

Tadpole-transporting frogs use stagnant water odor to find pools in the rainforest

Shirley J. Serrano-Rojas^{1,2*}, Andrius Pašukonis^{1,3**}

¹Department of Biology, Stanford University, 371 Jane Stanford Way, Stanford, CA 94305, USA

²Universidad Nacional de San Antonio Abad del Cusco (UNSAAC), Perú.

³CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

* serranoj@stanford.edu

** andrius.pasukonis@cefe.cnrs.fr

Abstract

Breeding sites are often a limited and ephemeral resource for rainforest frogs. This resource limitation has driven the evolution of diverse reproductive strategies that increase offspring survival. For example, poison frogs shuttle their tadpoles from terrestrial clutches to aquatic rearing sites, using various cues to assess pool suitability. Yet, how frogs find new pools is unknown. We tested the role of odor cues in the process of finding tadpole deposition sites by the poison frog *Allobates femoralis*. We created 60 artificial pools grouped into three conditions: stagnant water, tadpole water, and clean water control. Fifteen pools were discovered within six days, with more tadpoles and more frogs directly observed at pools with stagnant odor cues. Our findings suggest that frogs use odor cues associated with stagnant water for the initial discovery of new breeding pools. These cues may be good indicators of pool stability and increased likelihood of tadpole survival.

Key words: tropical amphibians, water finding, navigation, frog olfaction, chemical cues, reproductive strategy

1. Introduction

Most amphibians rely on water to successfully complete their life cycle (Wells, 2007). Therefore, the ability to find suitable water bodies is vital for amphibian survival (Duellman and Trueb, 1986; Wells, 2007). Most temperate-region amphibians use relatively stable streams and ponds at known localities and under predictable seasonal climatic conditions (Pernetta and Geltsch, 2007; Pittman et al., 2014; Sinsch, 2014). Chemical stimuli learned during larval development (i.e., natal site imprinting) and/or the ability to perceive long-distance cues from the breeding sites in part explain the ability of temperate-region amphibians to return to their natal ponds and find new ones (reviewed in (Ogurtsov, 2004; Ogurtsov and Bastakov, 2001; Sinsch, 1990; Sinsch, 2006)). In tropical environments, water is often abundant, but suitable breeding sites are rare because of high predator pressure (McKeon and Summers, 2013; Schulte et al., 2013) and high desiccation risk due to high evaporation rates (Poelman and Dicke, 2007; Schulte and Lötters, 2013). How amphibians find suitable water bodies in complex and unpredictable environments such as tropical rainforest is still largely unknown.

Tropical amphibians evolved to use a variety of aquatic ephemeral sites for reproduction, such as small standing-water bodies inside plant structures (i.e., phytotelmata) and temporarily flooded depressions, reducing the risk of predation by large predators present in permanent ponds or streams (Summers and McKeon, 2004). Quality of these small pools is variable (Fouilloux et al., 2021; Rudolf and Rödel, 2005) and the seasonality of tropical regions has a strong impact on breeding site availability (Bertoluci and Rodrigues, 2002; Gottsberger and Gruber, 2004). In response to the uncertainty of these breeding resources, frogs have

presumably evolved strategies allowing them to find, assess, and choose the appropriate rearing sites that maximize offspring survival (McKeon and Summers, 2013; Poelman et al., 2013; Ringler et al., 2013; Ringler et al., 2018).

Poison frogs, for example, have evolved parental care strategies, such as transporting tadpoles from terrestrial oviposition sites to widely-dispersed ephemeral tadpole-rearing pools (Pašukonis et al., 2019; Wells, 2007). This complex spatial behavior evolved along with the behavioral plasticity in tadpole deposition strategies that balance the benefits and risks involved. Pool choices are influenced by an interaction of many factors, such as physical and chemical characteristics (Brown et al., 2008; Fouilloux et al., 2021; McKeon and Summers, 2013; Poelman and Dicke, 2007; Poelman et al., 2013), distance to the territory (Erich et al., 2015; Pašukonis et al., 2019; Ringler et al., 2013; Ringler et al., 2018), predator presence, type and abundance (McKeon and Summers, 2013; Ringler et al., 2018; Schulte et al., 2013; Von May et al., 2009), conspecific presence (McKeon and Summers, 2013; Rojas, 2014; Schulte et al., 2013), and desiccation risks (Poelman and Dicke, 2007; Schulte and Lötters, 2013). Tadpole transporting parents can use chemical cues to detect the presence of predators or conspecific tadpoles (Rojas, 2014; Schulte and Lötters, 2013; Schulte and Lötters, 2014; Schulte et al., 2011; Schulte et al., 2015). Recent findings have also shown that some poison frogs use spatial memory to find and efficiently exploit pools tens to hundreds of meters away from the territory (Beck et al., 2017; Erich et al., 2015; Pašukonis et al., 2016; Pašukonis et al., 2019; Ringler et al., 2013), but how frogs find these pools in the first place is still unknown.

