

Factors influencing tadpole deposition site choice in a frog with male parental care: An experimental field study

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Abstract

Parents have evolved a variety of strategies to minimize risks to their offspring, including complex choices regarding suitable rearing sites, based on abiotic and biotic factors, which differentially affect offspring survival. Because availability and quality of these sites are variable, parents may have to choose between immediately available lower-quality rearing sites or extended search time. In some frog species with larval transport, parents are known to select bodies of water that are free of predators, cannibalistic tadpoles, or intraspecific competitors for larval deposition and rearing sites. We tested whether abiotic factors and the presence of predators and conspecific tadpoles affect tadpole deposition behavior in a population of smooth guardian frog, *Limnodynastes palauensis*, on the island of Borneo. Females lay eggs on land and males guard them until they hatch; after hatching, tadpoles climb onto the male's back and are subsequently transported to small pools of water on the forest floor, which are scarce and patchily distributed. We estimated the abundance of natural tadpole rearing sites in our study area and conducted experiments using artificial pools to test whether abiotic characteristics of these pools affect the probability of larval deposition. We also performed choice experiments to test whether males of *L. palauensis* avoid pools with conspecific tadpoles or predators. Lastly, we tested whether the tadpoles of this species exhibit cannibalism. The abundance of natural deposition sites was low, and males readily used artificial pools for tadpole deposition. Males were less likely to deposit tadpoles in artificial pools located in steep areas, and males did not avoid depositing tadpoles in pools with conspecifics or with experimentally introduced predators. Males exhibited clutch-partitioning behavior, dividing tadpoles between adjacent artificial pools. Pool availability, rather than the presence of potential competitors or predators in a pool, affects tadpole deposition decisions in this species.

KEYWORDS

amphibians, clutch-partitioning, conspecifics, *Limnodynastes palauensis*, parental care, predators

1 | INTRODUCTION

In species with parental care, parents have evolved a variety of strategies to maximize survival of their offspring (Clutton-Brock 1991, Royle, Smiseth, & Kölliker, 2012). Parents must choose when and where to breed and how much care to provide; such choices have direct consequences in terms of offspring life history and survival and ultimately impact parental reproductive success (Misenhelter & Rotenberry, 2000). In several vertebrate species, parents preferentially choose rearing sites that minimize risks to their offspring by selecting sites with low predation risk, low competition, protection from disease, and sufficient food resources (Martin 1993, Refsnider & Janzen, 2010; Spieler & Linsenmair, 1997; Welsh & Fuller, 2011). Shortage of rearing sites poses important selective pressures on the parents, as failure to find such places could incur in the complete loss of their offspring. Thus, natural selection will favor strategies that maximize the ability of parents to find suitable breeding or nesting sites (Jacot, Valcu, van Oers, & Kempenaers, 2009). While many birds and mammals provide parental care, with young remaining heavily dependent on parental provisioning or defense for extended periods (Reynolds, Goodwin, & Freckleton, 2002), amphibians exhibit parental care in only approximately 10% of all species (Royle et al. 2012), and our understanding of the costs and benefits of providing care is limited to only a handful of species.

In frog species with parental care, adults may place eggs or tadpoles in water-holding leaf axils of plants such as bromeliads, or in folds of fallen leaves, pitcher plants, and tree hollows (collectively termed “phytohelmata”), as well as in terrestrial sites such as small

forest floor rain pools and standing bodies of water near small streams (Wells, 2007). Although these small water bodies usually lack large predators, competition and predation from conspecific and heterospecific tadpoles and some small invertebrates can affect the suitability of these sites (Brown, Morales, & Summers, 2008; McKeon & Summers, 2013). For example, adult frogs of several species avoid depositing eggs in pools with conspecific tadpoles that cannibalize eggs or newly hatched tadpoles (Spieler & Linsenmair, 1997; Summers, 1999). Several experimental studies have demonstrated that, if given the choice, adult frogs preferentially deposit eggs or tadpoles in predator-free pools, independently of the type of predator present (i.e., anuran predatory larvae or non-anuran predators such as fish or aquatic invertebrates; Buxton & Sperry, 2017). These studies constitute evidence of the capability of anurans to assess the suitability or overall quality, of deposition sites with respect to predation risks. Moreover, permanency of these water bodies oscillates with variation in temperature and precipitation, rendering them ephemeral and therefore not always available. Indeed, factors such as desiccation or food availability may exert stronger selective pressures than the presence of predators or competitors (Crump, 1991). The ephemeral nature and unpredictable spatial occurrence of suitable water bodies makes them patchily distributed and difficult to find—all of which may place a premium on adults' ability to find and select high quality sites for aquatic larval development (Wells, 2007).

When suitable oviposition or larval deposition sites are scarce, parents may be faced with a trade-off between extended search time and depositing their offspring in low quality pools. Quality of



FIGURE 1 (a) Male of the smooth guardian frog, *Limnonectes palavanensis*, carrying four tadpoles on his back. (b) Experimental setup of artificial tadpole deposition sites implemented in our designated study area. (c) Natural tadpole deposition sites of *Limnonectes palavanensis* at the Ulu Temburong National Park study site in Brunei Darussalam, pool of an intermittent stream, (d) water-filled depression on a boulder next to a stream [Colour figure can be viewed at wileyonlinelibrary.com]

deposition sites may vary depending on water volume, variability of food resources, and presence or absence of competitors and/or predators (Murphy, 2003; Rojas, 2014). Depositing eggs or tadpoles in unsuitable pools can result in reproductive failure if the offspring is consumed by predators, but intense competition, desiccation risk, and decreased food availability also can affect offspring survival and other life history traits, such as time and size to metamorphosis and adult body size (Wilbur 1987). In the face of these challenges, adult frogs are expected to choose deposition sites with biotic and abiotic factors that minimize own risks and, at the same time, maximize offspring survival.

