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ORIGINAL ARTICLE

Hatching plasticity in a Southeast Asian tree frog mitigates submergence-induced mortality

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Abstract

Environmentally cued hatching has been well-documented in amphibians in response to a wide range of abiotic and biotic factors. The hatching of terrestrial amphibian eggs in response to flooding may be basal within the group, but amphibian lineages in tropical Asia and sub-Saharan Africa have not received as much attention as their Neotropical counterparts. We investigated submergence-induced hatching in *Feihyla hansenae*, a Rhacophorid tree frog with terrestrial eggs. We quantified natural rates of clutch submergence at our study site in Thailand. Using submergence experiments, we found that embryos are capable of hatching early to escape flooding, and that failure to hatch results in mortality. Among the embryos that were able to hatch early, only the earliest, youngest hatchlings experienced a trade-off in body size that persisted for 6 days, while later, older hatchlings were not significantly smaller than spontaneous hatchlings under control conditions. By incorporating our natural and experimental data into Monte Carlo methods to simulate and compare survival probabilities with and without hatching plasticity, we found an overall 3.1% increase in submergence survival due to hatching plasticity. Our findings support the idea that flooding-induced hatching is widespread across amphibians with terrestrial eggs and highlight the importance of researching understudied tropical regions. As climate change is projected to affect rainfall patterns, the ability of embryos to escape abiotic egg-stage threats may be an indicator of species' ability to flexibly navigate a changing environment.

KEYWORDS
anuran, egg adaptations, environmentally cued hatching, *Feihyla hansenae*, flood, Rhacophoridae, survival analyses, Thailand

1 | INTRODUCTION

Hatching, a life history transition between the embryonic and larval stage, marks a significant change in environment for an animal. This change in the immediate environment presents both benefits and constraints; eggs protect embryos and their resources during this vulnerable stage of development, but also restrict the acquisition of food and oxygen and the mobility of the organism (Warkentin, 2011a). Given these trade-offs, embryos of many species are known to exhibit adaptive plasticity in their hatching timing and hatching behavior. These variations in hatching can be seen in both the duration of the egg-stage and the developmental stage of the organism when hatching occurs (Warkentin, 2011a). Plasticity during this generally stationary stage of development allows for individuals to improve their chances of survival by being able to escape environmental threats such as flooding, desiccation, and predation (reviewed in Warkentin, 2011b). However, the benefits of escaping egg-stage mortality can come at the expense of hatchling development (Haramura, 2016), changes in juvenile phenotype (Capellán & Nicieza, 2007), and likelihood of survival to metamorphosis (Touchon et al., 2013). Evidence of both accelerated and delayed hatching have been found across various animal taxa, including plankton (reviewed in Oyarzun & Strathmann, 2011), echinoids (Armstrong (Armstrong et al., 2013), gastropods (Strathmann et al., 2010), arachnids (Li, 2002), fish (Wedekind & Muller, 2005), amphibians (reviewed in Warkentin, 2011b), reptiles (reviewed in Doody, 2011), and birds (Vedder, 2012), in response to a range of environmental cues (reviewed in Warkentin, 2011a).

Amphibians exhibit great diversity in their reproductive modes (Duellman & Trueb, 1986; Haddad & Prado, 2005), the environmental factors that cue hatching, and the magnitude and directionality of changes in hatching timing (reviewed in Warkentin, 2011b). The ancestral mode of reproduction in amphibians involves aquatic eggs and aquatic larvae; however, there are multiple, independent origins of terrestrial eggs within the lineage (Duellman & Trueb, 1986; Wells, 2007). Terrestrial amphibian eggs face the challenge of avoiding water loss but also obtaining sufficient oxygen through diffusion (Seymour, 1999). Given the importance of oxygen and water for the survival and development of amphibian eggs, it is not surprising that instances of terrestrial eggs with submergence-induced hatching are widely observed within the clade (reviewed in Warkentin, 2011b). Submergence-induced hatching in amphibians can be divided into two general categories: delayed hatching to wait for necessary flooding, and early hatching in response to deadly flooding (Warkentin, 2011b). An example of necessary flooding occurs in the marbled salamander, *Ambystoma opacum*, which deposits its eggs in dry basins of temporary pools or above the water line of pools that will later fill. Eggs develop and reach the stage where they are competent to hatch, and then can wait for months for the water levels to rise, after which they will hatch within minutes to hours (Petranka & Petranka, 1981). Most examples of hatching to escape deadly flooding come from arboreal-breeding, Neotropical frogs (Warkentin, 2011b). The majority of these species lay their egg clutches adjacent to or above bodies of water, which the tadpoles drop into after hatching. Because of their proximity to water, egg clutches may face the threat of flooding when water levels rise. In red-eyed tree frogs, *Agalychnis callidryas*, embryos die if submerged before hatching competence; however, once they are able to hatch they will do so to escape deadly flooding (Warkentin, 2007). For both *A. opacum*, which wait for flooding to hatch and *A. callidryas*, which hatch early to escape deadly flooding, the cue that triggers hatching is hypoxia (Petranka et al., 1982; Warkentin, 2007). While progress has been made in shedding light on the mechanism of submergence-induced hatching in some species, little is known about the frequency and severity of its occurrence in a natural setting. This gap in our knowledge requires substantial efforts to collect in situ observations of egg clutches to quantify the adaptive benefits of having the flexibility to hatch prematurely.