A study by Pašukonis et al. (2016) found that tadpole-carrying Brilliant-Thighed Poison Frogs, *Allobates femoralis*, were attracted to out-of-reach pools containing water with high concentrations of conspecific tadpoles and proposed that conspecific olfactory cues may facilitate the initial discovery of new deposition sites. However, an alternative hypothesis is that the pool water itself (and not the tadpoles in it) was responsible for attracting the frogs.

Allobates femoralis needs small pools of standing water that do not dry out for two to three months to complete tadpole development. Therefore, cues associated with standing water, such as decomposing leaf odor, that are indicators of pool stability may also provide a cue for finding new tadpole rearing sites.

To test these hypotheses, we first conducted a pilot study and found that pools with stagnant water or decomposing leaves were rapidly occupied by *A. femoralis* while pools with clean water and conspecific tadpoles remained empty. Based on these preliminary observations, we designed a large-scale field experiment to test the role of odor cues associated with standing water in the process of finding new pools by *A. femoralis*.

2. Material and methods

Study site and system

The study was carried out between 31st January and 06th March 2020 in the lowland rainforest near the field camp Saut Pararé (4°02' N, 52°41' W) at the Nouragues Ecological Research Station in the Nature Reserve Les Nouragues, French Guiana.

Allobates femoralis is a small diurnal leaf-litter frog distributed throughout the Amazonian basin and the Guiana Shield (Amézquita et al., 2009; Amézquita et al., 2017). Males vocally advertise territories to attract females and mating takes place inside the male's territory where clutches of approximately 20 eggs are laid in the leaf litter (Ringler et al., 2009; Roithmair, 1992; Stückler et al., 2019). Tadpole transport takes place after 15 – 20 days of larval development when primarily males carry 1 to 25 tadpoles to small pools (Ringler et al., 2013) (figure 1a). Frogs travel tens to hundreds of meters to deposit their tadpoles, spread them across several pools (Erich et al., 2015), and readily use artificial pools (Ringler et al., 2015). *Allobates femoralis* tadpoles are omnivorous but not predatory.

Experimental design

We placed three types of artificial pool conditions in the field (figure 1b–e): pools with stagnant water cues (stagnant water condition), pools with conspecific tadpole cues (tadpole water condition), and clean water (control condition). To produce the stagnant water odor, we used decaying leaf litter fermented in river water. Based on preliminary experiments, five liters of leaf litter were soaked for two weeks inside a mesh-covered plastic outdoor container with 40 liters of river water. This decomposing leaf litter was then used to prepare experimental pools.

Each artificial pool consisted of a brown plastic plant saucer (diameter 35 cm) filled with two liters of untreated water from a shallow groundwater well from the field station. For stagnant water condition, we surrounded the pool with two liters of wet decomposing leaf litter (figure 1c). For the tadpole water condition, we added ~18 (± 4) tadpoles of *A. femoralis* in different developmental stages collected from various pools in the area. For the latter and the control condition, we surrounded the pool with two liters of leaf litter from the forest floor making them visually similar to the stagnant water condition (figure 1d–e). Therefore, in the final setup the stagnant water pools only differed from the control pools in that the leaf litter immediately surrounding the pool was soaked in standing water for two weeks prior to the start of the experiment.

We set four non-simultaneous linear transects in areas of high frog density, separated from each other by at least 50 meters. Each transect had fifteen pools separated by 15 meters (Fig. 2b). Transects were located parallel to established trails but pools were placed at least two meters away from the trails. The order of the experimental pool conditions was randomized between every three consecutive pools within the transect.

Data collection

After the setup day, we recorded the number of new *A. femoralis* tadpoles deposited in each pool at end of the day (17:30 - 19:00) for six days. In addition, pools were checked every morning during the period of high tadpole transport activity (Beck et al., 2017; Ringler et al., 2013) to record the presence of *A. femoralis* visiting the pools. Each tadpole does not represent an independent sample of pool discovery because a single frog can deposit up to 25 tadpoles at once (Ringler et al., 2013) and the same frog can transport several times to the same pool. Since we cannot assess how many independent depositions happened in each pool, the only independent sample in our data is the presence or absence of tadpoles (probability of tadpole deposition). However, because frogs often split tadpoles between multiple pools (Beck et al., 2017; Erich et al., 2015) the number of tadpoles is a good indicator of pool preference among the pools that were found. Therefore, we use both the probability of tadpole deposition and the tadpole number in our data analysis with slightly different interpretations.

Finally, we used capture-recapture data to get some information on distances from which *A. femoralis* discover new pools. Male territory locations were determined as part of a long-term capture-recapture study and following previously established methods (Ringler et al., 2009; Ringler et al., 2013). In short, individuals are identified based on their unique ventral coloration patterns and capture locations mapped on a detailed GIS map (Ringler et al., 2016). Only the capture points in the area where the respective male showed territorial behavior (e.g., calling, courtship, phonotactic approach of a stimulated intruder) were considered for identifying the territories. To avoid disturbance we did not capture and identify the frogs seen at the pools during the main experiment, but several frogs were captured at the pools during the pilot experiments in 2017, which used similar pool conditions. In addition, some pools from the first transect in 2020 were left in place after six days to obtain additional data on how far the frogs traveled. We compiled these opportunistic observations to measure the linear distance from

the closest known territory point of each male to the pool where the respective male was captured. Rainfall data were provided by the Nouragues Ecological Research Station from an above-canopy weather station (Nouraflex: rainfall sensor Campbell ARG100).