A particularly appropriate species for investigating parental strategies and larval deposition behavior is the smooth guardian frog, *Limnonectes palavanensis*, a small, nocturnal, leaf-litter frog found in the primary rainforests of Borneo and the Palawan Island of the Philippines. In this species, mated pairs lay clutches of up to 21 eggs on land (Goyes Vallejos 2016). Males then attend eggs in terrestrial nests, and after hatching, tadpoles wriggle onto the male's back for subsequent transport to nearby bodies of water, where they complete their development (Inger & Voris, 1988; Inger, Voris, & Walker, 1986; Goyes Vallejos 2016; Figure 1a). *Limnonectes palavanensis* tadpoles occasionally have been found in pools of small intermittent streams, rock depressions, animal wallows, and puddles formed following rain, including pools in tree holes and fallen logs (Inger & Voris, 1988; this study). Despite the variety of sites used for tadpole deposition, such sites are scarce, ephemeral, and patchily distributed; thus, males may invest considerable time finding suitable sites. Together, the reproductive biology and life history attributes of *L. palavanensis* render the species a particularly well-suited study system with which to test hypotheses concerning trade-offs faced by parents when resources like suitable deposition sites are scarce and unpredictable.

In this study, we conducted four experiments using artificial pools to test whether specific abiotic and biotic factors (conspecifics and predators) influence parental choice of larval deposition sites, while experimentally controlling for differences in pool size, water volume, and location. We tested (1) whether habitat variables such as canopy cover, slope, distance to a stream, and amount of leaf litter affect the probability of males depositing tadpoles in artificial pools. These parameters are known to affect water temperature, relative humidity, amount of food resources, and pool permanency, factors likely to influence larval growth and survival (Semlitsch & Skelly, 2008; Williams, Rittenhouse, & Semlitsch, 2008). We predicted that males of *L. palavanensis* would choose sites with abiotic characteristics that maximize offspring survival. We also performed deposition site choice trials by experimentally manipulating the presence of (2) predators and (3) conspecifics. We predicted that males should prefer to deposit tadpoles in pools without predators or potential competitors. Finally, we also investigated whether (4) cannibalism occurs among tadpoles of *L. palavanensis*. Our study joins a nascent body of literature from anuran study systems, evaluating the importance of abiotic and biotic factors affecting parental selection of larval rearing sites.

2 | METHODS

2.1 | Characterization of natural tadpole deposition sites

This study took place in August–December 2014, in Ulu Temburong National Park, Temburong District, Brunei Darussalam. Ulu Temburong is the only national park in the country, comprising 50,000 ha of lowland mixed-dipterocarp rainforest. The Institute of Biodiversity and Environmental Research (IBER) manages the Kuala Belalong Field Studies Centre (KBFSC), a research facility adjacent to our study area (ca. 1.3 ha) located on the northern border of the park, at 115.1573°E, 4.5468°N, at elevations of 50–200 m. Yearly rainfall varies between 4,900 and 6,800 mm, with no well-defined dry and wet seasons, although the months of June to August show the least amount of rainfall (KBFSC Weather Station Data 2005–2014). Our characterization of natural deposition sites took place during the 2014 field season (August–December). We walked through the designated study area twice a week in the morning (0900 h–1200 h), identifying possible natural tadpole deposition sites such as rain-filled pools on the forest floor, water-filled tree holes, water-holding plants, husks, tree buttresses, and animal wallows. We also sampled along 300 m of a small stream adjacent to our study area, seeking small pools at the edge of the stream and holes in rocks. We checked for presence of *L. palavanensis* tadpoles, counted numbers of tadpoles when present, and noted presence of eggs or tadpoles of other frog species.

2.2 | Abiotic factors affecting tadpole deposition site choice

To determine which abiotic characteristics of tadpole deposition sites affect tadpole deposition choice in males of *L. palavanensis*, we used artificial pools (2.5 L plastic buckets, 24 cm diameter, 10 cm depth) placed in the study area and measured habitat characteristics surrounding them. Specifically, we measured leaf litter depth, canopy cover, slope, and distance to the nearest stream. In November 2013, we placed 24 artificial pools ("pools" hereafter) in transects perpendicular to the largest stream in the forest. Twenty artificial pools were placed along four transects. In each transect, four pools were placed at increasing distances from the stream (15, 30, 45, 60, and 90 m). Transects were separated by 15 m from each other. Four additional pools were placed haphazardly within the study area. Artificial pools were buried in the ground up to their rims and filled with stream water (Figure 1b). Because males of *L. palavanensis* were seen to vocalize and transport tadpoles in all areas where the artificial pools were located, we believe that the detection probability of the pools is equal among them.

Males of *L. palavanensis* did not use the artificial pools during the 2013 season (August–December), but the pools were left in place in the forest until August 2014, when we surveyed for *L. palavanensis* tadpoles by checking each pool every other day from August 18, 2014 to October 5, 2014. We recorded the presence or absence of tadpoles, and, if present, counted the number of tadpoles. Every time we found newly deposited tadpoles, we recorded it as a

“deposition event,” independent of the number of tadpoles deposited. When found, tadpoles were removed from the pools and taken to the field station laboratory for body measurements. We kept the tadpoles in the laboratory, in tanks supplied with leaf litter and stream water until the end of the survey, when they were released in natural pools. Tadpoles and eggs of other species were removed from artificial pools as well.

