Received: 10 July 2023 | Revised: 20 March 2024 | Accepted: 20 March 2024

DOI: 10.1111/btp.13326

bioTROPICA ASSOCIATION FOR TROPICAL BIOLOGY AND CONSERVATION WILEY

NATURAL HISTORY FIELD NOTE

Escape behavior lateralization of pointed-belly frog (*Leptodactylus podicipinus*) (Anura, Leptodactylidae) in the southern Pantanal

Sean Keuroghlian-Eaton[1,2] | Patrícia dos Santos Sousa[1] | Márcia Marrie Pinheiro Müller[1,2] | Diego G. Cavalheri[1,2] | Bruna E. Bolochio[1] | Diego José Santana[2]

[1]Programa de Pós-Graduação Em Biodiversidade, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", São Paulo, Brazil

[2]Mapinguari Lab, Instituto de Biociências, Universidade Federal de Mato Grosso Do Sul, Campo Grande, Brazil

Correspondence
Sean Keuroghlian-Eaton, Programa de Pós-Graduação Em Biodiversidade, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", São Paulo, Brazil.
Email: seanksad@gmail.com

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico; Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Associate Editor: Jennifer Powers
Handling Editor: Lisa M. Schulte

Abstract

Functional lateralization in directional preference for predator evasion was examined in 45 Pointed-belly frogs (*Leptodactylus podicipinus*). T-maze trials revealed a preference for rightward escape, though individual bias was minimal, at only 33%, to evade predictability. Sex and size didn't influence escape decisions, aligning with predator avoidance strategies.

Abstract in Portuguese is available with online material.

KEYWORDS
amphibians, brain asymmetry, defensive behavior, pointed-belly frog

1 | INTRODUCTION

Functional lateralization has been referred to as the asymmetry between the hemispheres of the brain and was initially believed to be a unique human feature (Vallortigara & Bisazza, 2002). However, studies have shown that it is present among many other vertebrates (Rogers & Andrew, 2002), with a particular focus on limb preference in numerous anuran species (e.g., Adler & Taylor, 1981; Borkhvardt & Ivashintsova, 1995; Dill, 1977; Malashichev & Robins, 2018; Pande, 1971). It is common for lateralization to occur at the population level, rather than having an equal number of individuals with a right or left bias (Rogers et al., 2013). However, any laterally biased population is not 100% skewed in one direction, as there are evolutionary benefits to having an asymmetrical population in response to interactions with other organisms, such as predator–prey interactions (Ghirlanda & Vallortigara, 2004). For example, if a population of anurans were all lateralized on the same side, it could be disadvantageous to individuals when escaping from predators, due to their escape direction being predictable (Ghirlanda et al., 2009; Ghirlanda & Vallortigara, 2004). Consequentially, we anticipate that

individual organisms will show lateralization, and the overall population should favor either the right or left when choosing an escape direction, all while preserving asymmetry.

Escape reactions can be influenced by many factors, including environmental temperature (Domenici et al., 2007; Wakeling, 2006), and morphological traits such as body size and shape (Langerhans, 2009; Wakeling, 2006). Functional lateralization is likely to result in a superior ability to escape predators (Ghirlanda et al., 2009; Ghirlanda & Vallortigara, 2004), as having fully bilateral symmetry between the hemispheres could actually slow the overall processing speed due to equal competition between them. For example, strongly lateralized individuals of the fish *Cymatogaster aggregata* exhibited higher escape reactivity, likely leading to an increased chance of escaping from predator attacks (Dadda et al., 2010). Nevertheless, while lateralization is recognized in most vertebrates (Vallortigara & Bisazza, 2002), in anurans, the effect of lateralization on escape behavior and survival is largely understudied.