Another major gap in our knowledge of environmentally cued hatching is the focus on Neotropical species, and, in comparison, a severe lack of information in other tropical regions, despite their high species diversity. While this discrepancy between tropical regions is by no means confined to the study of environmentally cued hatching (e.g. Deikumah et al., 2014; Sodhi et al., 2004), the underrepresentation of some lineages impedes our overall knowledge and understanding of this phenomenon. Southeast Asia, in particular, has long been neglected in comparison to the Neotropics, with fewer research articles published than should be expected, given its high biodiversity (Sodhi et al., 2004). In amphibians, for example, while a number of studies have documented and investigated hatching plasticity in Neotropical tree frogs in the Hylidae family (reviewed in Warkentin, 2011b), the same cannot be said for tree frogs in the Rhacophoridae family, which includes over 400 tree frog species that range from tropical Asia to sub-Saharan Africa (Frost, 2019). Similar to their Neotropical counterparts, Rhacophorid tree frogs exhibit a diversity of reproductive modes, with species laying arboreal gelatinous or foam eggs masses over lentic or lotic water (Grosjean et al., 2008), in tree holes, and in phytolems (Malkmus & Dehling, 2008). These embryos are presumably under the same constraints and pressures as other terrestrial amphibian eggs. However, while there has been evidence of environmentally cued hatching in Rhacophorid tree frogs in response to predation (Poo & Bickford, 2014) and high salinity (Haramura, 2016), the embryonic behavior within this family has gone largely understudied. Consequently, it is challenging to determine the prevalence of environmentally cued hatching across taxa and across geographical regions. Empirical investigations in historically understudied tropical regions are critical in helping us form a more complete understanding of the evolutionary drivers and adaptive value of this behavioral plasticity of embryos (Poo & Bickford, 2014).

In this study, we combined natural observations with experimental manipulations to quantify the role of hatching plasticity in increasing offspring survivorship in a Southeast Asian tree frog, *Feihyla hansenae*. Similar to many arboreal-breeding frogs, *F. hansenae* lays its egg clutches on vegetation overhanging ponds (Figure 1), rendering them vulnerable to flooding when the water level rises in

FIGURE 1 *Feihyla hansenae* egg clutch on vegetation above pond with female attending eggs.

periods of heavy rainfall. Predation is the most common threat that embryos face, and embryos are capable of hatching 22% early to escape attacks by katydids (Poo & Bickford, 2014). Submergence is the second highest cause of egg-stage mortality (Poo & Bickford, 2013). To measure how often flooding threatens this natural population, we monitored the background rate of clutch submergence in seasonal ponds over two breeding seasons. To quantify hatching plasticity that *F. hansenae* exhibit in response to deadly flooding, we conducted clutch submergence experiments at five time points along its embryonic development. We used our empirical results from natural clutch observation and clutch submergence experiments to model embryo mortality with and without hatchling plasticity and estimate its relative contribution to overall embryo survival. Based on previous findings of predator-induced hatching in *F. hansenae* (Poo & Bickford, 2014) and our general understanding of hatching plasticity in amphibians (see Warkentin, 2011b) we predicted embryos would be able to hatch early in response to submergence, increasing overall survivorship, but that it would come with a trade-off in tadpole size.

2 | METHODS

2.1 | Site and species

This study was conducted at the Sakaerat Environmental Research Station in Nakhon Ratchasima Province, Thailand (14°30′ N, 101°55′ E, elevation 250–762 m). *Feihyla hansenae* is a tree frog that breeds in temporary ponds during the rainy season in Thailand and parts of Cambodia (Aowphol et al., 2013; Cochran, 1927; Taylor, 1962). Eggs are deposited in a hemispherical gelatinous mass that is attached to vegetation or other substrates overhanging ponds, and clutches are attended by female frogs (Poo & Bickford, 2013). The embryonic period is 5 days on average, ending when the entire egg clutch detaches from the substrate, falls into the water below, and embryos hatch out of the gelatinous mass.

2.2 | Natural rates of clutch submergence

We conducted natural observations of 468 egg clutches from July to October in 2011 and 2012 in seasonal ponds within dry evergreen forests. We located newly laid egg clutches through visual encounter surveys conducted between 03:00 and 06:00 h to ensure that observations were made shortly after oviposition, which typically occurs between 21:00 and 03:00 h. For each clutch, we recorded the vertical distance from the center of the clutch to the surface of the pond (hereafter “clutch height”). Pond water level was recorded twice daily, at approximately 06:00 and 18:00 h until all clutches under observation were flooded or embryos had hatched.