For each of the artificial pools, we recorded depth of leaf litter, canopy cover, and slope. To determine leaf litter depth around each artificial pool, we took four measurements around it using a thin metallic rod to pierce through the leaves until it reached the soil; the four measurements were then averaged. To estimate canopy cover, we used a 60D EOS Canon camera with an 18-mm wide-angle lens and photographed the canopy at ground level with the camera centered on the artificial pool. We later converted the photographs to binary black and white images; using the software ImageJ, we estimated the percentage of canopy cover (black pixels) for each photo. To determine the slope where a given pool was located, we used the inclinometer function of a ©Bushnell Laser Rangefinder (Elite 1600 ARC), which provides degrees of elevation ($\pm 1^\circ$ error).

To determine which habitat variables affected the presence or absence of tadpoles in the artificial pools, we fitted generalized linear mixed-effect models using the package lme4 (Bates, Mächler, Bolker, & Walker, 2014) in R (R Development Core Team 2017). We modeled linear regressions ($y = \beta_0 + \beta_1 x$) with a binomial distribution using a logit link function for each of the parameters (leaf litter, canopy cover, slope, distance to the stream) and using “pool” as a random variable to account for repeated measures through time. We also included a global model with all covariates as fixed effects and a null model (no covariates). Predictor variables did not show strong correlations, in all cases with $|\rho| < 0.7$. We used Akaike's information criterion (AIC) to rank the six models and the values of $\Delta AIC < 2$ ($\Delta AIC_i = AIC_i - AIC_{min}$) to identify the best models.

2.3 | Tadpole deposition site choice experiments

In October 2014, we started a second set of experiments using paired artificial pools, to test whether *L. palavanensis* males' decisions about depositing their tadpoles were influenced by the presence or absence of conspecific tadpoles and/or predators. The conspecific tadpole experiment was conducted between October 6, 2014 and November 9, 2014, and the predator experiment between November 11, 2014 and December 13, 2014. For both experiments, we set up 10 pairs of artificial pools of the same size as in the tadpole surveys. The paired pools were 25–30 cm apart, placed opportunistically throughout the study area, with pairs of pools separated by at least 15 m. We covered the bottom of the pools with a fine layer of leaf litter and filled them with stream water.

To test whether the presence of conspecific tadpoles affects tadpole deposition by males (choice experiment 1), we deposited two tadpoles of *L. palavanensis* in one artificial pool and left the other empty. To test whether the presence of a predator affects tadpole deposition behavior by males of *L. palavanensis* (choice experiment

2), we added a dragonfly larva (order Odonata: suborder Anisoptera) to one of the pools, enclosed in a mesh bag ($7 \times 10 \times 1$ cm), whereas the other pool contained an empty mesh bag. The mesh bag allowed the dragonfly larva to swim freely inside of it, but prevented it from eating tadpoles. Dragonfly larvae were fed regularly with a *L. palavanensis* tadpole placed inside the mesh bag every 4 days or until the previous tadpole was eaten.

For both experiments, the paired artificial pools were checked every other day for the presence or absence of tadpoles and to record numbers of tadpoles. Any newly deposited tadpoles were removed and brought to the laboratory, where they were kept until the end of the experiment, so artificial pools were available for other males to deposit their tadpoles under the same experimental conditions. Pools with predators/conspecifics were switched each time after pools were checked, instead of switching predators/conspecifics from one artificial pool to another; this procedure removed any positional effects, but any odor or chemical cues present in the water remained in a given pool. We used a McNemar's chi-square test with continuity correction used for paired binary response data, with “no preference” as the null hypothesis. These tests were conducted in R (R Development Core Team 2017).

2.4 | Cannibalism experiment

From November 13 to December 13, 2014, we formed 18 pairs of tadpoles to test whether cannibalism occurs in tadpoles of *L. palavanensis*. Larvae were paired randomly using tadpoles found in the artificial pools from the choice experiments and tadpoles found in natural pools more than 500 m away, to ensure that the two tadpoles were not related. We placed a small tadpole (Gosner stage 25–26; Gosner 1960) with a large one (Gosner stage 25–36) and photographed them to permit subsequent measurement of size differences using ImageJ. Each pair of tadpoles was placed in a small plastic container ($16 \times 11 \times 6$ cm) on a dark background with a mesh lid. Each container was filled with 2 g of crushed leaf litter and 200 ml of water. We checked the pairs three times per day for four weeks to see if cannibalism had occurred (i.e., if one tadpole was missing).

2.5 | Ethical note

All behavioral observations and experimental methods were reviewed and authorized by the Institutional Animal Care and Use Committee at the University of Connecticut (Protocol No. A12-028) and followed the ABS guidelines for the treatment of animals in behavioral research and teaching.

3 | RESULTS

3.1 | Characterization of natural tadpole deposition sites

During our survey for natural deposition sites, we found only 10 natural pools throughout our study area that appeared to be suitable

for tadpole deposition. These pools were surveyed regularly for 5 months for the presence of *L. palavanensis* tadpoles. We found tadpoles of *L. palavanensis* only three times: in a pool of an intermittent stream ($N = 19$ tadpoles; Figure 1c), in a pig wallow ($N = 35$ tadpoles), and in a water-filled depression on a boulder next to a stream ($N = 30$ tadpoles; Figure 1d). Eggs and larvae of a microhylid frog, *Chaperina fusca*, were found in two of the remaining seven pools.