The Pointed-belly frog (*Leptodactylus podicipinus*) (Cope, 1862) is a common and abundant frog, found in Paraguay, Argentina, Bolivia, northwestern Uruguay, and central Brazil (Frost, 2021). In the Pantanal, the world's largest temporary wetland extending over Bolivia, Brazil, and Paraguay (Fraser & Keddy, 2005), *L. podicipinus* reproduces year-round, making it easy to find males, females, and juveniles in any season (Prado et al., 2005). Along with the high anuran abundance in the Pantanal (Ponce & Nunes da Cunha, 1993; Uetanabaro et al., 2008), these two factors make *L. podicipinus* ideal for behavioral studies. Our objective is to determine if there is a directional preference in the escape behavior exhibited by the captured *L. podicipinus* population.

Previous studies on vertebrates have established a link between sex and escape lateralization, (Pellitteri-Rosa & Gazzola, 2018; Rilea, 2008) However, males, females, and smaller individuals all experience moments when they are exposed to predators in the environment (Carrillo et al., 2022; Tuttle & Ryan, 1981; Frost, 2021), this leads us to hypothesize that size and sex will not exert any influence on lateralization. We also hypothesize that the population as a whole will have a directional preference, and that individuals will be highly lateralized (Ghirlanda & Vallortigara, 2004).

2 | METHODS

The study site is located in southern Pantanal, state of Mato Grosso do Sul, Brazil. The Pantanal is one of the largest wetlands in the world (Junk et al., 2014). The climate is humid to sub-humid, and tropical sub-warm, according to the Köppen classification (Köppen, 1884), with medium annual temperatures between 22°C and 26°C and maximum temperatures between 35°C and 40°C. Annual rainfall ranges from 1500 to 1750 mm, with a warmer wet season occurring between October and March, and a cooler dry season occurring between May and August (SEMAC, 2011).

On the 30 November 2021 we collected 45 *L. podicipinus* frogs at a pond (19°31′08.4000"S, 57°02′27.6000"W). We captured the frogs manually and placed them into a plastic bag, filled with some water and vegetation from the capture site, a standard method for capturing amphibians (Crump et al., 1994). They were then taken to the laboratory at Base de Estudos do Pantanal (BEP), owned by the Federal university of Mato Grosso do Sul (UFMS), and housed together in a 100-liter plastic vessel with vegetation and water at the bottom. The housing container was kept in the laboratory at ambient temperature (28°C on average) and the frogs remained in captivity for 26 h. No individual was harmed during the experiment, which was carried out under the Brazilian license of Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO 49080) and the ethical approval of the Universidade Federal de Mato Grosso do Sul Animal Ethics Committee (CEUA-UFMS 1.1096/2019).

We tested the 45 individuals of *L. podicicpinus*, 16 males, 13 females, and 16 nonsexed juveniles. To test the escape direction choice, we used a 1.7 cm diameter PVC pipe T-maze consisting in a 24 cm main pipe that ended in the middle of a transversal section measuring 6.5 cm (3.25 cm for each direction) (Figure 1). The central arm duct of the apparatus had a rear entrance for the frogs, while the opposite end was the cross-arm T section. Frogs were prevented from exiting the rear entrance by placing a prodding instrument made by wrapping tape around a stick until it was the size of the hole. The stick was used to gently force the individual to enter through the central arm of the maze, and then would continue forward gently forcing the frog only to the T section, then until the frog made the decision of going left or right. This method was used to simulate a predator cornering the frog, which may force it to make a decision to keep moving away. Each individual was passed through the T-maze 10 times, with 30 s intervals in a closed opaque box. We gave as much time as the frog needed to make a decision but it never took more than a minute for them to choose left or right. We noted

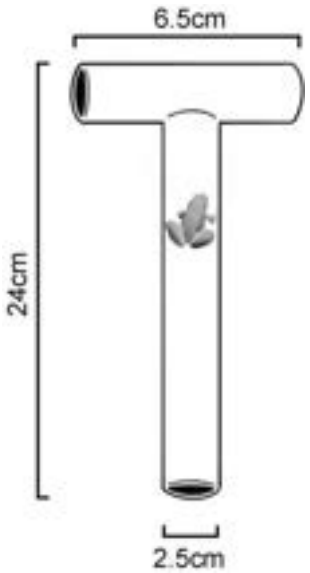

FIGURE 1 Design of the experimental apparatus used in this study, including the size and shape of the PVC pipe T-maze. The central arm duct of the apparatus had a rear entrance for the frogs, while the opposite end was the cross-arm T section.