Statistical analysis

We assessed if there were differences between pool conditions in: (1) the probability of tadpole deposition (yes = 1, no = 0) using a logistic regression by building a generalized linear mixed model (GLMM) with the complementary log-log (cloglog) link function and binomial error distribution; and (2) the number of tadpoles deposited in the artificial pools building a GLMM with log link function and Poisson error distribution. In both models, pool condition and cumulative rainfall were used as fixed effects, and pool ID was used as an observation-level random effect to model overdispersion in count data (Harrison, 2014). Models were built using the function *glmer* from the *lme4* R package (Bates et al., 2015). Overdispersion for count data was checked using the function *dispersion_glmer* from the *blmeco* R package (Korner-Nievergelt et al., 2015). Zero inflation was checked using the function *check_zeroinflation* implemented in the *performance* R package (Lüdecke et al., 2021). Likelihood ratio tests (LRTs) were used to determine the best-fit models. The proportion of variance explained by the best-fit models was calculated using the function *r.squaredGLMM* from the *MuMIn* R package (Bartoń, 2019; Nakagawa and Schielzeth, 2013). Tukey post-hoc pairwise comparisons were done with the function *glht* from the *multcomp* R package (Hothorn et al., 2008). All statistical analyses were performed in R (R Development Core Team, 2020). Data and custom scripts are available as electronic supplementary material.

3. Results

Of the 60 artificial pools created, 15 pools (25%) were used as deposition sites within six days. Most of them (73%) were occupied within the first two days. Frogs showed a clear differential pool usage, 11 out of 15 occupied pools were pools from the stagnant water condition (figure 2a). Out of the 253 tadpoles deposited, 212 tadpoles were found in the stagnant water condition, 23 in the tadpole water condition, and 18 in the control condition. Of the 18 adult frogs observed at the pools (four of which were transporting tadpoles), 15 were at the stagnant water condition. Sixteen frogs captured visiting pools with stagnant water condition had their closest known territory points between 3 – 50 meters (median = 12) from the pools where they were found.

The probability of tadpole deposition was affected by pool condition and cumulative rain (table 1; pool condition, $\chi^2 = 13.937$, $df = 2$, $p < 0.001$; rain, $\chi^2 = 10.501$, $df = 1$, $p = 0.001$) and fixed effects explained 57.37% of the variance. Pool condition and cumulative rain also had a significant effect on the number of tadpoles deposited in the artificial pools (table 1; pool condition, $\chi^2 = 13.246$, $df = 2$, $p = 0.001$; rain, $\chi^2 = 8.7149$, $df = 1$, $p = 0.003$) and fixed effects explained 45.41% of the variance. The stagnant water condition had, on average, a higher number of tadpoles per pool (figure 2b; mean \pm s.e.: 10.60 ± 2.73), compared to the tadpole water condition (1.15 ± 0.89), and to the control condition (0.90 ± 0.64).

4. Discussion

Although water is abundant in the rainforest, suitable water bodies for breeding are rare and difficult to discover by chance. Our findings suggest that frogs rely on olfactory cues associated with stagnant water to find and choose suitable pools and increase their offspring's chances of survival. This discovery provides a better understanding of the mechanisms used by tropical frogs to find new breeding resources in complex and unpredictable environments.

The hypothesis formulated by Pašukonis et al. (Pašukonis et al., 2016), that conspecific tadpole odor cues attract *A. femoralis* to novel breeding sites, was not supported by our findings. The presence and size of conspecific tadpoles can be an indicator of habitat permanence (Rojas, 2014; Rudolf and Rödel, 2005) and our results do not exclude the possibility that *A. femoralis* use this information. However, most pools with conspecific tadpoles remained unoccupied after six days suggesting that tadpole odor does not play a major role in the discovery of new pools.

The observed pattern is unlikely to be a result of frogs' avoidance to deposit in clean water, because frogs readily deposited in the clean water surrounded by olfactory stagnant water cues. In addition, most observed adults and all tadpole carriers were at the stagnant odor pools once again suggesting that frogs were more likely to discover these pools. Furthermore, most tadpole depositions occurred in the first two days after placing the pools, presumably because the odor attracting the frogs decreased as the leaf litter dried out and was washed by rain. Finally, our interpretation is in line with and further supported by tadpole carrier movements observed by Pašukonis et al. (2016), which showed frogs diverting from their normal path to investigate an area where out-of-reach pools with stagnant water and conspecific tadpoles were suspended above the ground. The high number of tadpoles found in stagnant water condition together with the observations mentioned above strongly suggest that our main effect is driven by the pool discoverability rather than a pool preference after discovery. Interestingly, most frogs directly observed at the pools did not carry tadpoles suggesting that frogs explore and evaluate the pools before the tadpole transport.

