



Breeding site choice in the Ecuadorian poison dart frog *Epipedobates aff. espinosai* (Anura: Dendrobatidae)

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Abstract. Breeding site selection is a critical determinant of reproductive success in amphibians, as larval survival depends on the suitability of the aquatic sites. Among the key ecological factors for developmental success, hydrological conditions are particularly important, with desiccation being lethal for tadpoles. Using artificial breeding sites with differing water retention capacities, we investigated the breeding site selection for tadpole deposition in *Epipedobates aff. espinosai*, a dendrobatid species exhibiting terrestrial oviposition, followed by tadpole transport to aquatic sites. Over a two-month period, we recorded tadpole depositions in 79 of 152 artificial breeding sites. Site selection was strongly associated with the water level and relative topographic position, with frogs consistently prioritizing sites containing more water and positioned at lower locations. Our results showed no association between site selection and indicators of long-term water stability, such as water level fluctuations or average water loss, suggesting that *E. aff. espinosai* relies primarily on immediately perceivable cues, rather than extended monitoring to assess breeding site suitability. Moreover, breeding sites already occupied by tadpoles were more likely to receive additional depositions, indicating potential conspecific attraction, possibly reflecting cues of site quality and predator absence. In a second experiment, we established artificial breeding sites at locations where the species was not previously recorded, and therewith demonstrate that frogs rapidly discover and use newly available breeding sites. This indicates both high exploratory efficiency and limited availability of suitable breeding sites. Our findings highlight that *E. aff. espinosai* makes rapid, ecologically informed decisions when selecting breeding sites, guided primarily by immediate water conditions and potentially by the presence of conspecific tadpoles.

Key words. Amphibia, Chocó, ecology, field experiments, forest regeneration, parental care.

Resumen. La selección del sitio de reproducción es un determinante crítico del éxito reproductivo en anfibios, ya que la supervivencia larval depende de la idoneidad de los sitios acuáticos. Entre los factores ecológicos clave para el éxito del desarrollo, las condiciones hidrológicas son particularmente importantes, siendo la desecación un riesgo letal para los renacuajos. Utilizando sitios de reproducción artificiales con diferentes capacidades de retención de agua, investigamos la selección del sitio de reproducción para la deposición de renacuajos en *Epipedobates aff. espinosai*, una especie de dendrobátido que exhibe oviposición terrestre, seguida del transporte de renacuajos a sitios acuáticos. Durante un período de dos meses, registramos deposiciones de renacuajos en 79 de 152 sitios de reproducción artificiales. La selección del sitio estuvo fuertemente asociada con el nivel del agua y la posición topográfica relativa. En concreto, las ranas priorizaron consistentemente los sitios que contenían más agua y estaban ubicados en lugares más bajos. Nuestros resultados no mostraron asociación entre la selección del sitio y los indicadores de estabilidad del agua a largo plazo, como fluctuaciones del nivel del agua o pérdida promedio de agua, lo que sugiere que *E. aff. espinosai* se basa principalmente en señales inmediatamente perceptibles, en lugar de un monitoreo prolongado, para evaluar la idoneidad del sitio de reproducción. Además, los sitios de reproducción ya ocupados por renacuajos tenían más probabilidades de recibir deposiciones adicionales, lo que indica una posible atracción conespecífica, posiblemente reflejando señales de la calidad del sitio y la ausencia de depredadores. En un segundo experimento, establecimos sitios de reproducción artificiales en lugares donde la especie no se había registrado previamente, y con ello demostramos que las ranas descubren y utilizan rápidamente los sitios de reproducción recientemente disponibles. Esto indica tanto una alta eficiencia exploratoria como una disponibilidad limitada de sitios de reproducción adecuados. Nuestros hallazgos resaltan que *E. aff. espinosai* toma decisiones rápidas y ecológicamente informadas al seleccionar sitios de reproducción, guiadas principalmente por las condiciones inmediatas del agua y, potencialmente, por la presencia de renacuajos conespecíficos.

Palabras claves. Amphibia, Chocó, ecología, experimentos de campo, regeneración forestal, cuidado parental.

Introduction

Fitness is a measure of the reproductive success of an individual, and it is measured through survival, mating success, and (surviving/reproducing) number of offspring (FUTUYMA & KIRKPATRICK 2017). Due to the typically high mortality rates among eggs and larvae, breeding site selection is a critical component of reproductive success in amphibians, directly influencing offspring survival and thus fitness (RESETARITS 1996, JANZ 2002, REFSNIDER & JANZEN 2010, PINTAR & RESETARITS 2017). The majority of anuran species undergo an aquatic larval stage, with many depositing their eggs in aquatic environments (WELLS 2007). However, various anurans exhibit terrestrial oviposition, wherein eggs are laid on land rather than in water (e.g. HADDAD & PRADO 2005, DUELLMAN 2007, CRUMP 2015). The evolution of this reproductive adaptation was potentially favored by reduced predation and competition, particularly from aquatic predators and other anuran larvae (MAGNUSSON & HERO 1991). Most species with terrestrial egg deposition are rainforest dwellers (WELLS 2007). The humid conditions of rainforests minimize the desiccation risk of terrestrial deposited eggs (SUMMERS & TUMULTY 2014). If larvae cannot finish metamorphosis terrestrially (WELLS 2007), they have to hatch (before metamorphosis) and either be able to move independently to water (OLDHAM 1977), or be transported by one or both of their parents to a suitable aquatic habitat. Terrestrial oviposition and parental care strategies, such as tadpole transport, is often linked in anurans (GOMEZ-MESTRE et al. 2012, RINGLER et al. 2023), and have gained considerable attention (e.g. RINGLER et al. 2013, PEIGNIERA et al. 2023), as obligatory tadpole transport provides the parents the possibility to select suitable breeding sites. For instance, PAŠUKONIS et al. (2016, 2019) showed that tadpole transporting frogs often bypass nearby pools and move to more distant sites. The authors therefore assume that tadpole transport is strategically planned and coordinated. Olfactory cues seem to play a critical role in the discovery and evaluation of breeding sites (SERRANO-ROJAS & PAŠUKONIS 2021). Chemical signals from predators, including conspecific, cannibalistic tadpoles, have been shown to play a major role in breeding site selection (SCHULTE et al. 2011, PAŠUKONIS et al. 2016, PEIGNIERA et al. 2023). The Neotropical poison frogs, Dendrobatidae, is a frog family where terrestrial oviposition and subsequent tadpole transportation to breeding sites, such as small streams, puddles or phytotelmata, is the rule. However, despite numerous advances in understanding the behavioral and physiological aspects of tadpole transport (RINGLER et al. 2013, BECK et al. 2017, FISCHER et al. 2019), the factors influencing breeding site selection in Dendrobatidae experienced relatively little research.

In this study, we aimed to investigate the influence of abiotic and biotic factors on the breeding site choice of an Ecuadorian dendrobatid species of the genus *Epipedobates* (see below for taxonomy). *Epipedobates* exhibits a reproductive strategy characterized by territorial behavior, terrestrial oviposition, and subsequent male-mediated tadpole transport to small bodies of water (LÖTTERS et al.

2007). However, the specific criteria and mechanisms underlying site selection in most *Epipedobates* remain unknown. In this study, we conducted a field experiment with artificial breeding sites in the Ecuadorian Chocó rainforest to address the following four questions: (1) Which habitat factors influence breeding site choice for tadpole deposition in *E. aff. espinosai*? (2) Does *E. aff. espinosai* assess the water holding capacity of breeding sites over time? (3) Does *E. aff. espinosai* avoid high tadpole densities to minimize competition? (4) Does *E. aff. espinosai* locate and use newly available breeding sites?