each frog's decision when they were put through the apparatus as *Left* or *Right*. The test sequence lasted 3–5 min for each frog, the total test time for all individuals was roughly 8 h. We kept artificial light two meters away from each side of the apparatus during the experiment in order to keep the same illumination. After the tests, we measured their snout-vent length (SVL) in mm.

To analyze our data we first conducted a Laterality Index (Bryden & Sprott, 1981) on each individual, testing for left and right decisions using the formula LI = (R−L)/(R + L) where "R" and "L" represent all right and left decisions made by one individual in the T-maze based on their 10 decisions. We performed this analysis for all 45 individuals. To determine whether individuals were significantly lateralized or not, we conducted a chi-square test on each individual's results from the Laterality Index test to observe any preference between left and right.

To test if there was a preference for right or left in the population as a whole, we performed a one-sample Wilcoxon signed rank test to observe if there was any significant difference in the right and left decisions of the sample population as a whole. The Wilcoxon test was done using the LI scores of each individual with a test value of 0 for the population. To test differences between males and females, we performed a two-sample paired Wilcoxon test also using the LI scores of every sexed individual (13 females and 16 males). Lastly there was a test to analyze if size affected left or right decisions in our population sample. "Left decisions" referring to instances when an individual chose the left exit in our T-maze, while "right decisions" refer to instances when an individual chose the right exit in our T-maze. To test the size effect on lateralization, which is an indirect approximation of ontogeny, we utilized the SVL measurements of each individual and conducted a binomial logistic regression test to observe whether there was any discernible pattern in left or right decision preferences when comparing it to various sizes of frogs. All of the analyses were carried out using R (R Core Team, 2022).

3 | RESULTS

Out of 450 choices, frogs preferred right 260 times (57.7%), and left 190 times (42.2%) (Figure 2). The one sample Wilcoxon signed rank test showed a significantly higher preference for right for all the choices as a whole ($Q^2 = 0.5$; $p = .02$). According to the individual chi-square tests, out of the 45 individuals tested, only 15 (33.33%) exhibited signs of lateralization.

Regarding the sexes, our two samples paired Wilcoxon test showed that there was no significant differences between females and males ($Z = 1.341$; $p = .500$). Males preferred right 160 times (59.3% of males), and females preferred right 130 times (56.1% of females).

About size differences affecting which side was chosen, analyzed by the binomial logistic regression, we found no significant difference ($Z = 0.669$; $p = .503$), meaning that the preference for left or right is not related to the individual's size (SVL).

4 | DISCUSSION

We found that our sampled population of *L. podicipinus* exhibited a lateralization pattern when escaping in a T maze towards right (Figure 2). On an individual level, the data indicates that approximately 33.33% of the population demonstrates lateralization. As we hypothesized though, there was no significant difference in left or right decisions between different sized individuals.

Functional lateralization as a whole provides to the individuals a rapid reaction time (Dadda et al., 2010). For escape performance to be advantageous to anurans, it is important that the decision is made as quickly as possible (Robins, 2005). If the entire population was greatly skewed to escaping in one direction, predators could easily predict which way *L. podicipinus* would escape (Ghirlanda