The stagnant odor cues used by frogs to find new breeding sites may also be an indicator of pool stability. Despite having been often noted for temperate-region amphibians (Ogurtsov, 2004; Sinsch, 1990; Sinsch, 2006), the possibility of tropical frogs using olfaction to find new breeding sites and evaluate the basic characteristics of pool stability has been largely

overlooked. So far, most studies implicating tropical frog olfaction have focused on detecting predators or conspecifics inside the pools. *Ranitomeya variabilis*, for example, uses olfactory cues to reduce the risk of predation, avoiding pools with cannibalistic conspecific tadpoles (Schulte et al., 2011). *Dendropsophus ebraccatus* detects aquatic egg predators and avoids laying eggs in habitats with fish (Touchon and Worley, 2015). However, very little is known about how frogs evaluate which pools are sufficiently stable for tadpole development. Our study raises a possibility that frogs could use chemical signatures of stagnant water as an indication of pool permanence. Wet decaying leaves, however, are omnipresent in the rainforest thus the frog attracting cue must be specific to chemical processes in stagnant water. Future studies should aim to elucidate what chemical signatures present in the stagnant water attract frogs and how they correlate with pool stability.

We recorded frogs arriving to our experimental pools from up to 50 meters, but *A. femoralis* and other poison frogs have been shown to find pools and navigate after translocations from hundreds of meters (Pašukonis et al., 2014; Pašukonis et al., 2018; Pašukonis et al., 2019; Ringler et al., 2013). It seems unlikely that odor cues alone could explain accurate navigation over longer distances in the rainforest understory, but in combination with well-developed spatial memory (Beck et al., 2017; Pašukonis et al., 2016) olfaction might be key when exploring new environments. Olfaction has been shown to play an important role in water-finding and navigation in temperate-region amphibians (reviewed in (Ferguson, 1971; Ogurtsov, 2004; Sinsch, 1990; Sinsch, 2006)), and several authors have suggested that amphibians might imprint on the odor of the natal water bodies (Ogurtsov, 2004; Ogurtsov and Bastakov, 2001; Oldham, 1967; Sinsch, 2014). It is possible that tropical frogs also imprint on the smell that represents suitable breeding sites. For example, a recent study found that *A. femoralis* prefer returning to their natal pools despite the presence of predators, and even when closer pools are available (Ringler et al., 2018). More generally, our results together with other recent studies in poison frogs (Beck et al., 2017; Pašukonis et al., 2016; Schulte et al.,

2011) indicate that olfaction might play a crucial but often overlooked role in tropical amphibian spatial behavior and reproduction.

Unpredictable environments such as those of tropical rainforests have driven the evolution of behavioral strategies and sensory abilities allowing exploitation of small and scattered resources of ephemeral nature. Our study provides the first evidence to suggest that poison frogs use stagnant water cues to discover novel breeding sites. Many tropical frogs rely on small pools for tadpole development, therefore similar mechanisms could be widespread among tropical amphibians and deserve further investigation.

Ethics

The study was approved by the scientific committee of the Nouragues Ecological Research Station and the Institutional Animal Care and Use Committee of Stanford University (Protocol ID 33211). We strictly adhered to the current U.S., French and European Union law, and followed the Association for the Study of Animal Behaviour's (ASAB) Guidelines for the use of live animals in teaching and research (ASAB (Association for the Study of Animal Behaviour), 2018).

Acknowledgments

This work is part of a partnership agreement between A. P. and the Nouragues Nature Reserve aimed at improving and spreading the knowledge about amphibians. We thank the staff of the Nouragues Nature Reserve for their commitment to preserving our natural world. We are grateful to the staff of the CNRS Nouragues Ecological Research Station for the logistic support and providing the meteorological data; Max Ringler for the logistic support in the field; Walter Hödl for his mentorship; Camilo Rodríguez and Luisa Arcila-Perez for collecting capture-

recapture data; Janne Valkonen and Camilo Rodríguez for advice on experimental design and statistics; Karen Warkentin and Bibiana Rojas for helpful comments on the manuscript. Finally, many thanks to Luisa Arcila Perez, Chloe A. Fouilloux, Camilo Rodríguez and Daniel Shaykevich for helping to set up the transects.

Competing interests

The authors declare no competing interests.

Author's contribution

S.J.S.R. Conceptualization (supporting); Methodology (equal); Investigation (lead); Data curation (lead); Formal analysis (lead); Writing – original draft (lead); Writing – review and editing (equal).

A.P. Conceptualization (lead); Methodology (equal); Investigation (supporting); Data curation (supporting); Formal analysis (supporting); Writing – original draft (supporting); Writing – review and editing (equal); Supervision; Funding acquisition.