We hypothesize that, in addition to predator presence, water level and stability of breeding sites are key determinants of site selection (1), as desiccation is lethal to the offspring. Consequently, we expect habitat factors associated with water stability to have the greatest influence on site selection. Several anuran species have demonstrated the ability to assess the water-holding capacity of breeding sites by monitoring potential sites repeatedly (SPIELER & LINSSENMAIR 1997, RUDOLF & RÖDEL 2005). Given the high desiccation risk of small, ephemeral water bodies, typically used by *E. aff. espinosai*, we hypothesize that this species can assess the water-holding capacity of breeding sites over time (2). Many dendrobatid species, including *E. aff. espinosai*, are specialized in utilizing very small bodies of water for tadpole deposition. In addition to the lethal risks of desiccation and predation, these microhabitats are typically low in nutrient availability (POELMAN et al. 2013). Therefore, we hypothesize that sites with high tadpole densities are avoided to reduce intraspecific competition (3). Although tropical rainforest habitats are typically characterized by high precipitation, our observations indicate that small water bodies, persisting long enough to support complete tadpole metamorphosis, are relatively scarce. We therefore propose that suitable breeding sites represent a limited and temporary resource, forcing individuals to search for and exploit novel breeding sites, even at considerable distances from established breeding areas (4).

Material and methods

Study species

Our study species is genetically closest, but probably not conspecific with *Epipedobates espinosai* (FUNKHOUSER, 1956). Recent investigations revealed that our populations likely comprise an undescribed, cryptic species, *E. aff. espinosai* fide LÓPEZ-HERVAS et al. (2024). Both species are small (snout–vent lengths ~ 20 mm), diurnal, terrestrial dendrobatid frogs, endemic to the rainforests of the Pacific coastal slopes of north-western Ecuador, from about sea level to 1000 m (ALMENDÁRIZ & CISNEROS-HEREDIA 2016, LÓPEZ-HERVAS et al. 2024, COLOMA & DUELLMAN 2025). We herein adapt the taxonomy of LÓPEZ-HERVAS et al. (2024). Morphologically our study species is intermediate between the descriptions provided for *E. espinosai* and *E. boulengeri* by ALMENDÁRIZ & CISNEROS-HEREDIA (2016), COLOMA et al. (2023a, b), and COLOMA & DUELLMAN (2025).

Reproduction of *E. aff. espinosai* at our study site occurs during the entire wet seasons, when females lay clutches in the leaf litter. These clutches are presumably guarded by a territorial male until the tadpoles hatch, whereafter the male transports the tadpoles (Fig. 1) on its back and deposits them in small water bodies for further development. We observed between one to more than 10 (then difficult to count without removing tadpoles) tadpoles transported at a time (Fig. 1). ALMENDÁRIZ & CISNEROS-HEREDIA (2016) assume that *E. espinosai* tadpoles develop in pools of slow flowing small streams. In our study populations we observed the frogs to deposit tadpoles in minute temporary puddles on the forest floor. Rarely they deposited tadpoles in rocks pools, close to but not connected to streams. The species was abundant in closed-canopy secondary and old-growth forest, but rare or absent in more open habitats (NEIRA-SALAMEA et al. 2026).

Study area

The study was conducted in the Canandé Reserve, managed by the Jocotoco Foundation (www.jocotoco.org.ec). The reserve is located in north-western Ecuador and encompasses a mosaic of old-growth and secondary lowland evergreen rainforests, as well as cacao plantations and pasture from 130–540 m above sea level (ESCOBAR et al. 2025, METZ et al. 2026). This habitat is part of the Chocó biome, an ecoregion stretching along the Pacific coasts of Panama, Columbia, and Ecuador. The Chocó rainforest is recognized as one of the world's biodiversity hotspots, representing an endangered ecosystem with high biodiversity (Myers 2000). This habitat experiences high humidity with annual precipitation of 4,400–5,500 mm, a pronounced rainy season from January to May, and a relatively drier period



Figure 1. Living male *Epipedobates* aff. *espinosai* transporting several tadpoles on its back in the Canandé Forest Reserve, Ecuador.

Table 1. Locations and elevations (in m above sea level) of the four plots with artificial breeding sites (compare Fig. 2) to examine tadpole deposition site selection of *Epipedobates* aff. *espinosai* in the Canandé Forest Reserve, Ecuador.

Plot	Coordinates	Elevation [m]
#1	0.52127°N, -79.21231°W	403
#2	0.52402°N, -79.21283°W	347
#3	0.52035°N, -79.21094°W	405
#4	0.52615°N, -79.21011°W	315

from July to November (RIVAS-TORRES & SIERRA 2020, NEWELL et al. 2026). Our study was conducted during the final phase of a warm El Niño–Southern Oscillation event. This weather phenomenon can significantly influence precipitation patterns in Ecuador's Pacific coastal rainforest, typically resulting in above-average rainfall (THIELEN et al. 2023).

Breeding site choice experiment

This experiment aimed to identify the key factors influencing breeding site selection in *E. aff. espinosai*, as well as to determine whether the species can differentiate between breeding sites with differing water holding capacity. Therefore, we established artificial breeding sites (aBS) and assessed several variables, known to be important for anuran development. This included water depth, water turbidity, presence of other species (i.e. predators) and conspecific tadpoles, sun exposure, forest floor vegetation density, elevation differences within a plot, and the distance to the nearest natural breeding site (nBS).

Four spatially separated plots were established (Table 1) within the secondary forest surrounding the research station. Plots were selected based on three main criteria: (1) the presence of an *E. aff. espinosai* population, (2) the availability of nBS, and (3) accessibility from the research station, i.e. ensuring the possibility for daily data collection. To ensure the independence of all plots, i.e. to cover different *E. aff. espinosai* populations, plots with the greatest spatial distance (plot center distances ranging between 190 and 700 m) have been chosen from all the suitable plots in the area. nBS within the plot areas primarily consisted of human and animal footprints in muddy sections of trails. The surface sizes of these breeding sites ranged from 95 to 700 cm², with depths varying from 3 to 8 cm. The areas were identified by listening for calling males and through observations of male *E. aff. espinosai* depositing tadpoles. The most persistent nBS in each plot was chosen as our plot center point (Fig. 2). This center was the reference point for all plot measurements. We used black rectangular plastic containers as aBS. These polypropylene containers have a volume of 1000 mL, a surface area of 250 cm², and a depth of 6.6 cm (exact measurements: 188 × 133 × 66 mm; www.packpack.de). These containers are manufactured for the use in the food industry and are therefore free of chemical

additives. This minimizes potential effects on tadpole development and reduces olfactory cues that may result from evaporation.

To simulate variable water-holding capacities, half of the aBS (N = 20 per plot) were modified prior to the plot setup. Modifications involved piercing one hole on each side of the container using a 0.8 mm medical cannula. These holes were positioned approximately 1 cm above the bottom of the container to allow water outflow while preventing complete desiccation and ensuring the survival of deposited tadpoles. Due to variation in soil structure and occasional clogging of the holes by sediment, the modified aBS showed differing degrees of reduced water-holding capacities. Consequently, we abandoned our original idea of comparing two discrete groups (good versus bad water holding aBS) and based our statistical analyses on actual, individual water level measurements for each aBS.