3.2 | Abiotic factors affecting tadpole deposition site choice

Given that natural deposition sites for tadpole deposition were scarce in our study area, we used artificial pools to increase the opportunity to observe tadpole deposition behavior. We predicted that if deposition sites were limited, male *L. palavanensis* would readily use artificial pools for tadpole deposition. Of the 24 pools surveyed in 2014, males of *L. palavanensis* used 18 of them at least once to deposit tadpoles, for a total of 56 tadpole deposition events (range = 1–17, median = 4; Figure 2). Other frog species also exploited these artificial pools as calling sites and breeding pools, with 20 pools used at night by *Chaperina fusca*, *Kalophrynus meizon*, and *Microhyla borneensis* (Microhylidae). Up to 10 adults of *C. fusca* were found in one artificial pool at a time, and up to two calling males of *K. meizon*. The frequency of pool usage by these species supports the idea that natural pools are a limited resource in this area and that several species (including *L. palavanensis*) rely on these resources

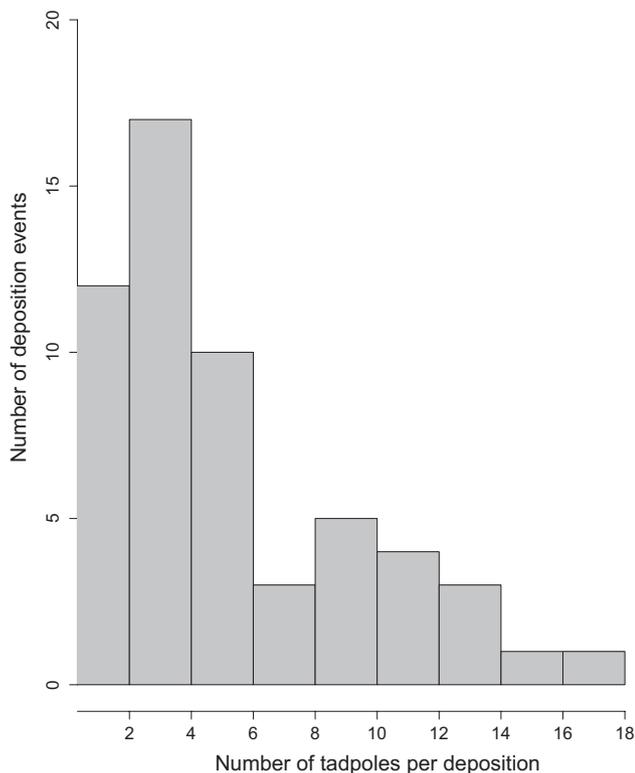


FIGURE 2 Number of *Limnectes palavanensis* tadpoles per deposition event found in artificial deposition sites (pools). Median number of tadpoles = 4 (range = 1–17)

TABLE 1 Summary of mean (\pm SD) leaf litter depth, slope, and canopy cover for each of the 24 artificial pools established in the study area

Variable	Mean \pm SD	Min-max
Leaf litter depth (cm)	2.7 \pm 1.1	1.6–5.5
Slope ($^{\circ}$)	20.8 \pm 13.1	0–44
Canopy cover (%)	87.0 \pm 7.5	65–97

for reproduction. Small dragonfly larvae (<1 cm) were found and removed from the pools on seven occasions.

The 24 artificial pools varied in depth of leaf litter, slope, and canopy cover surrounding them (Table 1). The model that included slope as a covariate was best supported by the data (Table 2), being significant ($\beta_1 = -0.51$, 95% CI = -0.94 to -0.08 , $p = 0.021$; Figure 3), indicating that slope is negatively correlated with the probability of tadpole deposition. None of the other variables (i.e., leaf litter depth, canopy cover, and distance to the stream) had a significant effect on the probability of finding tadpoles (all $p > 0.05$).

3.3 | Tadpole deposition site choice experiments

Males of *L. palavanensis* used seven of the ten pairs of experimental pools to deposit tadpoles, although we observed males carrying tadpoles at night (2030–0000 h) and depositing them in artificial pools on five occasions only. We counted the number of tadpoles carried by each of these males (number of tadpoles = 14, 15, 12, 14, and 11) and compared them with the number of tadpoles found in the artificial pools the next morning. In all five cases, our counts of number of tadpoles on males' backs matched the number of tadpoles found the following day. However, males did not deposit all of their tadpoles in a "higher quality" pool as expected (without conspecifics/predators); rather, they split their tadpoles between the paired pools.

In choice experiment 1, testing the effect of conspecific tadpoles, we recorded 21 tadpole deposition events, with a maximum

TABLE 2 Summary of model comparison and Akaike information criterion (AIC) values and Δ AIC values comparing different models reflecting the effect of leaf litter, slope, canopy cover, and distance to the stream on probability of tadpole deposition of *Limnectes palavanensis* in artificial pools, using "pool" as a random variable in all models

Models	k	AIC	Δ AIC	w_i
Model (tadpoles \sim slope + (1 pool))	3	324.0	0	0.55
Model (tadpoles \sim distance + (1 pool))	3	326.4	2.40	0.17
Model (tadpoles \sim 1 + (1 pool))	2	327.5	3.54	0.09
Model (tadpoles \sim litter + slope + canopy + distance + (1 pool))	6	327.7	3.74	0.09
Model (tadpoles \sim canopy + (1 pool))	3	328.6	4.60	0.05
Model (tadpoles \sim litter + (1 pool))	3	329.1	5.10	0.04