2.3 | Quantifying hatching plasticity

We conducted submergence experiments from July to October, 2013. We collected males and gravid female frogs from the ponds and paired them in glass aquaria (40 × 20 × 25 cm) that contained rocks and plants and were filled with 2 cm of aged tap water to simulate pond habitats. We monitored frogs and eggs at least twice daily, and sprayed them with rainwater to provide hydration. We recorded oviposition time for each clutch and submerged egg clutches at either 48, 60, 72, 84, or 96 h post oviposition (HPO). As a control, we left a subset of clutches undisturbed. Hatching of undisturbed clutches occurs around 5 days post oviposition at our study site (~ 115 HPO, Poo & Bickford, 2014). To submerge the clutches, we detached the leaf containing the egg clutch from the rest of the plant and carefully placed it into a bowl of aged rainwater, with the egg side facing down, so that the entire egg clutch was submerged in water. We minimized physical disturbance to egg clutches as much as possible and did not observe any eggs hatching during transportation. We counted the number of hatchlings that had emerged from the clutch every 5 min for up to 1 h. At 1 h post submergence, we took a photograph of 10 haphazardly selected tadpoles and measured their total length using ImageJ (Schneider et al., 2012). Clutches were left submerged for 24 h. At 24 h post submergence, the overall survivorship of the clutch was determined by counting the total number of hatched tadpoles and dead embryos. This count was also used to determine clutch size, as embryos either hatched or died after 24 h of being underwater. Subsequently, at 6 days post oviposition (144 HPO), we took another photograph of submergence-induced tadpoles and control tadpoles and measured their total length. We returned all animals to their original ponds once observations concluded. Methods followed the Institutional Animal Care and Use Committee approved protocol (B11/12) at the National University of Singapore.

2.4 | Statistical analyses

We estimated the likelihood of an egg clutch being submerged before 120 HPO, using a time-to-event analysis with data from the natural observations. Based on the initial height of the clutch above water level, and the measurements of fluctuations in pond water levels, we calculated the height of a clutch above water level every 12 h from 0–HPO. If the clutch height dropped to 0 cm and below, it was counted as submerged at that particular HPO. We ran a Cox proportional hazard regression analysis (Cox, 1972) to determine if there were year and site differences in submergence probabilities, as well as interactions between site and year as covariates, using the R package *survival* (Therneau, 2022). We then estimated the cumulative hazard of submergence at each HPO using a Kaplan–Meier model (Kaplan & Meier, 1958).

We tested effects of age at submergence on percent of clutch hatching in experimentally submerged clutches using a generalized linear model (GLM) with quasibinomial distribution for over-dispersed proportional data using the R package *stats* (R Core Team, 2021). We tested effects of age at submergence on tadpole size at 1 HPO and 144 HPO using GLMs with underlying lognormal distributions using the *glmmTMB* package (Brooks et al., 2017). We determined differences between submergence treatments using post hoc Tukey's Honest Significant Difference tests (Hothorn et al., 2008).

We estimated the effects of hatching plasticity on overall survival of egg clutches using results from both the natural observations and the submergence experiments, by comparing survival rates (1) with submergence mortality only, and (2) with submergence mortality and hatching plasticity. We ran Monte Carlo simulations of survival curves from 0 to 120 HPO for two scenarios. In the first scenario (null hypothesis), we simulated the fates of eggs from oviposition to submergence assuming that there is no hatching plasticity and that all eggs that are submerged before 120 HPO succumb to submergence-induced mortality. That is, all eggs that are submerged before 120 HPO immediately die. We ran this simulation 1000 times, randomly choosing from the distribution of egg survival (mean and standard deviation) from the Kaplan–Meier parameters outputted from our field observational data. For the second scenario, we simulated the fates of eggs from oviposition to submergence including hatching plasticity calculated from the experimental data. Unlike the first scenario, this second scenario takes into account hatching plasticity, where some eggs that are submerged early (i.e., before 120 HPO) have a chance of surviving submergence that is greater than zero, based on our experimental results showing submergence-induced early hatching. We ran this simulation 1000 times, randomly choosing from the distribution of egg survival (mean and standard deviation) from the submergence experiments described above, multiplied by the probability of submergence (Table S1). For both scenarios, we assumed all eggs that hatched at 120 HPO had a 100% survivorship. We compared the distributions of the two scenarios to estimate the relative contribution of hatching plasticity in affecting overall submergence-induced survival to hatching.

TABLE 1 Sample sizes (number of *Feihyla hansenae* clutches) for natural observations.

Year	Lower pond	Upper pond	Total
2011	25	209	234
2012	35	199	234
Total	67	408	468

We conducted all statistical analyses in the R programming environment (v. 3.6.0, R Core Team, 2021) using a significance level of $\alpha = .05$. Means are presented with standard errors.