All containers, both modified and unmodified, filled equally during rainfall events, whereafter the modified aBS generally exhibited a faster rate of water loss. Each plot contained 40 aBS, of which 20 have been modified. One modified and one non-modified aBS were always arranged in pairs. aBS pairs were arranged in a grid of five rows and four columns parallel to the trail, with the plot center in the middle. The distance between the center point and aBS

pairs, as well as between aBS pairs was 5 m. In total, each plot covered an area of 400 m² (Fig. 2). Each aBS was buried in the forest soil, until the upper aBS rim leveled with the surrounding soil. A small branch and a leaf were positioned reaching into each aBS to provide structural support for frogs during tadpole deposition and to facilitate exit of the aBS (Fig. 2).

The plots were established from 7 to 12 March 2024, and maintained until 13 May 2024. After establishment, the plots were left undisturbed for a minimum of two weeks to allow the aBS to accumulate rainwater and to provide time for the frogs to discover and accustom to the new breeding sites. Eight aBS (four modified and four unmodified) were excluded from data collection and analyses of plot 4 after they repeatedly merged into one big puddle after heavy rain, thus allowing tadpoles to freely disperse between the aBS. From 23 March to 13 May 2024, all plots were visited every three days to record the following parameters for each aBS: water level (water depth [cm]), the number of tadpoles, water turbidity, and the presence of other species. A small net was used to capture all macrofauna in each aBS. Tadpoles were counted and all other specimens were identified to the family level and classified regarding their ecological relevance for tadpoles (i.e. predators) before they were released back into the same aBS.

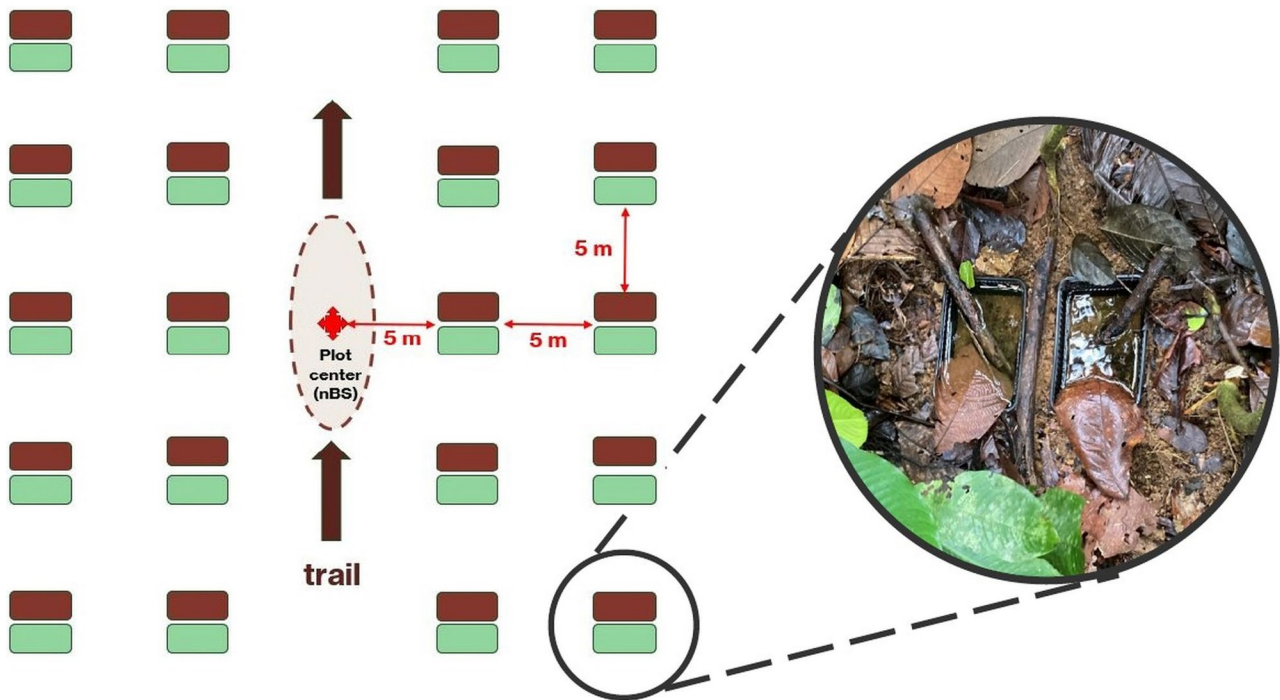


Figure 2. Scheme of the experimental design of artificial breeding sites for *Epipedobates* aff. *espinosai*. Left side: Plot setup with 40 artificial breeding sites. Each rectangle represents one breeding site. Brown colored squares represent modified artificial breeding sites (4 × 0.8 mm holes were drilled 1 cm above the bases to reduce water-holding capacity), green colored squares represent artificial breeding sites without modification. The natural breeding site is represented by the brown dotted ellipsoid with the red star indicating the plot center point. Red arrows illustrate the distance between artificial breeding site locations (5 m). Right side: Two artificial breeding sites (polypropylene container) within an established plot. The picture was taken directly after rainfall, thus both breeding sites (modified and unmodified) are filled equally with water.

Male *E. aff. espinosai* carry and deposited between one and more than 10 tadpoles at a time (Fig. 1). As the breeding sites were not continuously observed, we could not be sure if e.g., 10 additional tadpoles, on a consecutive visit, were due to one male depositing 10 tadpoles or, in extreme, 10 males depositing one tadpole each. We therefore registered each increase in the number of tadpoles between two visits of the site and within each individual aBS as a single deposition event.

Data about sun exposure, forest floor vegetation density (at a 1 m radius around each aBS), and elevation differences within the plot were collected for each aBS at the start of the data collection period. Sun exposure, vegetation density, and water turbidity were estimated in % and assigned to one of four categories (1 = 0–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%). To determine the elevation differences within the plot, i.e. the elevation difference between each aBS and the nBS at the plot center, a string was horizontally stretched in a straight line from the higher point to the corresponding lower point. The vertical distance [m] between the string and the ground at the position of the corresponding lower point was measured using a BOSCH UniversalDistance 40C digital laser rangefinder (Fig. 3). The linear distance [m] from each aBS to the central nBS was calculated using the known horizontal layout of the plot and the Pythagorean theorem:

$$c = \sqrt{a^2 + b^2} \text{ (Fig. 3).}$$

Colonization experiment

This experiment was designed to test our hypothesis that suitable breeding sites are a limited resource for *E. aff. espinosai*. Specifically, it aimed to assess whether, and how rap-

idly, novel sites are detected and used in areas previously lacking natural breeding sites for *E. aff. espinosai*.

Three experimental plots were established within an older secondary forest, in areas where our field work revealed no presence of *E. aff. espinosai* or other dendrobatid species. This monitoring took place between 14 and 26 March. During five monitoring walks we searched visually and acoustically for dendrobatid species in that area. The selected three sites lacked any aquatic habitats in the immediate surroundings (~ 100 m radius) and were spatially isolated from each other (distance between 300 and 600 m). Each plot comprised four unmodified aBS, identical to those described above. The containers were arranged in a square configuration with a 2 m distance between them, covering a total area of 4 m², and installed as described above.

The three plots were established on 27 March 2024, and checked until 13 May 2024. Observations were conducted on 15 April, 1 May, and 13 May 2024. The data collection (presence and numbers of tadpoles and the presence of other species) was conducted as in the breeding site choice experiment (see above). At the end of our study, all tadpoles in the aBS were released into the nearest nBS. Subsequently, all aBS were removed from the forest.