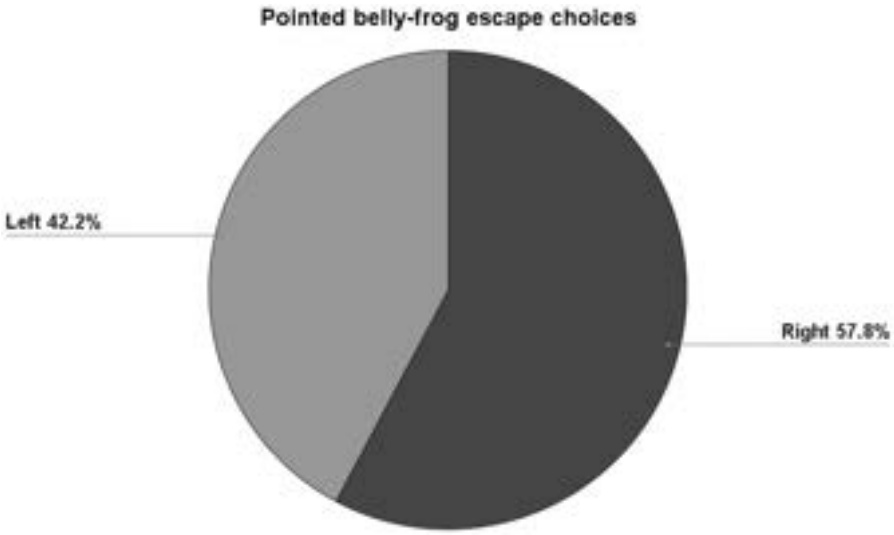

FIGURE 2 Left and Right escape choices of the *L. podicipinus* sample population through the T-maze experiment. Each frog underwent the maze 10 times, resulting in 260 choices for the right path (57.7%) and 190 choices for the left path (42.2%). Our one-sample Wilcoxon test revealed a significant preference for the right path within the population ($Q^2 = 0.5$; $p = .02$).

et al., 2009; Ghirlanda & Vallortigara, 2004). Therefore, having nearly equal choices for left and right when escaping is important to avoid predictability (Dadda et al., 2010). In addition, there should not be any size-specific side preference since virtually all animals need a form of escape behavior (Lecca et al., 2017). In a similar experiment, Romano et al. reported that adult locusts, exposed to a fowl-mimicking robot, exhibited greater lateralization compared to their young counterparts. This phenomenon was attributed to "the effect that the post-embryonic development of the neural system can play." The study also reported results similar to ours, indicating individual lateralization within the sample population, along with population-level lateralization (Romano et al., 2017).

We hypothesized that individuals would be highly lateralized, as observed in most vertebrates (Vallortigara & Bisazza, 2002). Additionally, we anticipated that the population as a whole would exhibit a directional preference, unaffected by size, or sex. We predicted that neither sex nor size would influence lateralization, given that all individuals had similar exposure to predators. For instance, males are exposed to predators in the environment when vocalizing (Tuttle & Ryan, 1981). On the other hand, *L. podicipinus* females also expose themselves to predators during reproduction periods when practicing parental care for their tadpole schooling (Carrillo et al., 2022). Finally smaller, or younger frog individuals have a tendency to be more focused on gaining energy and not being predated, meaning they also have a decent amount of exposure in the environment (Frost, 2021). At any given time of the year *L. podicipinus* can simultaneously have females during parental care, males vocalizing, and small individuals in the same region (Prado et al., 2005). Since males, females and small individuals have moments where they are exposed to predators if one of these groups had a specific side preference it could lead to predators being able to predict their escape if the, this may explain the lack of differences in different size escape behaviors.

A further study could be done timing each individual to observe if those with a higher lateralized preference had a faster or slower reaction time, when compared to the frogs that were ambidextrous in their left and right choices, demonstrating bilateral symmetry. In addition, future approaches in studying anuran lateralization should consider incorporating diverse methods to assess lateralization performance. For example, conducting the test on all individuals once and repeating the test at a later time. Nonetheless, we found that few individuals are well lateralized. Our experiment and analysis suggest that size does not affect *L. podicipinus* decision when escaping from a simulated predator attack, but the population as a whole does have a significant preference for the right side.

ACKNOWLEDGMENTS

SKE, PSS, MPM, DGC, and BEB thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES). DJS thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq processes 404239/2021-8, 402519/2016-7) and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT process 054/2017) for financial support. DJS also thanks CNPq for his research fellowship (CNPq 309420/2020-2).