3 | RESULTS

3.1 | Natural rates of clutch submergence

We obtained data from 468 unique *F. hansenae* egg clutches (Table 1). We detected no differences between survival probabilities between site and year or interactions between the two covariates (Wald Statistic = 3.88, *df* = 3, *p* = .27). A majority (0.91 ± 0.015) of clutches remained unsubmerged until 120 HPO (Figure 2a, b).

3.2 | Quantifying hatching plasticity

A total of 36 clutches were included in the submergence assay (*N* for 48 HPO = 5, 60 HPO = 6, 72 HPO = 7, 84 HPO = 5, 96 HPO = 6, and control = 7 clutches). Average clutch size for submerged clutches was 243 ± 9.2 eggs (range = 165–352 eggs). One hour after submergence, clutches that were submerged at 48 and 60 HPO had no eggs hatched, while those at 72, 84, and 96 HPO had 14.3 ± 10.7, 68.5 ± 7.2, and 71.4 ± 14.6% of eggs hatch, respectively (Figure 3a). The percentage of eggs that hatched 1 h after submergence was significantly different between treatment groups ($F_{4,24} = 12.96$, $p < .01$, Figure 3b). Hatching at 72 HPO was lower than 86 HPO ($z = 3.18$, $p = .01$) and lower than 96 HPO ($z = 3.43$, $p = .01$). However, no difference was found in the percent of eggs that hatched between 84 and 96 HPO ($z = 0.18$, $p = 1.00$). At 24 h after submergence, clutches that were submerged at 48 HPO had no eggs hatched, while those submerged at 60, 72, 86, and 96 HPO had 0.1 ± 0.2, 46.8 ± 15.3, 95.4 ± 3.7, and 97.7 ± 1.1% of eggs successfully hatch and survive, respectively. For clutches submerged at 60 HPO, only a single tadpole hatched across all six clutches (consisting of 1516 eggs). The percentage of eggs that hatched after 24 h of submergence was significantly different between treatment groups ($F_{4,24} = 34.87$, $p < .01$). Clutches that were submerged at 72 HPO had a significantly lower percentage of eggs that hatched successfully 24 h after submergence compared with those submerged at 84 HPO ($z = 3.16$, $p < .01$) and those submerged at 96 HPO ($z = 3.11$, $p < .01$),

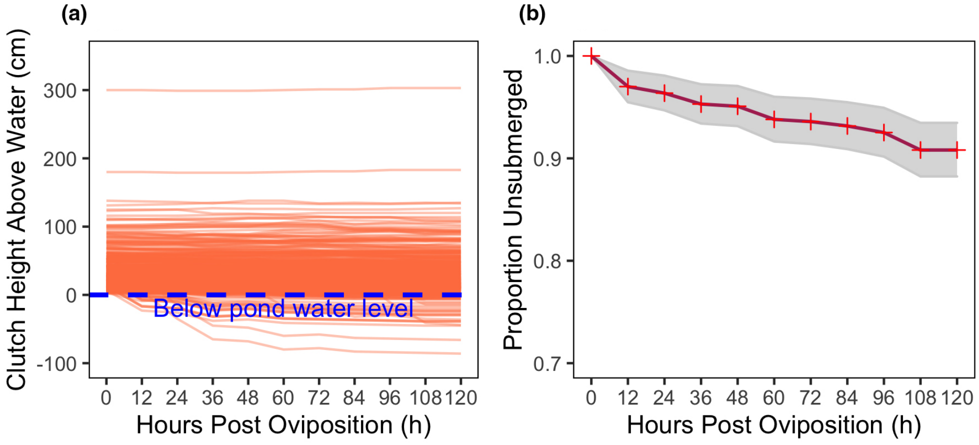


FIGURE 2 Clutch height above water (a) and Kaplan–Meier survival curve (b) for natural observations of 468 *Feihyla hansenae* egg clutches in 2011 and 2012 at Sakaerate Environmental Research Station. Note that the Y-axis for (b) starts at 0.7.