Statistical analysis

All statistical analyses were conducted in R v. 4.4.2 (R Core Team 2024) using the integrated development environment RStudio v2024.09.1 (Posit Team 2024). To address which are the key habitat factors in site selection and if *E. aff. espinosai* can assess the water-holding capacities of breeding sites over time, we performed two Generalized Linear Mixed Models (GLMM). The first model aimed to

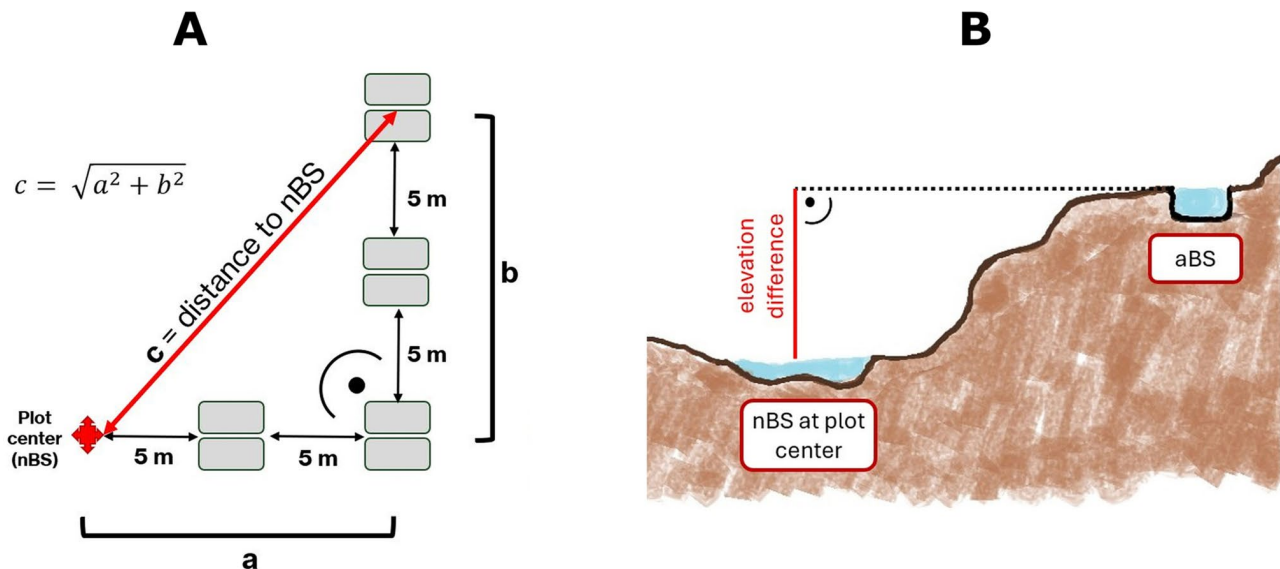


Figure 3. A graph and a sketch, illustrating (A) the calculation of distances between artificial breeding sites (aBS) and aBS and the natural breeding site (nBS), and (B) elevation differences between an artificial breeding site (aBS) and the natural breeding site (nBS); compare text. The grey boxes in (A) represent aBS within the plot layout (compare Fig. 2).

identify abiotic factors influencing individual tadpole deposition events. The GLMM was run with the individual tadpole deposition events in each aBS (yes = 1, no = 0) as a binomial response distribution, using the `glmer` function in the `lme4` package (BATES et al. 2015). It included the following predictor variables: water level, elevation differences within plot, distance to the nearest nBS, sun exposure, vegetation density, water turbidity, and previous presence or absence of tadpoles.

To account for spatial effects and nested data structure, “plot” (representing the four experimental plots) was incorporated as a random effect and “group” (representing each position where a modified and unmodified aBS are placed next to each other; Fig. 2) nested within “plot” as an additional random effect. The model was fitted using the ‘bobyqa’ optimizer to improve convergence. To evaluate model performance, a model comparison with a fitted null model was performed using the Akaike Information Criterion (AIC). A Likelihood Ratio Test (LRT) was used to assess the significance of model terms. Model diagnostics were conducted using the DHARMA package (Harting 2024). Simulated residuals were generated to evaluate model fit and perform diagnostics on residual distribution (examining a quantile-quantile plot), residual independence (examining a residuals- versus fitted values plot), overdispersion (using: `testDispersion()`), zero-inflation (using: `testInflation()`) and autocorrelation (using the autocorrelation function ACF). A test for collinearity was performed using the `vif` function from the `car` package, and a correlation matrix was created using the `cor` function to test correlations between the predictor variables.

The second model was based on a dataset in which all continuous observations (number of tadpole deposition events and water level) were summarized for each individual aBS. This approach allowed for the analysis of overall breeding site use frequency (number of tadpole deposition events) in relation to abiotic habitat characteristics. Additionally, it allowed the integration of derived hydrological variables such as the standard deviation of water level (water persistency) and mean water loss (desiccation risk) for each aBS. A GLMM was fitted using the `glmmTMB` function. The response variable, number of tadpole deposition events, was modeled using a negative binomial distribution (‘`nbinom2`’) to account for potential overdispersion in count data. The model included the following predictor variables: mean water level, standard deviation of the water level, mean water loss (all calculated for each aBS over the whole observation period), sun exposure, elevation differences within plot, vegetation density, and distance to the nearest natural breeding site. To account for spatial effects and nested data structure, nested random effects (“plot” and “group”) were incorporated identically to the analysis above. Presence/absence of tadpoles could not be included, as we were focusing on the summary of data of each aBS, and not on single deposition events. However, this approach allowed the analysis of tadpole deposition frequency in relation to hydrological site characteristics, specifically water stability indicators such as water level fluctuations

and average water loss. Model diagnostics were conducted identically to the first analysis.

In each of the four experimental plots, breeding site positions consisted of paired aBS, one modified and one unmodified, placed next to one another (Fig. 2); allowing tadpole transporting individuals to choose between breeding sites. In order to investigate the influence of water level on breeding site choice, water levels between used and unused sites were compared with a T-test. This analysis was restricted to those pairs in which deposition occurred in only one of the two aBS, thereby reflecting a presumed choice by the frogs.

Results

Breeding site choice experiment

Overall, 152 aBS were checked for water-holding capacity, with an equal number of modified and unmodified containers. The water-holding capacity was quite variable (Fig. 4). While the majority of unmodified aBS showed water levels around 5 cm, the modified aBS showed a wider range. However, due to factors such as soil structure and clogging of drainage holes, there was a large overlap between the two treatment groups. Consequently, we based our further analyses on the measured water levels of the individual aBS.

Overall, we observed tadpole depositions in 79 aBS, with 49 of them being used multiple times. In total, we recorded 175 tadpole deposition events, of which 73 occurred in modified aBS and 102 in unmodified aBS (Table 2).