CONFLICT OF INTEREST STATEMENT

Authors declare they have no conflicting or competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are publicly available from the corresponding author on github: https://github.com/Rhinella85/Keuroghlian-Eaton-et-al.-Lateralization.

ORCID

Sean Keuroghlian-Eaton https://orcid.org/0000-0001-5016-8985

REFERENCES

Adler, K., & Taylor, D. H. (1981). Toad orientation: Variability of response and its relationship to individuality and environmental parameters. *Journal of Comparative Physiology*, *144*(1), 45–51. https://doi.org/10.1007/BF00612796

Borkhvardt, V. G., & Ivashintsova, E. B. (1995). Arciferal pectoral girdle of amphibians—An instrument for recognizing right-and left-handedness? *Russian Journal of Herpetology*, *2*(1), 34–35.

Bryden, M. P., & Sprott, D. A. (1981). Statistical determination of degree of laterality. *Neuropsychologia*, *19*(4), 571–581.

Carrillo, J. F., Santana, D. J., & Prado, C. P. A. (2022). Body condition off emales during tadpole attendance and its potential costs in a neotropical foam nesting from (Leptodactylus podicipinus). *Ethology Ecology and Evolution*, *34*, 1–14. https://doi.org/10.1080/03949370.2022.2026481

Crump, M. L., Scott, N. J., Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. C., & Foster, M. S. (1994). Measuring and monitoring biological diversity: Standard methods for amphibians. *Visual Encounter Surveys*, *364*, 84–92.

Dadda, M., Koolhaas, W. H., & Domenici, P. (2010). Behavioural asymmetry affects escape performance in a teleost fish. *Biology Letters*, *6*(3), 414–417. https://doi.org/10.1098/rsbl.2009.0904

Dill, L. M. (1977). 'Handedness' in the Pacific tree frog (Hyla regilla). *Canadian Journal of Zoology*, *55*(11), 1926–1929.

Domenici, P., Lefrancois, C., & Shingles, A. (2007). Hypoxia and the antipredator behaviors of fishes. *Philosophical Transactions of the Royal Society B*, *362*, 2105–2121. https://doi.org/10.1098/rstb.2007.2103

Fraser, L. H., & Keddy, P. A. (2005). *The world's largest wetlands: Ecology and conservation*. Cambridge University Press.

Frost, D. R. (2021). Amphibian species of the world: an online reference. version 6.1. *http://research. amnh. org/vz/herpetology/amphibia*.

Ghirlanda, S., Frasnelli, E., & Vallortigara, G. (2009). Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *364*(1519), 861–866. https://doi.org/10.1098/rstb.2008.0227

Ghirlanda, S., & Vallortigara, G. (2004). The evolution of brain lateralization: A game-theoretical analysis of population structure. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1541), 853–857. https://doi.org/10.1098/rspb.2003.2669

Junk, W. J., Piedade, M. T. F., Lourival, R., Wittmann, F., Kandus, P., Lacerda, L. D., … Agostinho, A. A. (2014). Brazilian wetlands: Their definition, delineation, and classification for research, sustainable management, and protection. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(1), 5–22. https://doi.org/10.1590/S2179-975X7619

Köppen, W. (1884). Die Wärmezonen der Erde, nach der Dauer der heissen, gemässigten und kalten Zeit und nach der Wirkung der Wärme auf die organische Welt betrachtet. *Meteorologische Zeitschrift*, *1*(21), 5–226.

Langerhans, R. B. (2009). Morphology, performance, fitness: Functional insight into a post-Pleistocene radiation of mosquitofish. *Biology Letters*, *5*, 488–491. https://doi.org/10.1098/rsbl.2009.0179

Lecca, S., Meye, F. J., Trusel, M., Tchenio, A., Harris, J., Schwarz, M. K., Burdakov, D., Georges, F., & Mameli, M. (2017). Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior. *eLife*, *6*, e30697.