Funding

This project was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 835530 and the Austrian Science Fund (FWF) project J3827-B29. A.P. and S.J.S.R. were also supported by Lauren A. O'Connell with Stanford University and the National Science Foundation (IOS-1845651) funds. The meteorological data and logistic support were provided by the Nouragues Ecological

Research Station (managed by CNRS), which benefits from the Investissement d'Avenir funds of the ANR (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01).

Data accessibility

Raw data and a data handling protocol containing all data analysis can be accessed as electronic supplementary material.

References

- Amézquita, A., Lima, A. P., Jehle, R., Castellanos, L., Ramos, Ó., Crawford, A. J., Gasser, H. and Hödl, W.** (2009). Calls, colours, shape, and genes: A multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biol. J. Linn. Soc.* **98**, 826–838.
- Amézquita, A., Ramos, Ó., González, M. C., Rodríguez, C., Medina, I., Simões, P. I. and Lima, A. P.** (2017). Conspicuousness, color resemblance, and toxicity in geographically diverging mimicry: The pan-Amazonian frog *Allobates femoralis*. *Evolution (N. Y.)*. **71**, 1039–1050.
- ASAB (Association for the Study of Animal Behaviour)** (2018). Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* **135**, 1–X.
- Bartoń, K.** (2019). MuMIn: Multi-Model Inference, Version 1.43.6. *R Packag.* 1–75.
- Bates, D., Mächler, M., Bolker, B. and Walker, S.** (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **67**, 1–48.

- Beck, K. B., Loretto, M. C., Ringler, M., Hödl, W. and Pašukonis, A.** (2017). Relying on known or exploring for new? movement patterns and reproductive resource use in a tadpole-transporting frog. *PeerJ* **5**, e3745.
- Bertoluci, J. and Rodrigues, M. T.** (2002). Seasonal patterns of breeding activity of Atlantic Rainforest anurans at Boracéia, Southeastern Brazil. *Amphib. Reptil.* **23**, 161–167.
- Brown, J. L., Morales, V. and Summers, K.** (2008). Divergence in parental care, habitat selection and larval life history between two species of Peruvian poison frogs: An experimental analysis. *J. Evol. Biol.* **21**, 1534–1543.
- Duellman, W. E. and Trueb, L.** (1986). *Biology of Amphibians*. New York, U.S.A.: McGraw-Hill Book Company.
- Erich, M., Ringler, M., Hödl, W. and Ringler, E.** (2015). Brood-partitioning behaviour in unpredictable environments: hedging the bets? *Behav. Ecol. Sociobiol.* **69**, 1011–1017.
- Ferguson, D. E.** (1971). The sensory basis of orientation in amphibians. *Ann. N. Y. Acad. Sci.* **188**, 30–36.
- Fouilloux, C. A., Serrano Rojas, S. J., Carvajal-Castro, J. D., Valkonen, J. K., Gaucher, P., Fischer, M. T., Pašukonis, A. and Rojas, B.** (2021). Pool choice in a vertical landscape: Tadpole-rearing site flexibility in phytotelm-breeding frogs. *Ecol. Evol.* **11**, 9021–9038.
- Gottsberger, B. and Gruber, E.** (2004). Temporal partitioning of reproductive activity in a neotropical anuran community. *J. Trop. Ecol.* **20**, 271–280.
- Harrison, X. A.** (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2014**, e616.
- Hothorn, T., Bretz, F. and Westfall, P.** (2008). Simultaneous inference in general parametric models. *Biometrical J.* **50**,

Korner-Nievergelt, F., Roth, T., von Felten, S., Guélat, J., Almasi, B. and Korner-Nievergelt, P.

(2015). *Bayesian Data Analysis in Ecology Using Linear Models with R, BUGS, and Stan*.

Elsevier Inc.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P. and Makowski, D. (2021). performance:

An R Package for Assessment, Comparison and Testing of Statistical Models. *J. Open*

Source Softw. **6**, 3139.

McKeon, C. S. and Summers, K. (2013). Predator driven reproductive behavior in a tropical

frog. *Evol. Ecol.* **27**, 725–737.

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R² from

generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142.

Ogurtsov, S. V. (2004). Olfactory Orientation in Anuran Amphibians. *Russ. J. Herpetol.* **11**, 35–

40.

Ogurtsov, S. V. and Bastakov, V. A. (2001). Imprinting on Native Pond Odour in the Pool Frog,

Rana Lessonae Cam. In *Chemical Signals in Vertebrates 9*, pp. 433–438. Springer US.

Oldham, R. S. (1967). Orienting Mechanisms of the Green Frog, *Rana Clamitans*. *Ecology* **48**,

477–491.

Pašukonis, A., Warrington, I., Ringler, M. and Hödl, W. (2014). Poison frogs rely on experience

to find the way home in the rainforest. *Biol. Lett.*

Pašukonis, A., Trenkwalder, K., Ringler, M., Ringler, E., Mangione, R., Steininger, J.,

Warrington, I. and Hödl, W. (2016). The significance of spatial memory for water finding

in a tadpole-transporting frog. *Anim. Behav.* **116**, 89–98.