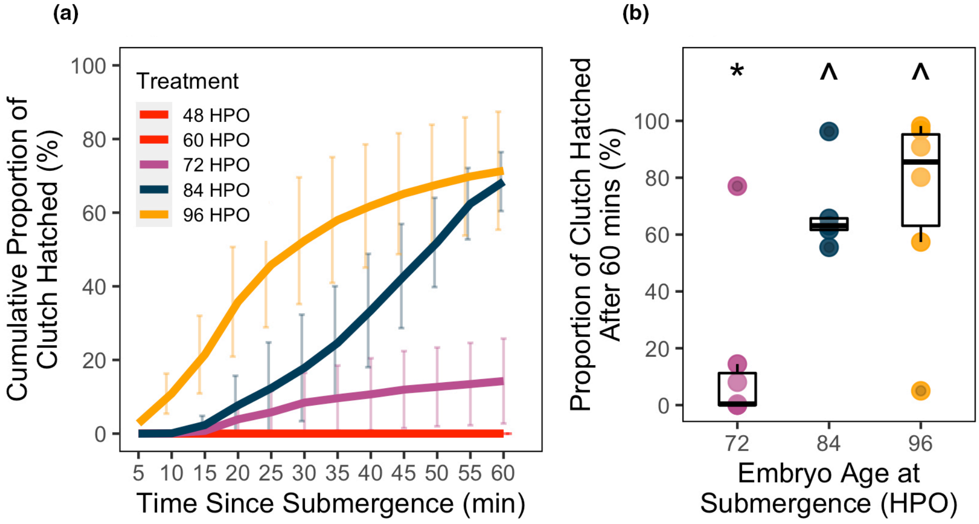


FIGURE 3 Proportion of *Feihyla hansenae* egg clutch hatched at 10 min time intervals within the first hour of submergence (a) and at 1 h after submergence (b) when submerged at 48, 60, 72, 84, and 96 h post oviposition. Error bars in (a) represent standard error. Horizontal line in (b) denotes mean, and the box represents the interquartile range. Characters (*, ^) represent statistically significant groupings ($p < .05$).

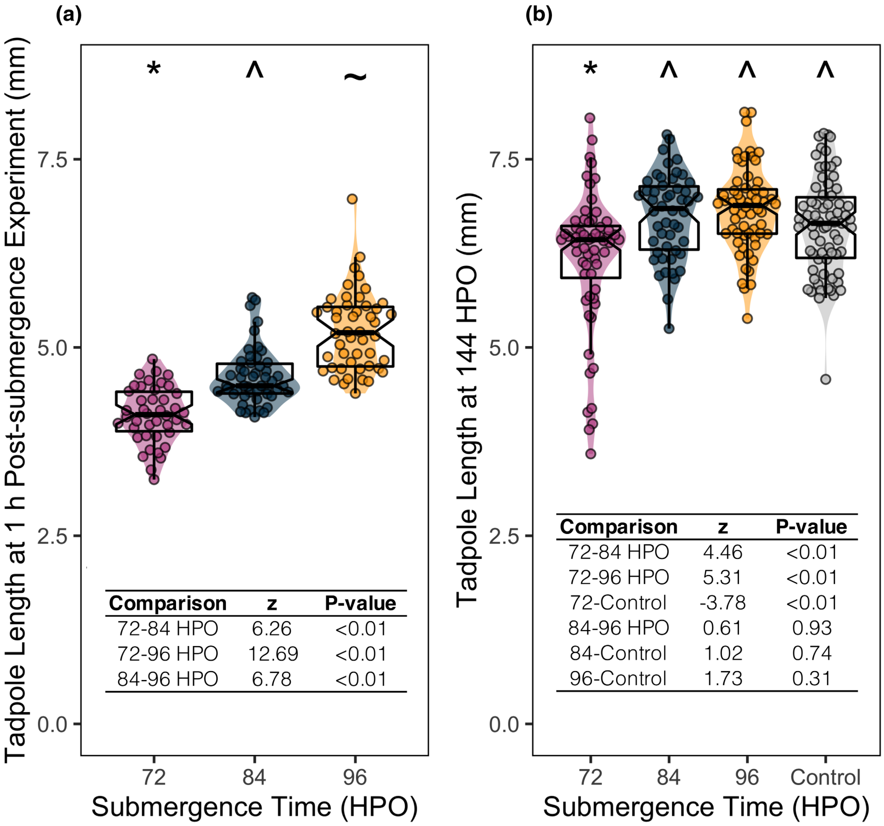
while no difference was found between the 84 and 96 HPO treatment groups ($z = 0.46$, $p = .99$). All embryos that did not hatch within 24 h of submergence were confirmed to be dead.

Total length 1 h post submergence was measured for tadpoles that hatched at 72, 84, and 96 HPO ($N = 42$, 50, and 48 tadpoles, respectively). There were no hatchlings after 1 h of submergence from clutches that were submerged at 48 and 60 HPO, and therefore these two treatment groups were removed from tadpole analyses. Hatchling size was significantly different between treatment groups ($\chi^2_{2,140} = 161.46$, $p < 0.01$, Figure 4a). Total length of hatchlings submerged at 72 HPO (4.11 ± 0.05 mm) was significantly lower compared with their counterparts at 84 HPO (4.61 ± 0.05 mm) and 96 HPO (5.17 ± 0.07 mm). Tadpoles that hatched at 84 HPO were also significantly smaller than those that hatched at 96 HPO (Figure 4a).

