (Lorenz, 1949), but there is a wide gap between ecological knowledge and behavioral biology (Kelleher *et al.*, 2018). This gap represents an ample opportunity to understand intrinsic processes on behavioural variations of individuals for diverse populations, or under specific characteristics of the environments they inhabit, and even how we can contribute to avoid the vulnerability of populations by understanding their behavioural patterns (de Azevedo and Young, 2021).

SHYNESS – BOLDNESS AND RISK-TAKING EVIDENCE

Our results clearly showed a relationship between the behavioral traits studied and their relationship with the habitats they occupied, specifically when we differentiated the relationship between the sexes we could observe that males showed a greater boldness, leaving the shelter in less time than females, which in a natural environment would be translated as a greater tendency to expose themselves to risk. This behavior would otherwise allow the individual to find better territories for foraging (Rodda, 1992; Stephens *et al.* 2008; Manenti *et al.*, 2013; Koprivnikar and Penalva, 2015; Winandy and Denoël, 2015), shelter (Navas, 1996; Schwarzkopf and Alford, 1996; Seebacher and Alford, 2002;

González-Bernal *et al.*, 2016), thermoregulation (Lillywhite *et al.*, 1973; Dupré and Petranka 1985; Hoppe, 2018) and mating (Tetley and O'Hara, 2012; Kelleher *et al.*, 2018).

Some biological characteristics that have a direct implication with boldness are sexual maturity and hormone levels associated with the time of the life cycle that the individual is at (Ishii *et al.*, 1995; Ott *et al.*, 2000; Oers, 2004; Idler, 2012), also the variation in the composition of the venom may be evolutionarily related to the time of facing predators giving a greater advantage to males over females in snakes (Barros *et al.*, 2022). In contrast to what we found in this study, Zamora-Camacho, (2022), found greater Boldness in females of *Epidalea calamita*, in all tests performed demonstrating a greater tendency to take risks when exposed to a new environment.

In addition to their clear differences in habitat structure and composition, there were no differences in boldness and tendency to take risks in a new environment among individuals belonging to each habitat type, but toads from wooded areas were the most extroverted, followed by those from urban areas and finally those from agricultural areas. This may be related to innate behavioral characteristics associated with this type of habitat and the presence or absence of predators that allow this degree of extroversion to be the most frequent within the population studied. This case is also evidenced in other taxa groups as an innate behavioral characteristic (Baxter-Gilbert et al., 2019) where individuals from both forested and urban areas have similar boldness and it turn different in semi-urban populations. For urban areas, the individuals found possibly have a home range where contact with dangerous situations and predators is not as frequent as in agricultural areas but enough to keep them less exposed than individuals in forested habitats (Swanson et al., 2018). If we analyze this behavioral trait in urban areas as a learned response, we can intuit that interaction with novel stimuli does not necessarily have to represent an apparent danger. And the possibility of being able to forage, find a better shelter and potential mates may allow the individual to learn this behavior, disassociating a potentially dangerous response in other circumstances with a real benefit (DeMaynadier and Hunter, 1995, Dukas, 1998; Sol et al., 2013; Kelleher et al., 2018; Burmeister, 2022). In this type of habitat some of the found path were in front of streetlights. This type of environment within urban habitats may be the most dangerous to be present because predator presence and roadkill possibility is higher, but even so we frequently found our study animals near this type of environment (Hels and Buchwald, 2001; Sol et al., 2013;). For Rhinella marina, R. rubescens and R. diptycha opportunistic behaviours taking advantage of the food resources attracted by lighting have been recorded, giving them a probabilistic disadvantage of being roadkill (González-Bernal *et al.*, 2016; Fraga and Wiederhecker, 2021). The lower extroversion presented by individuals from agricultural areas may be related to farm worker's activity and machinery of active plantations, delimiting animal exposition by the risky probability of encounters with humans or agricultural related predators (Lemckert and Brassil, 2000; Toma, 2004; Lara-Tufiño *et al.*, 2019; Hegde *et al.*, 2019; Shine *et al.*, 2021).