- Pašukonis, A., Loretto, M. C. and Hodl, W.** (2018). Map-like navigation from distances exceeding routine movements in the three-striped poison frog (*Ameerega trivittata*). *J. Exp. Biol.* **221**, jeb169714.
- Pašukonis, A., Loretto, M. C. and Rojas, B.** (2019). How far do tadpoles travel in the rainforest? Parent-assisted dispersal in poison frogs. *Evol. Ecol.* **33**, 613–623.
- Pernetta, A. P. and Geltsch, I. C.** (2007). The reproductive dynamics of temperate amphibians: a review. *North. West. J. Zool.* **3**, 127–144.
- Pittman, S. E., Osbourn, M. S. and Semlitsch, R. D.** (2014). Movement ecology of amphibians: A missing component for understanding population declines. *Biol. Conserv.* **169**, 44–53.
- Poelman, E. H. and Dicke, M.** (2007). Offering offspring as food to cannibals: Oviposition strategies of Amazonian poison frogs (*Dendrobates ventrimaculatus*). *Evol. Ecol.* **21**, 215–227.
- Poelman, E. H., van Wijngaarden, R. P. A. and Raaijmakers, C. E.** (2013). Amazon poison frogs (*Ranitomeya amazonica*) use different phytotelm characteristics to determine their suitability for egg and tadpole deposition. *Evol. Ecol.* **27**, 661–674.
- R Development Core Team** (2020). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing.
- Ringler, M., Ursprung, E. and Hödl, W.** (2009). Site fidelity and patterns of short- and long-term movement in the brilliant-thighed poison frog *Allobates femoralis* (Aromobatidae). *Behav. Ecol. Sociobiol.* **63**, 1281–1293.
- Ringler, E., Pašukonis, A., Hödl, W. and Ringler, M.** (2013). Tadpole transport logistics in a Neotropical poison frog: Indications for strategic planning and adaptive plasticity in anuran parental care. *Front. Zool.* **10**, 1–10.

- Ringler, M., Hödl, W. and Ringler, E.** (2015). Populations, pools, and peccaries: Simulating the impact of ecosystem engineers on rainforest frogs. *Behav. Ecol.* **26**, 340–349.
- Ringler, M., Mangione, R., Pašukonis, A., Rainer, G., Gyimesi, K., Felling, J., Kronaus, H., Réjou-Méchain, M., Chave, J., Reiter, K., et al.** (2016). High-resolution forest mapping for behavioural studies in the Nature Reserve ‘Les Nouragues’, French Guiana. *J. Maps.*
- Ringler, E., Szipl, G., Harrigan, R. J., Bartl-Binder, P., Mangione, R. and Ringler, M.** (2018). Hierarchical decision-making balances current and future reproductive success. *Mol. Ecol.* **27**, 2289–2301.
- Roithmair, M. E.** (1992). Territoriality and Male Mating Success in the Dart-poison Frog, *Epipedobates femoralis* (Dendrobatidae, Anura). *Ethology* **92**, 331–343.
- Rojas, B.** (2014). Strange parental decisions: Fathers of the dyeing poison frog deposit their tadpoles in pools occupied by large cannibals. *Behav. Ecol. Sociobiol.* **68**, 551–559.
- Rudolf, V. H. W. and Rödel, M. O.** (2005). Oviposition site selection in a complex and variable environment: The role of habitat quality and conspecific cues. *Oecologia* **142**, 316–325.
- Schulte, L. M. and Lötters, S.** (2013). The power of the seasons: Rainfall triggers parental care in poison frogs. *Evol. Ecol.* **27**, 711–723.
- Schulte, L. M. and Lötters, S.** (2014). A danger foreseen is a danger avoided: How chemical cues of different tadpoles influence parental decisions of a Neotropical poison frog. *Anim. Cogn.* **17**, 267–275.
- Schulte, L. M., Yeager, J., Schulte, R., Veith, M., Werner, P., Beck, L. A. and Lötters, S.** (2011). The smell of success: Choice of larval rearing sites by means of chemical cues in a Peruvian poison frog. *Anim. Behav.* **81**, 1147–1154.

- Schulte, L. M., Schulte, R. and Lötters, S.** (2013). Avoiding Predation: The Importance of Chemical and Visual Cues in Poison Frog Reproductive Behaviour. In *Chemical Signals in Vertebrates 12*, pp. 309–321. Springer New York.
- Schulte, L. M., Krauss, M., Lötters, S., Schulze, T. and Brack, W.** (2015). Decoding and discrimination of chemical cues and signals: Avoidance of predation and competition during parental care behavior in sympatric poison frogs. *PLoS One* **10**,.
- Sinsch, U.** (1990). Migration and orientation in anuran amphibians. *Ethol. Ecol. Evol.* **2**, 65–79.
- Sinsch, U.** (2006). Orientation and navigation in Amphibia. *Mar. Freshw. Behav. Physiol.* **39**, 65–71.
- Sinsch, U.** (2014). Movement ecology of amphibians: From individual migratory behaviour to spatially structured populations in heterogeneous landscapes. *Can. J. Zool.* **92**, 491–502.
- Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W. and Ringler, E.** (2019). Spatio-temporal characteristics of the prolonged courtship in brilliant-thighed poison frogs, *Allobates femoralis*. *Herpetologica* **75**, 268–279.
- Summers, K. and McKeon, C. S.** (2004). The Evolutionary Ecology of Phytotelmata use in Neotropical Poison frogs. In *Ecology and Evolution of Phytotelm-breeding Anurans* (ed. RM, L.), pp. 55–73. Museum of Zoology, University of Michigan.
- Touchon, J. C. and Worley, J. L.** (2015). Oviposition site choice under conflicting risks demonstrates that aquatic predators drive terrestrial egg-laying. *Proc. R. Soc. B Biol. Sci.* **282**, 1–7.