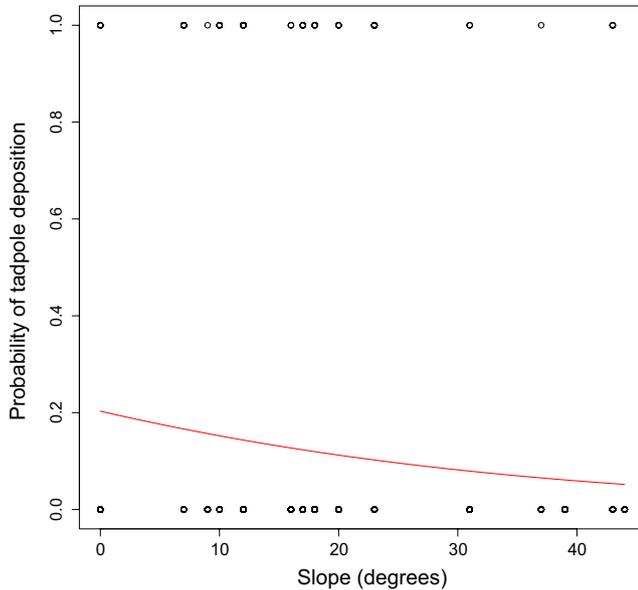


FIGURE 3 Effect of slope on the probability of a male depositing tadpoles in an artificial pool. The circles indicate data obtained in the field, and the line shows the predicted probabilities using a binomial logistic regression [Colour figure can be viewed at wileyonlinelibrary.com]

of five repeated deposition events per pair of artificial pools (range = 2–5; Table 3). Consecutive tadpole deposition events in the same pair of pools occurred always within 5 days. On average, it takes 10 days for a clutch of eggs to hatch (Goyes Vallejos 2016); therefore, it is unlikely that the same male returned to the same pair of pools to deposit his tadpoles. Males did not exhibit any preference between the empty pool and the pool occupied by conspecific tadpoles (McNemar's $X^2 = 0.13$, $df = 1$, $p = 0.72$). In total, for choice experiment 1, we found evidence suggestive of clutch-partitioning behavior on 13 occasions, because we detected tadpoles in both pools, with a maximum combined number of tadpoles of 27 (median = 12, range = 3–27). To determine whether tadpoles found in the same pair of artificial pools

belonged to the same clutch, we tested for differences in total length between the tadpoles found in the empty pool and the pool with the conspecific tadpoles (when the number of tadpoles was >2 in both pools); in all but one case, we found no significant differences in size (all $p > 0.1$).

In the exceptional case, 27 tadpoles were deposited, 7 tadpoles in the occupied pool and 20 tadpoles in the empty pool. In *L. palavanensis*, the average clutch size is 15 eggs (range = 10–21; Goyes Vallejos 2016), so it was unlikely that all 27 tadpoles belonged to the same clutch. Rather, after inspection of the development of the ventral intestinal coil and overall body size, we sorted the tadpoles into three groups that differed in developmental stage; these included 7, 8, and 12 tadpoles each, with significant differences in size among these groups ($p < 0.05$), suggesting that three different males deposited the tadpoles in the two artificial pools over a span of 2 days (Appendix 1).

In choice experiment 2, testing the effect of a predatory dragonfly nymph on tadpole deposition behavior, we recorded 12 deposition events, with a maximum of three repeated deposition events per pair of pools throughout the experiment (range = 1–3; Table 3). Males did not show a preference for depositing tadpoles in artificial pools with or without a predator (McNemar's $X^2 = 2.28$, $df = 1$, $p = 0.13$) and deposited at least three tadpoles in the pool with the predator in 50% of the deposition events (Appendix 2). In 5 of 12 deposition events, males also divided their clutches between the empty pool and the one with the dragonfly larva in it, with a combined maximum number of tadpoles of 14 (median = 11, range = 6–14). The largest clutch size observed in this species is 21 eggs, so we assumed that the tadpoles found in the two pools belonged to the same clutch. To confirm this supposition, we compared the developmental stage and total length of the tadpoles found in the two pools; in all cases, tadpole sizes were not distinguishable (all $p > 0.1$). The mean ($\pm SD$) number of tadpoles found per deposition event was 11 ± 5 (range = 2–27) in the presence of conspecific tadpole experiment, and 9 ± 5 (range = 1–15) in the predator experiment, which were not significantly different ($p > 0.05$).

TABLE 3 Number of tadpole deposition events in experiment 1 (choice of pool with or without conspecifics) and experiment 2 (choice of pool with or without a predator)

Pool pair #	Experiment 1			Experiment 2		
	With conspecifics	w/o conspecifics	Both pools	With predator	w/o predator	Both pools
1	0	0	2	0	0	1
2	0	0	2	0	0	2
3	1	1	0	1	1	1
6	1	1	2	0	1	1
7	2	0	0	0	2	0
9	0	0	4	0	1	0
10	1	1	3	0	1	0
Total	5	3	13	1	6	5

Note. Males did not show preferences for a particular pool (McNemar's chi-square test, $p > 0.1$, both experiments). "Both pools" refers to cases in which males exhibited clutch-partitioning behavior involving deposition of tadpoles in both artificial pools treatments.