EXPLORATION AND STRAIGHTNESS OF DISPLACEMENT

We were able to observe an interesting relationship between animals from urban areas and how much they move in the open field arena. They presented the highest values of exploration in a new environment and a lower straightness coefficient. In the same way as with extroversion traits it is likely to be associated with a conditioned tendency by the low interaction with predators in their home range (Anholt *et al.*, 1996; Mogali *et al.*, 2011; Mogali, 2018; Zamora-Camacho *et al.*, 2018). This type of behavior is also positively related to high dispersal and a positive relationship with resource acquisition (Dingemanse *et al.*, 2003; Dall *et al.*, 2004; Bonte, 2012). Exploration and straightness coefficient, which explains why individuals who explore more have a greater tendency to cross points close to already explored areas (Benhamou, 2004).

The Exploration explains separation and classification into the first principal component for individuals in each of the habitat types and also served as a predictive model to explain the size of the area used (more exploration = larger used area), similar results were found by Gruber *et al.*, 2017, with *Rhinella marina*, where toads that explored more occupied larger areas. This personality trait may vary with age and size, and in adults this trait will be related to greater fitness consequences (Dingemanse *et al.*, 2003).

In the same way the straightness coefficient also served as a model to explain the size of used area by individuals, understanding that the lower the coefficient (more tortuous - less straight) the smaller the used area polygon, straight paths and constant trajectories serve to explain escape movements (Benhamou, 2004; Barraquand, and Benhamou, 2008; Codling *et al.*, 2008), the less straight an individual's path through the area, the greater the tendency of the animal to be able to pass close to the starting area which indicates that the animal was not trying to escape from the starting point (Bartón *et al.*, 2012, Beardsworth *et al.*, 2021).

Evidence shows that the most tortuous behaviours are related to foraging (Benhamou, 2004, Brehm *et al.*, 2019, Beardsworth *et al.*, 2021), exploration, microhabitat and resource search (Barraquand, and Benhamou, 2008; Imirzian *et al.*, 2019). The resources distribution in many cases determines the direction and straightness of an individual's movement within its home range (Pittman *et al.*, 2014), and the heterogenic resource availability in urban habitats, possibly tends to lead individuals to move more torturously even in a new environment (Brehm *et al.*, 2019), which also shows us a higher comfort of being in a new place (Baxter-Gilbert *et al.*, 2021), as the case of individuals from forest and agricultural areas that had straighter paths which could be explained by a little more discomfort in a new habitat (Bartón *et al.*, 2012; Baxter-Gilbert *et al.*, 2021; Beardsworth *et al.*, 2021).

Although there were no records of amplexi or reproductive events during the study, in breeding seasons males participating in active chorus nights are known to express greater exploratory activities compared to non-participating males and females (Dodd and Cale, 1998; Jaquiéry *et al.*, 2010). Another relationship of activity patterns is with the positive relationship of food intake and the likelihood of prey encounters in more exploratory individuals (Wells, 2007). A variation at individual levels can have positive consequences and important trade-offs for survival, individuals with high and constant exploratory activity may have a higher predation risk and/or a higher rate of resource acquisition (Sih *et al.*, 2004; Sih *et al.*, 2015), conversely high levels of activity raise metabolic rates affecting growth if the individual does not have access to food resources. This scenario may be present in anthropised habitats with established pest control such as plantations and urban or agricultural areas that do not allow a high abundance of insects as prey for toads (Sih *et al.*, 2015).

Although it is not known that variation in metabolic rate underlies some personality traits that affect energy expenditure (such as activity or exploration) in amphibians (Careau *et al.*, 2008; Biro and Stamps 2010; Sih *et al.*, 2015), this approach will need to be studied in depth to understand the role of certain personality types in predatory dynamics in the ecosystems they inhabit and their trophic effect.