Von May, R., Medina-Müller, M., Donnelly, M. A. and Summers, K. (2009). Breeding-site selection by the poison frog *Ranitomeya imitator* in amazonian bamboo forests: An experimental approach. *Can. J. Zool.* **87**, 453–463.

Wells, K. D. (2007). *The Ecology and Behavior of Amphibians*. Chicago, IL: University of Chicago Press.

Figures

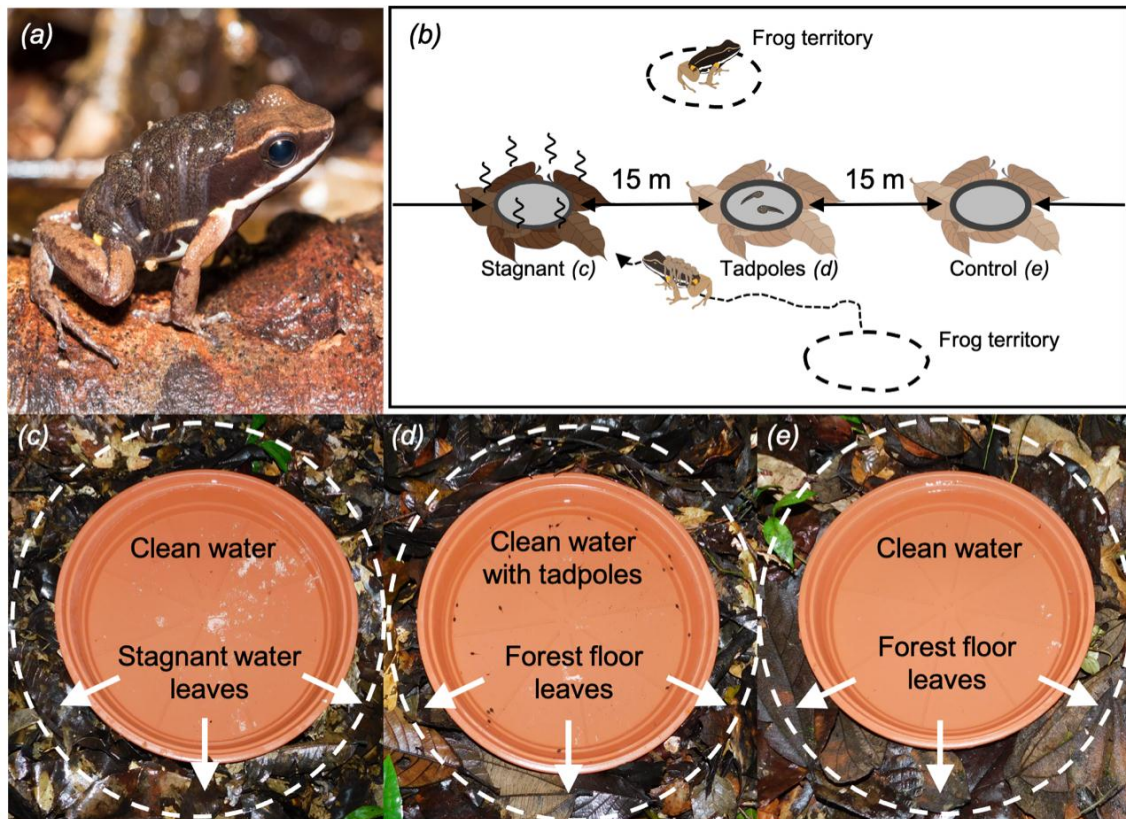


Figure 1. (a) Photograph of a male *Allobates femoralis* transporting tadpoles on his back. (b) Schematic representation of the experimental setup. Photographs of each experimental pool condition are shown below in panels (c) for stagnant water cues (stagnant water condition), (d) for conspecific tadpole cues (tadpole water condition), and (e) for clean water (control condition). The dashed white circle and white arrows highlight the leaf litter surrounding the pools, which was either soaked (c) or not (d, e) in stagnant water for two weeks prior to setting up the transect. Same clean water was used in all three conditions with *A. femoralis* tadpoles added to the tadpole condition (d).