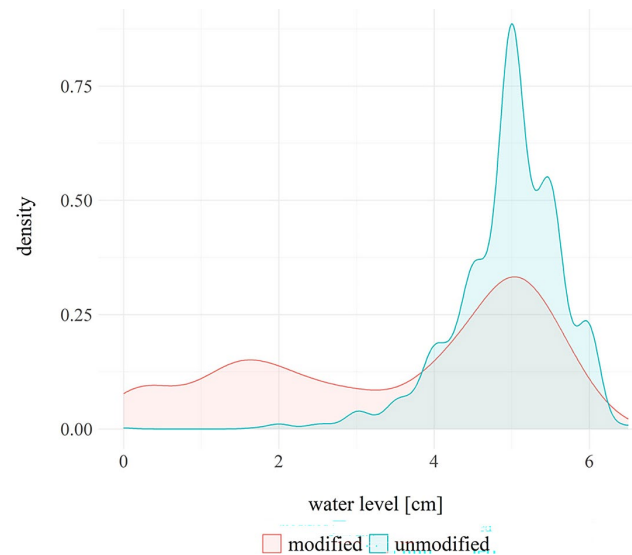


Figure 4. Kernel density estimates of all recorded water level measurements for modified (red; compare text) and unmodified (blue) artificial breeding sites (aBS). The x-axis gives water level (i.e., water depth) in cm, the y-axis represents the estimated probability density. Each curve reflects the distribution of water levels within its respective aBS group (modified or unmodified), with the area under each curve normalized to one.

Apart from *E. aff. espinosai* tadpoles, only two other taxa of macrofauna were detected, larvae of Culicidae and small Dytiscidae (< 5 mm). These taxa were persistently found throughout all plots and likely did not prey on tadpoles. Consequently, we do not take them further into consideration.

With the first GLMM model we aimed to identify factors influencing the probability of tadpole deposition. The model included habitat characteristics and continuous measurements of abiotic habitat variables recorded for each aBS, on each control visit, as well as a tadpoles' presence or absence prior to the deposition event. Table 3 summarizes the range (minimum and maximum), mean and variance for all predictor variables included in the analysis. In the GLMM three factors revealed significant effects on the probability of tadpole deposition (Table 4). The presence of tadpoles revealed a positive effect, indicating that aBS that already contained tadpoles were more likely to receive further tadpole depositions. The water level had a positive effect, indicating that aBS with higher water levels were more likely to receive tadpole depositions (Fig. 5). Despite a non-uniform data distribution, with a pronounced density peak at 5 cm water level, the values showed a sufficient range (Table 3) to support this effect. The elevation differences within a plot revealed a negative effect, indicating that *E. aff. espinosai* preferred aBS at lower elevations, relative to the respective nBS in the plot center (Fig. 5). This variable ranged from -1.7 to 2.3 m, with the density distribution showing a peak at 0 m. The distance to the nearest nBS, with a relatively high variance of 7.95 m, revealed only a small and non-significant effect on tadpole deposition, suggesting that the distance between aBS and nBS did not influence site selection. The measured abiotic factors (Table 3), sun exposure, vegetation density, and water turbidity, all showed low variance and revealed no significant effect on tadpole deposition probability (Table 4).

The LRT revealed that adding a nested random effect to the GLMM did not significantly improve the model ($p = 1$). However, based on AIC and deviance, the model with the nested random effect structure was chosen as the best-fitting one. The corresponding null model was rejected by comparing AIC values ($AIC_{GLMM} = 1179.83$; $AIC_{NULL} = 1217.60$). The model diagnostics revealed no deviations from the 45-degree line in the quantile-quantile plot, no signs of overdispersion (dispersion parameter = 1.0256, $p = 0.8$), and no evidence for zero-inflation (observed-to-simulated ratio = 0.9976, $p = 0.8$). An ACF plot of the residuals indicated no significant autocorrelation or collinearity (all values < 1.1), or pairwise correlation (all values < 0.2).

With the second GLMM, we investigated the frequency of aBS use (predicted tadpole depositions) by summarizing all observations across the monitoring period for each individual aBS. This approach made possible the analysis of tadpole deposition frequency in relation to hydrological site characteristics, specifically water stability indicators such as water level fluctuations and average water loss (Table 5). The model revealed a significant negative effect of elevation differences within plots, and a significant positive

Table 2. Number of all monitored artificial breeding sites (aBS), modified and non-modified ones (see text). "used aBS" represents the breeding sites in which at least once *Epipedobates aff. espinosai* tadpoles have been deposited. "number of tadpole depositions" represents the total number of tadpole deposition events.

	Total	Modified	Unmodified
number of aBS	152	76	76
used aBS	79	38	41
aBS used more than once	49	21	28
number of tadpole depositions	175	73	102

Table 3. Value range of all predictor variables included in the first GLMM model; tadpoles were included as present versus absent (1/0); for further details compare text.

	Min. value	Max. value	Mean	Variance
Water level [cm]	0	6.50	4.25	2.36
Elevation differences within plot [m]	-1.70	2.30	0.26	0.84
Distance to nearest nBS [m]	5.00	14.10	10.10	7.95
Sun exposure [4 categories]	1.00	3.00	1.88	0.53
Vegetation density [4 categories]	1.00	4.00	2.39	0.53
Water turbidity [4 categories]	1.00	4.00	1.27	0.37

Table 4. Results for all predictor variables of the first GLMM model using individual *Epipedobates aff. espinosai* tadpole deposition as binomial response and the random nested effects "plot" and "group" (nested within "plot"). The predictor variables tadpoles (presence/absence), water level and elevation differences within plot showed significant effects on the tadpole deposition, while no effect was observed for the other variables. Data distributions of predictor variables are listed in Table 3.

Generalized Linear Mixed Model (binomial)				
	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-415.487	0.7866	-5.282	< 0.0001
tadpoles present (yes/no)	0.6669	0.2156	3.094	0.0020
water level	0.2759	0.0759	3.634	0.0003
elevation differences within plot	-0.4355	0.1540	-2.829	0.0047
distance to nearest nBS	-0.0251	0.0442	-0.567	0.5706
sun exposure	0.0513	0.1719	0.298	0.7655
vegetation density	0.0123	0.1727	0.071	0.9432
water turbidity	-0.0733	0.1458	-0.503	0.6151

effect of mean water level on aBS use frequency. These results indicated that aBS located at lower elevations, relative to the central nBS within a plot, as well as those maintaining higher average water levels, were more frequently used for tadpole deposition. None of the additional predictor

variables, including water level standard deviation, mean water loss, sun exposure, vegetation density, and distance to the nearest nBS, showed a significant effect (Table 5). These results suggest that variability in water stability (water level standard deviation and mean water loss), sun exposure, vegetation density, and the distance to the nearest nBS did not affect on how often a given aBS was used for tadpole deposition.

The model diagnostics confirmed an adequate fit of the data and showed no signs of overdispersion (dispersion parameter = 0.514, $p = 0.4$) and no evidence for zero-inflation (observed-to-simulated ratio = 0.999, $p = 1.0$). An ACF plot of the residuals demonstrated no significant autocorrelation. A high correlation was observed between mean water loss and water level standard deviation (correlation value = 0.769); however, no critical collinearity could be detected (vif for water level standard deviation = 3.15). As these two predictor variables represent distinct and biologically relevant aspects of hydrological BS conditions – specifically, the rate of water loss and the degree of fluctuation in water levels – both variables were kept in the model based on their relevance.

We further compared the influence of water level of aBS pairs (the two aBS directly adjacent to each other) on the breeding site choice in *E. aff. espinosai*. This analysis was restricted to aBS pairs in which tadpoles were deposited in only one of the two aBSs ($n = 101$). The water level differences between paired aBS ranged from 0 to 5.5 cm, with a mean of 1.6 cm and a variance of 2.6 cm. The comparison revealed that aBS used for tadpole deposition showed significantly higher water levels than the unused neighboring sites ($p = 2 \times 10^{-6}$; $n=101$; Fig. 6).