3.4 | Cannibalism experiment

Total length of the small tadpoles ranged from 14.6 to 20 mm (mean \pm SD = 17.3 \pm 1.7 mm), whereas the total length of the big tadpoles ranged from 16.8 to 23.6 mm (mean \pm SD = 21.3 \pm 2.0 mm). Paired tadpoles differed in total length by 0.73–7.69 mm and by 0.13–1.50 mm in width. We did not observe cannibalism in any of the pairs of tadpoles.

4 | DISCUSSION

Here, we have shown that the low availability of suitable larval deposition pools appears to be the primary limiting factor affecting parental choice of rearing sites for tadpole development in *L. palavanensis*. We found that natural tadpole deposition sites are scarce throughout the forest—and that, apparently, not all natural forest floor pools are equally suitable for aquatic larval development. Several characteristics may influence the suitability of offspring deposition sites in anurans, including the permanence and duration of these aquatic microhabitats. In some species of frogs where adults deposit their eggs in small bodies of water, parents show a preference for sites that can hold water for several weeks (Rudolf & Rödel, 2005).

In our study area, the type of soil in the forest floor and the generally hilly terrain does not allow for accumulated rainwater pools to last very long, rendering potential tadpole deposition sites ephemeral. Of three natural pools observed to have developing *L. palavanensis* tadpoles, the unambiguously common feature inferred was substrate type, promoting pool permanency. The rocky depression near the permanent stream found in our study area was maintained by frequent rainfall, while the pool in the intermittent stream was sustained by continuous trickling from a larger, adjacent stream. In these cases, the rocky substrate at the bottom prevents water filtering or percolation through underlying soils, rendering them relatively “permanent.” Our third pool, the animal wallow, was the only natural pool used by *L. palavanensis* that showed substantial fluctuations in water level. During heavy rains, the wallow increased in size and held water for over a week, but in the absence of precipitation, became a series of several smaller pools, each containing between 2 and 16 tadpoles of *L. palavanensis*. Males of *L. palavanensis* carry between 8 and 15 tadpoles at a time (median = 13; Goyes Vallejos 2016). The larger numbers of *L. palavanensis* tadpoles found in natural pools (19–35 tadpoles) suggest that multiple males deposited tadpoles in these pools, which we consider consistent with the interpretation of limited number of natural tadpole deposition sites in our study area.

In our survey of artificial pools, the probability of a male depositing tadpoles in one of the artificial pools did not depend on the distance to the stream, the amount of leaf litter around the pool, or the amount of canopy cover. However, the slope at the location of the artificial pool did influence tadpole deposition. Males apparently avoid depositing tadpoles in pools found in steep areas because inclination of the forest floor may promote downhill flow of contained

water, resulting in desiccation in the dry season or flushing of larvae downstream in rainy months (Brown & Iskandar, 2000). Our artificial pools were readily used as oviposition sites by three other species of frogs. The presence of eggs and larvae of these species did not seem to affect tadpole deposition by *L. palavanensis* males. This immediate and frequent colonization of artificial pools by all four local species could also indicate scarcity of deposition sites at our study site.

To date, it is unknown how *L. palavanensis* and heterospecifics find natural or experimental water bodies on the forest floor. Males of *L. palavanensis* do not call while carrying the tadpoles, but it remains conceivable that they could use heterospecific acoustic cues to find pools. Studies in temperate frogs that breed in ephemeral pools suggest that calls from the first-arriving males provide orientation cues for subsequently arriving males (Buxton, Ward, & Sperry, 2015). In addition to using acoustic cues, males of *L. palavanensis* may learn their locations over time and return repeatedly to deposit their offspring, as observed in species with tadpole transport behavior in New World anurans (Pasukonis et al., 2016; Pašukonis, Warrington, Ringler, & Hödl, 2014).

We observed a wide range in the number of tadpoles deposited in each artificial pool (1–17, median = 4). In *L. palavanensis*, males transport the entire clutch after the eggs hatch (up to 21 tadpoles). A few cases of clutches with low survival rates have been observed in the lab in the absence of terrestrial predators, but most clutches had a survival rate of 75% or higher (Goyes Vallejos 2016). Thus, we cannot explain the low number of tadpoles per deposition in *L. palavanensis* without further studies in offspring survival and larval transport logistics. In other taxa with larval transport, larval survivorship is high and parents sometimes carry entire clutches and distribute their offspring in different pools when available, but also travel long distances if suitable sites are rare (Ringler, Pasukonis, Hödl, & Ringler, 2013). These unknown aspects of the tadpole transport behavior of *L. palavanensis* provide numerous opportunities for future studies.

In species with parental care, offspring benefit from parental decisions that provide protection against competitors, predators, and desiccation (Wells, 2007). Several studies in poison frogs have shown that adults avoid depositing tadpoles in pools with predators (conspecific and heterospecific), competitors, and cannibalistic tadpoles (Brown et al., 2008; Summers, 1999; Summers & Symula, 2001). Thus, we expected that *L. palavanensis* males must choose tadpole deposition sites with low-risk characteristics that will positively affect offspring survival. However, in our study, males of *L. palavanensis* did not avoid pools with conspecific tadpoles or predatory dragonfly larvae and often split their clutches between the two available artificial pools. As such, whether males split clutches may depend on pool availability and awareness of the location of neighboring pools. Nonetheless, clutch-partitioning behavior observed here may be advantageous if males maximize their fitness by depositing small numbers of tadpoles in different deposition sites, thereby minimizing risks associated with particular pools or “hedging” bets across variation in biotic and abiotic pool characteristics (Erich, Ringler, Hödl, & Ringler, 2015).