Total length at 144 HPO (6 days post oviposition) was measured for tadpoles that hatched at 72, 84, and 96 HPO and control tadpoles that hatched without disturbance ($N = 60$, 50, 60, and 70 tadpoles, respectively). Tadpole length was significantly different overall ($\chi^2_{3,240} = 161.46$, $p < .01$), but not between those hatched at 84 HPO, 96 HPO, and control tadpoles that hatching naturally (6.75 ± 0.08, 6.84 ± 0.07, 6.61 ± 0.08 mm, respectively, Figure 4b). However, tadpoles from the aforementioned groups were all significantly larger when compared to those hatched at 72 HPO (6.18 ± 0.12 mm, Figure 4b).

3.3 | Simulations combining submergence probability with hatching plasticity

Our simulations culminated in a mean 3.05% (range 95% CI 2.94–3.17%) increase in overall submergence survival if hatching plasticity is included (Figure 5, Table S2).



Comparison	z	P-value
72-84 HPO	6.26	<0.01
72-96 HPO	12.69	<0.01
84-96 HPO	6.78	<0.01

Comparison	z	P-value
72-84 HPO	4.46	<0.01
72-96 HPO	5.31	<0.01
72-Control	-3.78	<0.01
84-96 HPO	0.61	0.93
84-Control	1.02	0.74
96-Control	1.73	0.31

FIGURE 4 (a) Tadpole length at 1 h post submergence for *Feihyla hansenae* eggs submerged at 72, 84, and 96 h post oviposition (HPO). Note that tadpoles are 73, 85, and 97 HPO, respectively. (b) Tadpole length for the same submergence treatments and control tadpoles, which hatch naturally, at 144 HPO. Characters (*, ^, ~) represent statistically significant groupings ($p < .05$). The control group in (b) were not submerged, and allowed to hatch naturally.

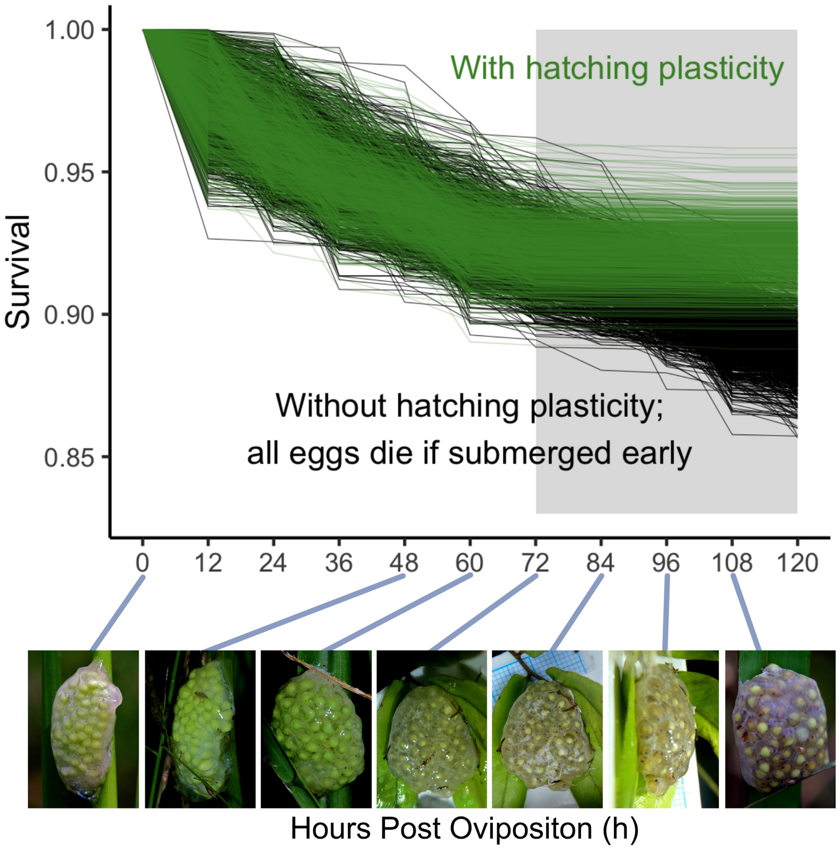


FIGURE 5 Simulation results with and without hatching plasticity (1000 iterations for each condition). Area shaded in gray indicates hours post oviposition at which simulations with hatching plasticity differ significantly from simulations without hatching plasticity (72–120 HPO). Note that the y-axis begins at 0.85.

4 | DISCUSSION

With few examples of in situ observations of submergence-induced early hatching, it can be difficult to quantify the benefits of plasticity in this trait. By incorporating data from natural observations and experimental manipulations into a statistical model, we provide insights into adaptive hatching plasticity in response to egg submergence in an arboreal-breeding frog. We show that natural submergence of egg clutches happens relatively infrequently (Figure 2). However, despite this infrequent occurrence, embryos beyond 72 HPO were able to respond to being submerged by hatching shortly after submergence (Figure 3), thereby shortening the egg stage by approximately 34% from the average hatching time of 115 HPO for clutches that hatched without disturbance (Poo & Bickford, 2013). Early hatching resulted in an obvious trade-off in size at the time of hatching that correlated with the age of the individual. However, for early hatchlings that have passed a threshold in their development (in this case 84 HPO), the negative effects of early hatching on size are quickly overcome (Figure 4). Through simulations of egg survival with and without hatching plasticity, we show the presence and maintenance of submergence-induced hatching plasticity as a trait increases egg survivorship modestly (3.05%), but significantly (Figure 5, Table S2).