We originally hypothesized that *E. aff. espinosai* avoids sites where tadpoles are already present, in order to reduce potential intraspecific competition. However, we observed repeated tadpole depositions in 49 aBS (62% of all used aBS), and actually could show (above), that tadpole presence increased the probability of new tadpole depositions. Among occupied breeding sites, the number of tadpoles ranged from one to 27 individuals (mean = 5.81). Corresponding tadpole density ranged from 0.8 to 18 tadpoles per liter (mean = 4.7). Within these 49 aBS we detected no statistically significant difference concerning the number of deposited tadpoles, relative to the number of already

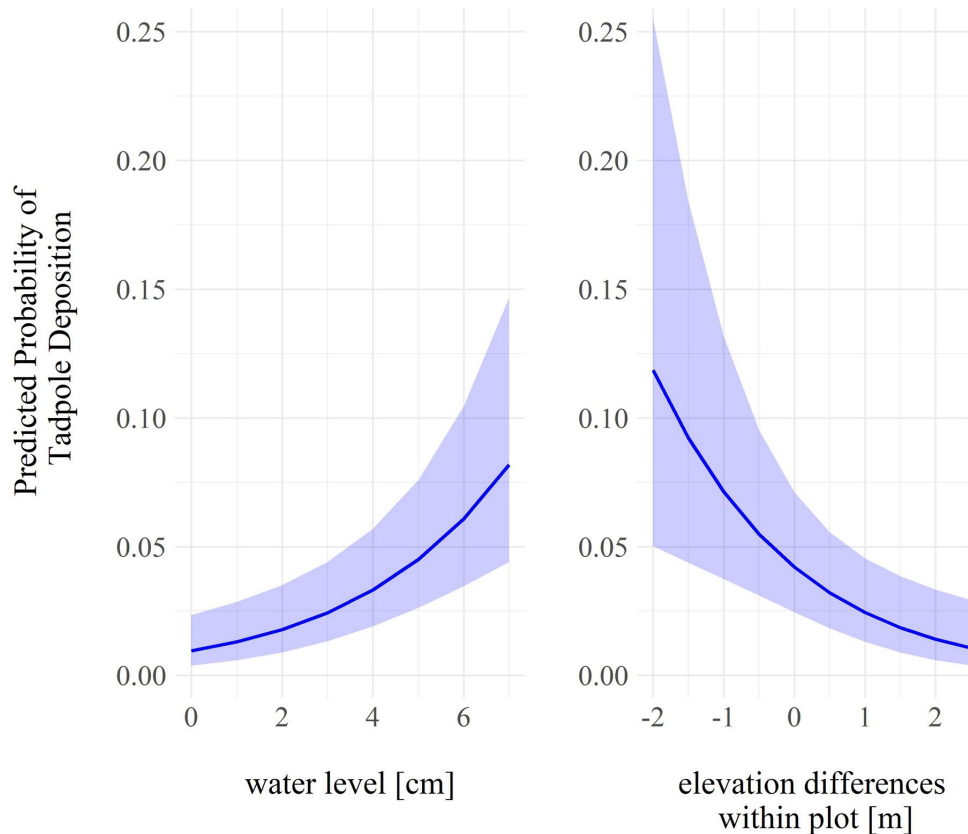


Figure 5. Marginal effect plots visualizing the influence of the abiotic predictors “water level” and “elevation differences within plot” on the probability of *Epipedobates aff. espinosai* tadpole deposition. The water level showed a strong positive effect, with the predicted probability increased at higher water levels. The elevation differences within plot, i.e. the elevation difference between the individual artificial breeding site towards the natural breeding site at the plot center, exhibited a negative trend, indicating higher deposition probabilities at lower sites. Confidence bands are represented in light blue and indicate the certainty of the prediction. The y-axis was standardized across all plots to facilitate direct visual comparison of effect strengths.

Table 5. Results for all predictor variables of the second GLMM model using the number of *Epipedobates aff. espinosai* tadpole depositions as response and the random nested effects “plot” and “group” (nested within “plot”). A significant effect was observed for mean water level and elevation differences within plot, indicating that artificial breeding sites located at lower elevations relative to the central natural breeding site, as well as those maintaining higher average water levels, experienced a higher number of tadpole deposits. No significance was observed for the other variables.

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-1.496.925	1.083.396	-1.382	0.1671
mean water level	0.3489	0.1456	2.396	0.0166
water level standard deviation	0.1090	0.3316	0.329	0.7423
mean water loss	0.1525	0.9632	0.158	0.8742
sun exposure	0.0025	0.1925	0.013	0.9895
elevation differences within plot	-0.4537	0.1655	-2.741	0.0061
vegetation density	-0.0212	0.1929	-0.11	0.9124
distance to nearest nBS	-0.0349	0.0491	-0.711	0.4771

present tadpoles. However, there was a trend that aBS containing already a high number of tadpoles, tended to receive a higher number of tadpoles in the following deposi-

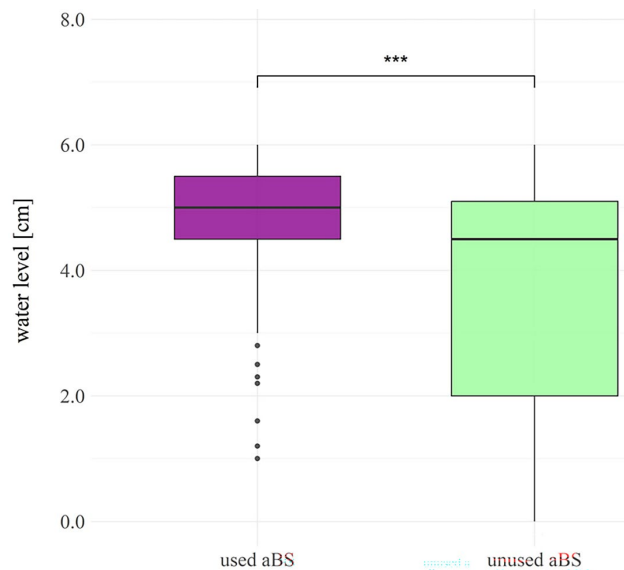


Figure 6. Box plot comparing water levels between artificial breeding sites (aBS) between BS pairs ($n = 101$ pairs; compare Fig. 2). The analysis was restricted to events in which *Epipedobates aff. espinosai* tadpoles were deposited in only one of the two neighboring aBS. The purple boxplot (used aBS) represents water levels of aBS that received tadpoles, while the green boxplot (unused aBS) represents the corresponding unused aBS. Statistical analyses were performed using a T-test. Frogs significantly preferred the aBS with higher water level ($p < 0.0001$ $n = 101$).

tion event. On average, aBS with fewer than five tadpoles prior to deposition received two or fewer additional tadpoles. In contrast, sites already containing 6, 7, 10, or 21 tadpoles received a minimum of three new tadpoles on average. The highest number of newly deposited tadpoles (11) was recorded in aBS that contained 10 tadpoles prior to the deposition event. These results at least indicated that *E. aff. espinosai* may tend to deposit a larger number of tadpoles in breeding sites that already contain (a high number of) conspecific tadpoles.

Finally, we analyzed the temporal patterns of repeated tadpole depositions. The majority of repeated depositions occurred shortly after the previous event; 65.9% took place within six days, including 34 depositions within three days and 20 depositions within six days. Beyond a six-day interval, the number of depositions remained consistently low, with fewer than six depositions per time point. This may indicate that *E. aff. espinosai* preferentially selects breeding sites containing conspecific tadpoles of similar developmental stages over sites with more mature tadpoles.