Malashichev, Y., & Robins, A. (2018). Lateralized motor responses in anuran amphibians—An overview of methods and perspectives of studies. *Biomedical Communications*, *63*(4), 210–242. https://doi.org/10.21638/spbu03.2018.402

Pande, B., & Singh, I. (1971). One-sided dominance in the upper limbs of human fetuses as evidenced by asymmetry in muscle and bone weight. *Journal of Anatomy*, *109*, 457.

Pellitteri-Rosa, D., & Gazzola, A. (2018). Context-dependent behavioural lateralization in the European pond turtle, Emys orbicularis (testudines, Emydidae). *Journal of Experimental Biology*, *221*(20), jeb186775. https://doi.org/10.1242/jeb.186775

Ponce, V. M., & Nunes da Cunha, C. (1993). Vegetated earthmounds in tropical savannas of Central Brazil: A synthesis: With special reference to the Pantanal at Mato Grosso. *Journal of Biogeography*, *20*, 219–225.

Prado, C., Uetanabaro, M., & Haddad, C. (2005). Breeding activity patterns, reproductive modes, and habitat use by anurans (amphibia) in a seasonal environment in the Pantanal, Brazil. *Amphibia-Reptilia*, *26*(2), 211–221. https://doi.org/10.1163/1568538054253375

R Core Team (2022). *R: A language and environment for statistical R foundation for statistical computing*. Version 4.1.1. R Foundation for Statistical Computing. https://www.R-project.org/

Rilea, S. (2008). A lateralization of function approach to sex differences in spatial ability: A reexamination. *Brain and Cognition*, *67*, 168–182. https://doi.org/10.1016/j.bandc.2008.01.001

Robins, A. (2005). Development lateralized visual processing in anurans. *Eurekah Bioscience*, *1*(5), 462–473.

Rogers, L. J., & Andrew, R. (Eds.). (2002). *Comparative vertebrate lateralization*. Cambridge University Press.

Rogers, L. J., Vallortigara, G., & Andrew, R. J. (2013). *Divided brains: The biology and behaviour of brain asymmetries*. Cambridge University Press.

Romano, D., Benelli, G., & Stefanini, C. (2017). Escape and surveillance asymmetries in locusts exposed to a Guinea fowl-mimicking robot predator. *Scientific Reports*, *7*(1), 12825.

SEMAC (2011). Secretaria de Estado de Meio Ambiente,do Planejamento, da Ciência e Tecnologia. Caderno Geoambiental das Regiões de Planejamento de MS – 5. Região do Pantanal. Governo do Estado de Mato Grosso do Sul. Campo Grande, MS.

Tuttle, M. D., & Ryan, M. J. (1981). Bat predation and the evolution of frog vocalizations in the neotropics. *Science*, *214*(4521), 677–678. https://doi.org/10.1126/science.214.4521.677

Uetanabaro, M., Prado, C. P. A., Rodrigues, D. J., Gordo, M., & Campos, Z. (2008). *Guia de campo dos anuros do Pantanal e planaltos de entorno*. Editora UFMS.

Vallortigara, G., & Bisazza, A. (2002). How ancient is brain lateralization? In L. J. Rogers & R. J. Andrew (Eds.), *Comparative vertebrate lateralization* (pp. 9–69). Cambridge University Press.

Wakeling, J. M. (2006). Fast-start mechanics. In R. E. Shadwick & G. V. Lauder (Eds.), *Fish biomechanics: Fish physiology* (Vol. *23*, pp. 333–368). Academic Press.

How to cite this article: Keuroghlian-Eaton, S., dos Santos Sousa, P., Marrie Pinheiro Müller, M., Cavalheri, D. G., Bolochio, B. E., & Santana, D. J. (2024). Escape behavior lateralization of pointed-belly frog (*Leptodactylus podicipinus*) (Anura, Leptodactylidae) in the southern Pantanal. *Biotropica*, *56*, e13326. https://doi.org/10.1111/btp.13326