As far as is known, conspecifics do not present a direct threat because *L. palavanensis* tadpoles do not engage in cannibalism. In some species, high tadpole densities in small pools trigger cannibalistic behavior (Caldwell & de Araujo, 1998). This does not seem to be the case in *L. palavanensis*, where large numbers of tadpoles at times have been observed in a single pool. Thus, males of *L. palavanensis* choose to deposit tadpoles in pools with conspecifics because their presence may be an indicator of availability of food resources and water stability (Poelman & Dicke, 2007; Schulte & Lötters, 2014). Water permanence may be a critical limiting abiotic resource, depending on the time needed to complete the larval period and reach metamorphosis. Field studies investigating the duration of the larval period of *L. palavanensis* have yet to be performed, but tadpoles used as “residents” for this experiment were judged to be approximately a week old, based on our familiarity of size. In approximately three weeks, all residents developed hind legs (Gosner stage 39–40), and by the end of the experiment (~1 month), their bodies elongated and forelimbs started to emerge. This indicates that natural pools must persist for a minimum of 30 days for successful larval development. Nonetheless, we are aware of the fact that males of *L. palavanensis* may not have been capable of detecting the presence of the two resident tadpoles by movement alone, as the tadpoles are very inactive, resting among leaves at the bottom of the pool. As a result, males did not discriminate between the experimental pools employed in this study. However, in the Peruvian poison frog, *Ranitomeya variabilis*, it has been demonstrated that adults use chemical cues to discriminate between pools with cannibalistic and non-cannibalistic, and even deposit more larvae in pools with non-cannibalistic conspecifics (Schulte et al., 2011). Likewise, females of the African dicroglossid, *Hoplobatrachus occipitalis*, avoid depositing eggs in rock pools inhabited by conspecific cannibalistic tadpoles (Spieler & Linsenmair, 1997). Whether males of *L. palavanensis* can detect the presence of conspecific tadpoles by means of chemical cues remains unknown—and presents another opportunity for future studies.

Males did not discriminate between predator-free artificial pools versus ones to which a predator was experimentally introduced. We assume males would be expected to make choices that maximize offspring survival; therefore, we found it surprising that males of *L. palavanensis* deposit some, and in other cases all, of their tadpoles in the artificial pool with a predatory insect. We propose a few explanations of why males of *L. palavanensis* chose to deposit tadpoles in the artificial pool with a predator present. First, if finding larval deposition sites in a patchy environment is more pressing than the threats imposed by predators, males might choose to deposit tadpoles in any available pool despite the presence of predators. This has been observed in other anuran species where parents choose to deposit their offspring in pools with conspecific and heterospecific predators due to higher selection pressures such as site availability, amount of resources, and desiccation risk (McKeon & Summers, 2013; Rojas, 2014). Second, predation risk may be low even in the presence of a predator because tadpoles of *L. palavanensis* seem to rely heavily on camouflage and inactivity for protection, feeding on the bottom of the pool under the leaf litter without swimming

actively. Hence, predation is minimized, because many species of odonate larvae rely on movement for prey capture (Chovanec, 1992). Third, males of *L. palavanensis* might be unable to effectively detect odonate predator chemical cues in the water, and therefore, they do not discriminate against the predator artificial pool. The latter explanation seems unlikely given that many anuran species have the ability to detect predators present in water bodies, even when they have never been in contact with a particular predator (Touchon & Worley 2015; Downie, Livingstone & Cormack; 2001). The ability of adults to detect potential predators is under strong selection since depositing their offspring in rearing sites with a high predation risk results in the total loss of their reproductive input, and species with larval transport where adults do not provide any additional care after depositing their offspring must have evolved the ability to reliably detect predators (Crump, 1991; Ringler et al., 2018). Therefore, *L. palavanensis* males may be able to detect the presence of dragonfly larvae in the artificial pools but the unpredictability and the possibility of not finding a tadpole deposition site represents a higher risk (Laufer, Vaira, Pereyra, & Akmentins, 2015; Spieler & Linsenmair, 1997). In addition, the seemingly bad choice of depositing some of the tadpoles in a pool with a predator present may be counteracted by the clutch-partitioning behavior observed in this species.

Finding suitable deposition sites in an unpredictable environment such as the ones found in tropical forests presents several challenges for parents looking to maximize offspring survival. In addition, when found, these deposition sites vary in their stability and quality, and therefore, parents must be able to assess potential risks and favor pools that optimize the associated trade-offs. While the risks posed by competitors and predators are clear, our results suggest that low density of deposition sites and water permanency may be the main drivers affecting tadpole deposition site choice by males of *L. palavanensis*. The few available sites in the field must be sufficiently permanent to allow larval development. Males then should deposit all of their tadpoles in one pool when the probability of finding another pool is low or requires traveling long distances, which may increase predation risk, adult energy reserve depletion, and/or dehydration of tadpoles or depletion of their yolk reserves. However, given the opportunity, males divide the clutches between two nearby pools, which may increase the probability of survivorship otherwise detrimentally decreased by desiccation of deposition sites, or negative biotic interactions with taxa not studied here. Other aspects such as pool size and availability of food resources in natural and/or experimental pools should be studied further to fully understand the deposition behavior of *L. palavanensis*.