Throughout our study, we observed metamorphosis of 15 specimens. The average developmental time from first observation in an aBS to metamorphosis was 37 days. Given that monitoring occurred in three-day intervals, this estimate encompasses a potential margin of error of $\pm$ two days.

Colonization experiment

With this experiment we aimed to investigate whether and how fast *E. aff. espinosai* can detect and utilize newly available breeding sites, located far from established ones. We therefore established aBS at three sites (plots) where the species in previous surveys was not observed. Nineteen days after plot establishment, tadpoles were observed in two aBS within plot 1. By day 35, all plots contained aBS with tadpoles. At the final visit, 47 days after plot establishment, all but one aBS contained deposited tadpoles (Fig. 7). While plot 1 and 3 showed a continuous but gradual increase in deposited tadpoles, a rapid and substantial increase in tadpole numbers could be observed in aBS 1 and 4 of plot 2 (Fig. 7). Thus, all plots were quickly discovered and repeatedly used for tadpole deposition.

Discussion

In this study we investigated the tadpole deposition site selection of *Epipedobates aff. espinosai*. Specifically, we examined the influence of abiotic factors on site choice, the species' potential ability to assess water-holding capacity over time, and the role of conspecific tadpoles. Using a controlled setup of artificial breeding sites within a natural rainforest environment, we analyzed both the likelihood of tadpole deposition and the frequency of site use in relation to key environmental variables. Our results identify water level, microtopographic position and the presence of

conspecific tadpoles as primary predictors of breeding site choice. We found no evidence that *E. aff. espinosai* assesses long-term hydrological stability, and the positive effect of conspecific tadpoles was in contrast to our original hypothesis that frogs may avoid those sites.

Breeding site choice experiment

We hypothesized that water level and stable hydrological conditions are key determinants for the breeding site selection in *E. aff. espinosai*. This hypothesis was based on desiccation being a major mortality factor for aquatic tadpoles developing in ephemeral waters (e.g. SMITH 1983, NEWMAN 1988, MURPHY 2003, TOUCHON 2012). Accordingly, we expected both the actual water level and variables related to water stability to significantly influence breeding site selection.

Our findings supported the hypothesis that water level is a critical factor in breeding site choice. Breeding sites with higher water levels were significantly more likely to receive tadpole depositions and were used more frequently over time. This conclusion was further supported by a direct comparison of water levels of aBS pairs at the time of tadpole deposition. Focusing exclusively on events where tadpoles were deposited in only one of the two neighboring sites, frogs significantly more often chose the aBS with a higher water level for tadpole depositions. This finding is consistent with previous research on another dendrobatid frog, *Ranitomeya biolat*, which showed that tadpole depositions were significantly more likely in breeding sites with high water levels (MAY et al. 2009).

Contrary to our expectations, variables associated with hydrological stability (e.g. water level fluctuation and mean water loss) did not influence breeding site selection. As these parameters can only be assessed through repeated observations over time, our findings suggest that *E. aff. espinosai* males primarily relay on immediate, perceivable environmental cues assessable during the process of tadpole transport and deposition, rather than on prior monitoring of water holding capacity of different sites. This finding is further discussed below. Also in contrast to our original hypothesis was the positive effect of conspecific tadpoles on breeding site choice. We assumed that, in order to avoid competition for limited food resources, males would avoid sites which contain tadpoles. We discuss this further below. Other habitat variables, including sun exposure, vegetation density in the surrounding of breeding sites, and water turbidity, were not found to influence neither the likelihood of tadpole deposition nor the frequency of breeding site use. This lack may however, be attributable to limited variance in these variables within our experimental setup. Previous other research revealed these factors to be relevant under different conditions. FOUILLOUX et al. (2023) for instance demonstrated that certain Aromobatidae, a sister group to Dendrobatidae, prefer clear water for tadpole depositions.

Our data also did not reveal an effect of the distance to the nearest natural breeding site on tadpole deposition patterns. Although home range sizes in Dendrobatidae may vary widely, e.g. ranging in *Phylllobates vittatus* from 6 to 55.7 m² (SUMMERS 2000, GARRIDO-PRIEGO et al. 2004), each of our experimental plots covered 400 m², with many distances to the nearest nBS far exceeding typical home range dimensions. Although we have not measured terri-

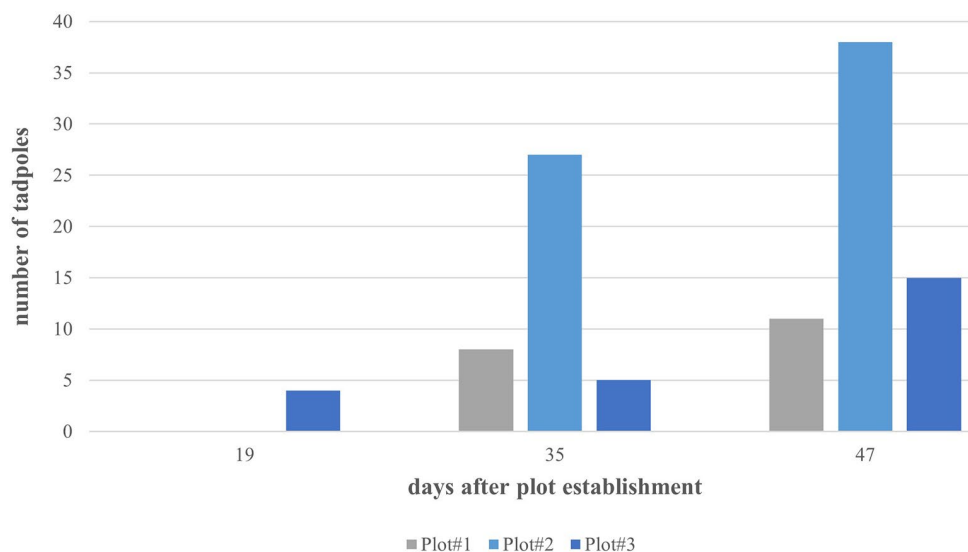


Figure 7. Cumulative number of *Epipedobates aff. espinosai* tadpoles recorded on three experimental plots (each with four artificial breeding sites) in areas where the species previously was not recorded. Each plot was visited three times in order to check for tadpoles. The x-axis indicates the three observation days, represented by the number of days since plot establishment. The y-axis gives the number of observed tadpoles. Each bar represents the sum of tadpoles recorded in all four artificial breeding sites within a given plot at a given observation day.

tory sizes, our results suggest that *E. aff. espinosai* males frequently use breeding sites beyond their territory. This interpretation is in line with research by PAŠUKONIS et al. (2016, 2019), demonstrating that tadpole-carrying *Ameerega trivittata* navigate several hundred meters beyond their established territories to deposit their tadpoles.

An unexpected result was that both GLMMs revealed a significant negative effect of relative elevation, meaning that frogs preferentially deposited tadpoles in sites located at lower elevations within the plots. This effect is likely linked to influence of topography on hydrology. In the steep terrain of the Canandé Rainforest Reserve, elevated areas experience rapid water runoff, while depressions or lower-lying areas are more likely to receive and retain water. However, in the aBS, water levels were independent of local elevation differences due to the usage of polypropylene containers impeding water runoff and limiting the water level to a defined maximum. The measured preference for lower positioned sites suggests that frogs may use micro-topography as an indirect cue for water availability and stability. We therefore assume that micro-topography is a relevant factor for breeding site choice in *E. aff. espinosai*.