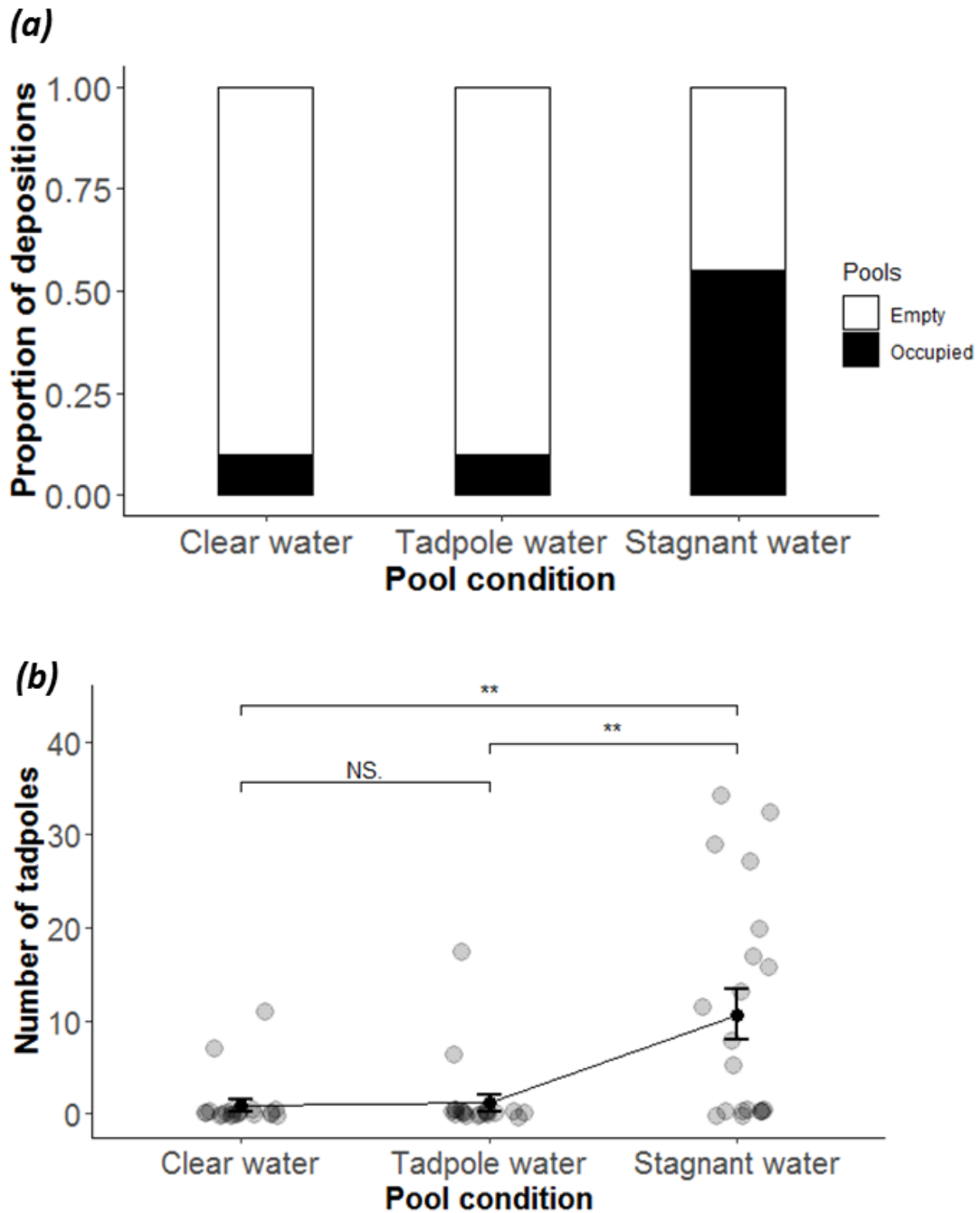


Figure 2. Poison frogs use stagnant water cues to discover novel breeding sites. *(a)* Proportion of occupied pools in each condition after six days. *(b)* Number of *A. femoralis* tadpoles deposited per pool condition after six days. The plot depicts the mean number of tadpoles deposited (black bold circles) and the associated standard errors (whiskers). The grey-filled circles show the number of tadpoles for each pool.

Table 1. Results of the generalized mixed models (GLMM) testing the effect of pool condition and cumulative rain on (i) the probability of tadpole depositions and (ii) the number of

Fixed effects	estimate	s.e.	z value	P value
(i) Probability of tadpole depositions				
Intercept (control condition)	-4.264	1.405		
Tadpole water condition	0.000	1.182	0.000	0.999
Stagnant water condition	3.154	1.235	2.554	0.010 *
Cumulative rain	0.037	0.013	2.742	0.006 **
(ii) Number of tadpoles deposited				
Intercept (control condition)	-6.182	1.905		
Tadpole water condition	0.049	1.832	0.027	0.979
Stagnant water condition	4.954	1.634	3.033	0.002 **
Cumulative rain	0.051	0.018	2.866	0.004 **

Significance codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; >0.1 ''
tadpoles deposited by the frog *A. femoralis*. p-values in bold are significant at <0.05