Ultimately, we propose a scenario in which availability, patchiness, and overall quality of natural ephemeral water bodies may influence the mating system of *L. palavanensis*. For example, in some species of anurans with tadpole transport behavior found in the Neotropics, water volume and amount of food resources of larval deposition sites lead to the evolution of elaborate mating systems (Brown, Morales, & Summers, 2010). Shortage of deposition sites would be expected to increase the time a male carries tadpoles before a suitable site is found. In *L. palavanensis*, egg attendance takes

an average of ten days (Goyes Vallejos 2016) and any additional time spent traveling to and identifying deposition sites will prevent them from acquiring additional mates, and females will be subject to low availability of males, thus reducing their mating opportunities. This additional cost imposed on males could have important implications for *L. palavanensis*' mating system. It could, for instance, lead to reversal of the usual sex roles in mate searching, courtship, and communication (Goyes Vallejos 2016). Additional experimental characterization of the mating strategies of males and females in this species will provide valuable insights into the costs of parental care and tadpole deposition site availability on mating systems.

In summary, we show that contrary to our predictions, the scarcity of natural deposition sites may have a greater influence in the choice of deposition sites by males of *L. palavanensis* than direct or indirect threats such as competition or the presence of predators. Only a handful of frog species from South-East Asia are known to provide parental care (Poo & Bickford, 2013). The lack of suitable tadpole deposition sites, particularly the absence of bromeliads, is likely to be a major limiting ecological factor in South-East Asia. Our understanding of the evolution of parental care behaviors can be enhanced by performing experimental studies on these few South-East Asian species to test the universality of theories developed from observations done mostly in the Neotropics.

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APPENDIX

Appendix 1 Tadpole deposition data for the experiment using conspecific tadpoles. The numbers indicate the total number of tadpoles per deposition event in each artificial pool. Tadpole total length at deposition (TTL) in mm, measured from the snout to the tip of the tail. Pool ID indicates the transect number (MT) and distance to the stream in meters.

Pair	Pool ID	Tadpoles		Total	Mean \pm SD TTL (mm)
		Present	Absent		
1	MT1 60	3	2	5	12.0 \pm 1.2 (11.2–14.2)
1	MT1 60	13	1	14	13.8 \pm 0.9 (11.6–14.9)
2	MT1 45	7	6	13	13.4 \pm 0.9 (11.9–14.9)
2	MT1 45	4	11	15	13.9 \pm 0.4 (13.2–14.8)
3	MT2 90	12	0	12	12.3 \pm 0.9 (10.7–14.0)
3	MT2 90	0	11	11	14.4 \pm 0.6 (13.3–15.4)
6	MT3 45	8	0	8	13.9 \pm 0.6 (12.7–14.5)
6	MT3 45	0	2	2	13.6 \pm 0.1 (13.6–13.7)
6	MT3 45	1	2	3	13.2 \pm 0.2 (12.9–13.5)
6	MT3 45	6	6	12	12.7 \pm 0.4 (11.9–13.3)
7	MT3 30	7	0	7	12.9 \pm 0.7 (11.4–13.7)
7	MT3 30	7	0	7	13.8 \pm 0.4 (13.1–14.3)
9	MT4 60	7	1	8	15.4 \pm 0.5 (15.0–16.0)
9	MT4 60	2	19	21	14.2 \pm 0.9 (12.3–15.8)
9	MT4 60	7	10	17	13.4 \pm 0.4 (12.0–14.1)
9	MT4 60	5	6	11	14.5 \pm 0.8 (12.6–15.3)
10	MT4 45	17	0	17	13.3 \pm 0.4 (12.2–14.2)
10	MT4 45	6	4	10	15.1 \pm 0.3 (14.4–15.7)
10	MT4 45	3	2	5	14.6 \pm 0.4 (14.1–15.1)
10	MT4 45	0	12	12	13.9 \pm 0.6 (12.5–14.8) ^a
10	MT4 45	7	8	15	14.7 \pm 0.5 (14.0–15.3, N = 7) ^{a,b}
					15.7 \pm 0.6 (14.6–16.3, N = 8) ^{a,b}

^aThese three groups of tadpoles were found in the same day and same artificial pool pair, but differences in body size and intestinal ventral coil development indicate that each size group was an independent deposition event belongs to a different male. ^bSignificant differences ($P < 0.05$) between tadpoles deposited in the pool with tadpoles ($N = 7$) and the pool without the tadpoles ($N = 8$).

Appendix 2 Tadpole deposition data for the predator experiment. Numbers indicate the total number of tadpoles per deposition event in each artificial pool. Tadpole total length at deposition (TTL) in mm, measured from the snout to the tip of the tail. Pool ID indicates the transect number (MT); distance to the stream is given in meters.

Pair	Pool ID	Predator		Total	Mean \pm SD TTL (mm)
		Present	Absent		
1	MT1 60	4	2	6	14.1 \pm 0.5 (13.4–14.7)
2	MT1 45	6	5	11	13.6 \pm 0.9 (12.1–14.7)
2	MT1 45	3	4	7	12.0 \pm 0.6 (11.2–12.7)
3	MT2 90	6	6	12	13.7 \pm 0.5 (12.6–14.4)
3	MT2 90	0	1	1	15.9
3	MT2 90	12	0	12	14.5 \pm 0.4 (13.6–15.2)
6	MT3 45	9	5	14	14.0 \pm 1.0 (12.2–15.6)
6	MT3 45	0	14	14	14.2 \pm 0.4 (13.4–15.0)
7	MT3 30	0	15	15	12.8 \pm 0.8 (11.0–14.1)
7	MT3 30	0	4	4	13.7 \pm 0.1 (13.5–13.8)
9	MT4 60	0	7	7	13.9 \pm 0.9 (12.4–14.8)
10	MT4 45	0	6	6	13.1 \pm 1.2 (11.1–14.7)