We hypothesized that *E. aff. espinosai* can assess not only the current water level, but also the water holding capacity or long-term water stability of potential breeding sites – a potentially adaptive behaviour in response to the high desiccation risk associated with ephemeral bodies of water. For several anuran species, including the savanna dwelling *Hoplobatrachus occipitalis* and the tree-hole breeding *Phrynobatrachus guineensis*, it has been shown that individuals assess the water holding capacity of breeding sites by monitoring and revisiting potential sites (SPIELER & LINSSENMAIR 1997, RUDOLF & RÖDEL 2005). If *E. aff. espinosai* would do that as well, we expected the number of tadpole depositions to be associated with low water level fluctuations and reduced mean water loss.

However, our findings did not support this expectation. While the mean water level exhibited a weak positive effect on deposition frequency, neither water level fluctuation nor average water loss between (our) plot visits, both indicators of long-term water stability, significantly influenced site use. This suggests that *E. aff. espinosai* does not assess long-term hydrological stability. Instead, the species appears to select breeding sites based on immediate, directly perceivable factors that are indirectly linked to water availability, such as actual water level and micro-topography. Dendrobatids typically remain within defined home ranges, however, our results indicate that many males transport their tadpoles beyond the borders of their territories. If that is true, breeding sites probably cannot be visited regularly, and the frogs would need to rely on directly perceivable environmental cues for assessing site suitability. Water level and topography might be such easy to assess cues. Recent research on dendrobatids has also highlighted the role of chemical cues, including olfactory signals related to stagnant water, in breeding site detection (PEIGNIER et al. 2023). This strategy may be an adaptive response to the highly dynamic breeding site conditions of rainforest

habitats, where frequent and unpredictable fluctuations in water availability make continuous monitoring of breeding sites both inefficient and energetically costly. However, we could not test for chemical cues in our study.

An important aspect in breeding site selection is detecting the presence or absence of predators and competitors (BUXTON & SPERRY 2027, DIMITRIE & BENRAD 2023). Conspecific individuals will be particularly strong competitors, as they depend on identical resources (CHESSON 2000, DAVIS et al. 2011). Indeed, various studies on different anuran species have shown that ovipositing frogs avoid habitats containing conspecific eggs or larvae to minimize intraspecific competition (e.g. RESETARITS & WILBUR 1989, CRUMP 1991, SPIELER & LINSSENMAIR 1997). As the small breeding sites used by *E. aff. espinosai*, likely do not comprise a wealth of nutrients, we hypothesized that the males avoid sites with high conspecific tadpole densities and we expected fewer tadpole depositions in aBS already containing tadpoles.

Contrary to this prediction, we found no evidence for males avoiding sites with conspecific tadpoles. In fact, we observed that breeding sites already containing conspecific tadpoles were more likely to receive a high number of additional tadpoles. Moreover, repeated depositions at the same site occurred frequently and typically followed closely in time after a previous deposition event. Our results thus indicate that *E. aff. espinosai* may use the presence of conspecific larvae as a positive sign for favorable breeding site conditions. The strategy of selecting breeding sites based on the presence of conspecific larvae (conspecific attraction) is widespread across various taxa, including anurans, birds, butterflies, and mosquitoes. This behavior is usually interpreted that the presence of conspecific individuals indicates favorable feeding conditions and/or the absence of predators (WARD & SCHLOSSBERG 2004, RUDOLF & RÖDEL 2005, RAITANEN et al. 2014, BUXTON et al. 2020, SHARMA et al. 2025). Conspecific attraction likely is relevant in those dendrobatid species whose tadpoles do not exhibit carnivorous and cannibalistic behavior (FOLT et al. 2018, BUXTON et al. 2020). In the usually small breeding sites used by dendrobatids, predators typically could devour all offspring in little time. Thus, frogs may prioritize predator-free environments over minimizing competition, accepting the cost of increased food competition in exchange for a higher likelihood of offspring survival. As shown in recent studies chemical signals, including signals from tadpoles, may play a crucial role in the discoverability and quality discrimination of breeding sites (SCHULTE et al. 2011, PAŠUKONIS et al. 2016, SERRANO-ROJAS & PAŠUKONIS 2021, PEIGNIER et al. 2023). Consequently, breeding sites with strong olfactory cues resulting from high tadpole abundance may be more easily discovered by tadpole transporting individuals.

We found that tadpole deposition events at the same site, frequently occurred close in time after a previous deposition event. Given that younger and smaller tadpoles are generally outcompeted by older and larger conspecifics (RICHTER-BOIX et al. 2007, ASQUITH & VONESH 2012), it is likely that individuals preferentially selected breeding sites

containing recently deposited tadpoles of similar age. The observation that repeated depositions typically occurred within a seven-day interval supports this interpretation.

Colonization experiment

We hypothesized that suitable breeding sites for tadpole deposition are a limited resource. Dendrobatids use a large range of different breeding sites, from extremely small bodies of water within leaf axils of plants (phytotelmata), often inhabited by oophagous tadpoles (KAHN et al. 2016), to small temporary, or more permanent water bodies next to streams. Small water bodies may comprise water filled mammal footprints in muddy areas, or fallen palm leaves that accumulate water. Numerous, predictable breeding sites, such as mammal footprints on frequently used trails, often comprise a high and consistent density of poison dart frogs throughout the breeding period (authors pers. obs.). Most dendrobatids seem to be territorial, typically males defend territories that encompass optimal calling and reproductive sites (PRÖHL 1999; 2005). However, predictable breeding sites may be unexpectedly rare, even in a humid rainforest habitat. A study on *Limnonectes palavanensis*, a frog species exhibiting parental care and depositing tadpoles in pools, comparable to those of *E. aff. espinosai*, identified only 10 natural pools within a 1.3 ha study area in the high-precipitation rainforest of Borneo (VALLEJOS et al. 2019), indicating the scarcity of suitable sites. Because these sites are both rare and characterized by intense intraspecific competition of the tadpoles for food, we expected that some individuals would disperse into the forest and thereby detect novel sites, including some located at substantial distances from established populations.

Our results supported this assumption. In newly established breeding sites, at places the absence of *E. aff. espinosai* had been confirmed before, the first tadpoles were deposited within 19 days after plot establishment. We found tadpoles in all three plots after 35 days. Although showing different rates of colonization, the artificial breeding sites of all plots were successfully located and used for tadpole deposition, with a consistent increase in the number of tadpoles being observed over time. The observed variation in colonization rates among the three experimental plots is likely attributed to differences in local environment and topography, probably resulting in differences in numbers of floating *E. aff. espinosai*. Although all plots were positioned at a considerable distance (~ 100 m radius) from known breeding sites, plot 1 was located approximately 130 m from an area containing several small and stable breeding sites that comprised an established of *E. aff. espinosai* population. We also observed one *E. aff. espinosai* approximately 120 m from plot 3. When this plot was established we encountered no open water bodies. However, palm trees where present and in other places we have found dendrobatids (*Hyloxalus toachi*) using these leaves for breeding when they accumulated rainwater. No potential breeding sites were observed in the vicinity of plot 2, making it the

most isolated one. Still this plot was detected and used as well. After being detected this plot showed a rapid and pronounced increase in deposited tadpoles. The fast detection of our new sites and their intensive use, supports our hypothesis that such sites are a limited resource. However, our results also show that *E. aff. espinosai* is able to detect and colonize newly established sites in very short time.

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