HABITATS AS A MODEL FOR EXPLAINING DISPLACEMENT

Finally, habitat type was a determinant model to establish how much individuals moved within each kind of environment. At a structural and functional level, anthropic habitats have structures that can be used by biodiversity, their characteristics in terms of resource availability limit and conditioning the movement patterns of animals and may block them (Marsh *et al.*, 2005; Bissonette & Adair, 2008; Shepard *et al.*, 2008; Beyer *et al.*, 2016; Panzacchi *et al.*, 2016) or facilitate them (Beier & Noss 1998; Dodd & Cale, 1998; Bissonette & Adair, 2008) depending on the biology of the species.

For amphibians, these characteristics are determinant for understanding movement patterns; specifically for bufonids, plasticity has been reported for *Rhinella marina*, where behavioural characteristics play a fundamental role (Tingley and Shine, 2011; Cabrera-Guzmán *et al.*, 2013; Brown and Shine, 2014; Gonzalez-Bernal *et al.*, 2014; Gruber *et al* 2017; Gruber *et al.*, 2018). Such tolerance has allowed it to become one of the most widely distributed bufonids in the world and at the same time to be an invasive species in a few countries (Brown *et al.*, 2015; Tingley *et al.*, 2017; Hudson *et al.*, 2020).

In urban environments the studied individuals did not move as much as individuals from forest and agricultural areas, it is important to mention that it is the most common bufonid in urban areas in the whole region of Cachoeiras de Macacu, (Almeida-Gomes *et al.*, 2014; Dorigo *et al.*, 2018), as well as in *R. marina* the set of behavioral characteristics that allow for facilitated movements and survival in a wide variety of habitats are also present in other *Rhinella* species, such as *Rhinella ornate*, modulating exploration in these environments.

Although the structural differences that make up the study areas demonstrate significant differences, future studies will need to focus on what kind of environments this type of bufonid manages to exploit for various activities related to its reproductive biology, foraging and shelter selection based on personality traits. It is possible that if there would have been more connectivity between study areas, individuals could have shown different patterns of movement and even different behavioural traits, as individuals born in anthropic habitats may have moved to agricultural or forest areas, and vice versa. This type of flow could also allow behavioural heterogeneity for different traits (Baguette and Dyck, 2007), although our focus was to understand daily activity and personality, in order to obtain data related to home range and migration capacity, spool and line studies will not be sufficient, and the necessary approach will be to use telemetry/gps to be able to track for a longer period of time and without path loss.

CONCLUSIONS

We were able to observe a remarkable variation in the behavioural traits studied demonstrating a specific variation related to habitat types and structural variation of the habitat.

- Males of *Rhinella ornata* have a greater tendency to take risks than females and this variation becomes more intense when we look at habitat type.
- On average, individuals from agricultural areas moved shorter distances and females from these habitats were the timidest organisms in the study when introduced to a new environment.
- Exploration (expressed as total distance travelled in a new environment) was the best determinant for separating the various habitat types based on their behavioural characteristic.
- There are two models that allow us to understand the size of the area occupied in their natural habitat (Straightness coefficient and Exploration).
- More structural metrics are needed to determine the internal variation of the habitat used to further understand the needs of the species in relation to environmental structure and how these variables are related to personality and movement.

Rhinella ornata is an excellent biological model for determining behavioural traits and dispersal dynamics in amphibians, but studies with more time for field analysis per individual will be needed to understand movement patterns and connectivity between habitat types associated with the animal personality, as well as the distribution of these traits deeply within populations by habitat type, and to understand variables such as stress hormone levels and physical condition as suggested by Janín *et al.*, (2012).

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Supplementart materials



Sup.mat 1 : One of our individuals with fixed spool and line device in the forest understory



Sup.mat 2 : Urban individual moving in front of a house garden



Sup.mat 3 : Details on the trail left by the spool and line device on the understory.



Sup.mat 4 : Details on the spool and line in 3D image (https://poly.cam/capture/44F48B6B-13A5-4E71-B48E-E600438CC448)



Sup.mat 5 : Mapping of the existing points in the area traveled by one of the individuals during one day, for an agricultural area.



Sup.mat 6 : Side view of the arena for the OFT



Sup.mat 7 : Visualization by means of tracktor for movement analysis