Although hatching due to hypoxia or flooding is one of the most common and widespread forms of hatching plasticity in amphibians (Warkentin, 2011b), submergence-induced hatching is rarely observed and quantified in situ. In tropical monsoon regions, however, terrestrial eggs are presumably under higher risks of seasonal flooding following periods of heavy rainfall. By conducting observations of over 400 egg clutches in situ, we present a relatively large dataset on the risk of submergence in a tropical arboreal-breeding frog. We quantify the frequency of egg submergence due to pond level rises under natural settings, and show that roughly 10% of egg clutches end up underwater before they complete their regular embryonic development (Figure 2). While few studies have demonstrated the frequency of submergence under natural circumstances, observations of the Neotropical hourglass tree frog, *Dendropsophus ebraccatus*, indicated that around 25% of terrestrial eggs became desiccated during development and around 19% were submerged in water (Touchon & Warkentin, 2009, 2010), which is a similar rate of submergence to what we observed in *F. hansenae*. In the case of *D. ebraccatus*, however, eggs are capable of developing in either air or water and adults can lay eggs aquatically or terrestrially depending on rainfall patterns (Touchon, 2012), with only a small number of eggs suffering mortality from submergence (Touchon & Warkentin, 2009).

While adaptive hatching plasticity can reduce egg-stage mortality risks, there can be a trade-off between hatching early to avoid egg mortality and increased mortality risks at the larval stage (Johnson et al., 2003; Warkentin, 1995), as early hatchings are often smaller in size and less developed (Haramura, 2016). We observed this trade-off in our submergence trials, where embryos that hatched at 72 HPO were significantly smaller than those that hatched at later time points, both at time of hatching (Figure 4a) and at 144 HPO (Figure 4). Although we did not record the developmental stage of the tadpoles at hatching or specifically measure the costs of this difference, smaller tadpoles are known to be less efficient in prey consumption (Crossland, 1998) and more susceptible to predation (Formanowicz, 1986). As such, hatching early, depending on the post-hatching environment, can negatively affect survivorship during the larval stage (Warkentin, 1995; Willink et al., 2014) and can even carryover into subsequent life stages (Touchon et al., 2013). However, there is also evidence that the negative effects of early hatching can be relatively short term. For instance, in the East African reed frog, *Hyperolius spinigularis*, early hatchlings grew more rapidly and had higher survival at metamorphosis (Vonesh & Bolker, 2005). In *D. ebraccatus* while submergence-induced hatchlings were less developed and had a higher baseline mortality, difference in tadpole mortality dissipated after 10 days, with no long-term costs on late-stage tadpoles or metamorphic frogs (Touchon & Warkentin, 2010). Our findings in *F. hansenae* show an initial difference in size at hatching, which presumably would negatively impact larval survival. However, after a relatively short period of time (by 144 HPO) the effects of early hatching on tadpole size were no longer apparent in tadpoles that hatched at 84 and 96 HPO (Figure 4b). In comparison, tadpoles that hatched at 72 HPO were still smaller in size compared with the other treatment groups and the control group. This suggests that there may be a threshold, whereby once an individual reaches a certain point in development, the effects of hatching early are less pronounced.

Through statistical simulations we combined data from natural observations of the frequency of deadly flooding with experimental observations of submergence-induced hatching across developmental stages. We estimated the survival curves of *F. hansenae* under two scenarios: with and without hatching plasticity in response to submergence. We found a modest but significant difference between the two scenarios, with hatching plasticity increasing submergence survival by 3.05% (Figure 5, Table S2). One limit to our model is that it is focused on embryo survival and does not take into consideration tadpole stage mortality. The model assumed 100% survivorship for all individuals that hatched at 120 HPO, because this time point was past the average time of spontaneous hatching from undisturbed clutches in previous observations (Poo & Bickford, 2014). The model also assumes all tadpoles that hatched are able to survive. However, we know from our submergence trials that earliest hatchlings were smaller in size compared with tadpoles that hatched later. Therefore, if we take the potential disadvantages of hatching early on tadpole survival into consideration, our model could be slightly overestimating the benefit of plasticity. However, as discussed below, the adaptive value of hatching plasticity in *F. hansenae* is not limited to the context of inundation. We do not know of other studies that have incorporated data from in situ observations of threats into models of survival probability with and without plasticity in this way. Therefore, although our model has limitations, it also provides valuable insight into quantifying the benefit of plasticity in natural contexts.

We observed natural rates of flooding to be relatively low (~10% of clutches). However, *F. hansenae* also hatches early to escape attacks from predators, which account for ~30% of clutch mortality (Poo & Bickford, 2014). The evolutionary benefit of hatching plasticity likely accrues across contexts when embryos can hatch early to escape multiple types of threats. Plasticity is typically beneficial in heterogeneous environments when a predictive cue is present, but the ability of an organism to achieve the optimal match between phenotype and environment may be limited. DeWitt et al. (1998) discussed potential costs and limits to adaptive plasticity that would constrain its evolution. The limits most relevant to hatching plasticity include information reliability and the lag time between cue and response; both of which can hinder the ability of organisms to correctly match their phenotype to the environment (DeWitt et al., 1998). In the case of submergence, hypoxia provides a sustained and reliable cue, unlike the complex physical disturbances produced during predator attacks. Furthermore, embryos have a greater lag time between cue and hatching response when submerged than they do during predation. In our submergence experiments, some embryos were capable of surviving underwater in the clutch for 1 h or more before hatching. Embryos that did not hatch within 24 h, on the contrary, experienced 100% mortality at all ages of submergence. If the costs and limits of hatching plasticity in the context of deadly flooding are likely to be quite low, a small but modest benefit would favor the evolutionary maintenance of this ability, especially in a species such as *F. hansenae*, which hatches early to escape multiple threats.

Our submergence experiments revealed the earliest environmentally cued hatching thus far reported in *F. hansenae*, in terms of percentage of overall duration of the egg stage. Direct predation was previously shown to induce immediate hatching behavior in eggs between 89 and 91 HPO (Poo & Bickford, 2014); however, embryos were not tested at earlier stages. The present study is the first to systematically examine the ability of *F. hansenae* eggs to respond to hatching cues periodically throughout their development and shows that eggs are able to hatch much earlier than previously known. In future studies, it would be beneficial to systematically test for the onset of predator-cued hatching in *F. hansenae*. In *A. callidryas*, for example, the onset of flooding-cued hatching is known to precede the onset of predator-cued hatching (Warkentin, 2007). Overall, hatching in response to necessary or dangerous flooding appears to be widespread across diverse amphibian lineages (Warkentin, 2011b) and may be an ancestral ability or trait for the group. Documenting flooding-induced hatching in species that have previously been shown to hatch in response to other environmental cues provides additional evidence to support this hypothesis.

As intensity and consistency of rainfall become more unpredictable and more variable due to climate change, the selective landscape for adaptive traits will also fluctuate. While plasticity can play a role in mitigating the threats of climate change (Urban et al., 2014), there are still large areas of uncertainty due to lack of data across taxa and across different geographical regions. With the uncertainties of weather patterns, one assumption is that amphibian populations from ephemeral environments, which have presumably evolved mechanisms to cope with environmental heterogeneity, would be more resilient than those from more stable environments (Matthews et al., 2012). Predictive population modeling requires incorporating climate change-induced effects on survivorship across all amphibian life stages, including the embryonic and larval stages. However, the interaction between climate change and adaptive developmental plasticity remains poorly studied, with a few exceptions. For example, hatchlings of the pond-breeding gliding tree frog, *Agalychnis spurrelli*, have been shown to be affected by even subtle changes in humidity, with eggs hatching prematurely in response to a reduction in humidity of only 3% (González et al., 2021). Whereas in the boreal chorus frog, *Pseudacris maculata*, which can breed in permanent or ephemeral water bodies, findings are more varied. Some studies indicate tadpoles lacked a plastic response to hydroperiod reductions, suggesting an inability to metamorphose in time if breeding pools start drying up at a faster rate (Amburgey et al., 2012), while others show tadpoles were able to accelerate metamorphosis in response to a shortened hydroperiod, but at a cost of an overall increase in tadpole mortality (Amburgey et al., 2016). With the clear need for data to increase the accuracy of our predictions, our results add to the growing body of literature surrounding climate-correlated effects on poorly-studied amphibian populations.

While there is increasing evidence of hatching plasticity across taxa and within amphibians in particular, there is still a paucity of studies from biodiversity hot spots such as Southeast Asia (Sodhi et al., 2004). This lack of research focus is in stark contrast with the rapid rates of biodiversity loss within the region (Rowley et al., 2010; Sodhi et al., 2004). By combining natural observations with experimental manipulations to quantify the role of hatching plasticity, our study shows how this plasticity can be adaptive in a Southeast Asian tree frog and provides an important comparison to known examples of such behavior in other tropical regions.

AUTHOR CONTRIBUTIONS

SP conceptualized and supervised the project. SP, AKC, KLC, and ADS wrote the original draft of the manuscript. SP, FTE, SAM, BDN, AFM, JJR, JSS, ARA, HERJ, and LLJ conducted the investigation process, collected data, and curated data. SP and ADS conducted formal analysis and data visualization. All authors were involved in reviewing and editing the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Github at https://github.com/devansong/Poo_et.al_hatching_plasticity.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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