Androgenic shifts and external cues mediate parental care versus infanticide in mimic poison frogs

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Abstract

Infanticide is widespread across the animal kingdom. However, the physiological mechanisms underlying infanticide versus care or neglect are relatively unexplored. Here, we identified salient environmental and physiological antecedents of care and infanticide in the mimic poison frog (*Ranitomeya imitator*), a biparental and monogamous amphibian in which female parents feed their tadpoles with unfertilized eggs. Specifically, we explored potential environmental cues by evaluating changes in the frequency of food provisioning and tadpole mortality after cross-fostering tadpoles within and between morphs and displacing tadpoles within the terraria of their parents. After analyzing trophic egg deposition and observing tadpole mortality in the latter condition, we concluded that changes to offspring location reduce care and increase infanticide. Following this observation, we investigated whether steroid hormones relate to infanticide in an unfamiliar, resource-limited setting. Infanticidal behavior towards fertilized eggs and hatchlings included cannibalism and was associated with lower testosterone concentrations upon displacement to the new environment, while corticosterone concentrations did not correlate with infanticide at any sampling event. Ratios of testosterone to corticosterone were overall lower in infanticidal individuals. Overall, these findings support earlier investigations of environmental cues for offspring-directed behavior in poison frogs, while offering a novel perspective on temporal endocrine correlates of infanticide.

Highlights

- Offspring location drives parental decisions of care vs. infanticide.
- In novel territories, adults cannibalize conspecific, unrelated young.
- Lower circulating T in novel territory is associated with infanticide.
- Infanticidal adults exhibit lower T/CORT ratios.

1. Introduction

Animal parents exhibit remarkable behavioral flexibility, performing parental care (e.g. feeding, protecting, and transporting young), neglect, and infanticide, regardless of kinship (Hrdy, 1979; Royle et al. 2012; Lukas and Huchard, 2014; Lukas and Huchard, 2019). As care often represents a significant energy investment, parental decision-making is sensitive to evolutionary trade-offs dependent on ecological and physiological constraints (Clutton-Brock, 1991; Alonso-Alvarez and Velando, 2012). Moreover, parental behavior is often informed by environmental cues relevant to discriminating between related and unrelated offspring (Waldman, 1988). Therefore, parents provide a valuable framework for studying flexible social decision-making at multiple levels. While behavioral ecologists have reported numerous adaptive rationales for infanticide across diverse taxa, including mammals, fish, amphibians, insects, and birds (Hrdy, 1979; Ebensperger and Blumstein, 2007; Bose, 2022), efforts to link this behavior to its hormonal substrates is limited, especially beyond mammals. By connecting endocrine states to the performance of parental care versus infanticide, we can clarify how evolutionary innovations in physiology enable rapid responses to social challenges.

Steroid hormones present a compelling avenue to investigate the physiological basis of infanticide due to their well-established role in the proximate facilitation of social behaviors, especially those relating to aggression and parental care. For example, acutely elevated glucocorticoids promote social aggression in mammals and non-mammals alike (Summers et al., 2005; Haller, 2014), but are also positively associated with parental care in many vertebrates (Bales et al., 2006; O'Connor et al., 2009; Crossin et al., 2012). There are also reports of a positive association between infanticide and postnatal testosterone in rodents, although this relationship appears inconsistent, possibly owing to differences in genetic backgrounds and prenatal exposures (Elwood and Stolzenberg, 2020). More generally, across the animal kingdom, increases in male aggressive behavior sometimes co-occur with increases in circulating androgens during periods of social instability, such as intraspecific competition for

territory or mates (Wingfield et al., 1990; Hirschenhauser and Oliveira, 2006; Rodriguez et al., 2022). Accordingly, testosterone is generally hypothesized to be lower in adult males during parental care, although research suggests a more complicated relationship, as the trade-off between sexual and paternal behavior is variably detrimental to lifetime fitness across ecological contexts (Wingfield et al., 2001; Hirschenhauser and Oliveira, 2006). There also exists emerging support for a link between testosterone, parental care, and aggression in female vertebrates, including mammals, birds, lizards, and fishes (Rosvall, 2020). Additionally, proneness to social aggression may be better understood in terms of hormonal interactions, as proposed by the dual hormone hypothesis (Mehta and Prasad, 2015), where higher testosterone/cortisol ratios are generally associated with dominance attainment, risk-taking, and social aggression in some mammals including humans (Sapolsky, 2005; Terburg et al., 2009; Goll et al., 2023), but this trend has yet to be directly explored in other vertebrates.

Hormonal transitions between care and infanticide are unpredictable across species. Although some work on this topic has emerged in mammalian models (Elwood and Stolzenberg, 2020), the lack of comparable investigations in other taxa impedes the development of a comparative, predictive framework for parental decision-making. Poison frogs demonstrate extraordinary diversity in care strategies, including unimaternal, uniparental, and biparental care, in addition to a range of parental behaviors, namely tadpole transport, maternal egg provisioning, and cannibalism (Weygoldt, 1987; Summers and Tumulty, 2014). Therefore, these amphibians present an ideal system to study how changes in physiology resulting from environmental challenges influence parental decisions. Previous work has shown that, in poison frogs, offspring location, territorial status, and recent reproductive activity can be determining factors for whether adults cannibalize young or not (Stynoski, 2009; Ringler et al., 2016; Ringler et al., 2017; Spring et al., 2019). Specifically, work by Ringler et al., found that in the brilliant-thighed poison frog, *Allobates femoralis,* territorial status defines parental care or infanticidal decisions in males, whereas females perform care dependent on exact location of offspring

within their territory (2016, 2017). These findings have established a framework for investigating environmental cues for infanticide in other poison frogs, but the concurrent hormonal states underlying this behavior remain unexplored.

We used mimic poison frogs (*Ranitomeya imitator*) to better understand how hormonal shifts in response to environmental changes may elicit changes in parental behavior. Mimic poison frogs are biparental and sexually monogamous, forming pair bonds that last several months and performing sex-specific parental roles to cooperate in the care of their eggs and tadpoles (Brown et al., 2008). Males of this species transport tadpoles after egg hatching, and females provision trophic eggs to their tadpoles. By studying a monogamous, biparental amphibian, we contribute to a comparative framework for infanticide relating to the evolution of mating systems and parental care. Based on prior work in poison frogs (Stynoski, 2009; Ringler et al., 2016; Ringler et al., 2017), we hypothesized that care versus infanticide decisions in this species depend more on tadpole location than direct recognition cues. We performed kin recognition assays including cross-fostering and displacement within parents' terraria, then identified differences in the frequencies of egg provisioning and tadpole mortality. Following the identification of cues for care versus infanticide, we sought to uncover hormonal correlates of infanticidal behavior by moving individual adults to a novel environment with an unrelated fertilized egg or hatchling and measuring water-borne testosterone and corticosterone concentrations. We reasoned that testosterone and corticosterone should be reflective of perceived social competition and stress, respectively, and that individuals would react to these challenges by eliminating unrelated young.

2. Methods

2.1 Animal husbandry

All *R. imitator* eggs and tadpoles used in this study were captive-bred in our poison frog colony. Adults were purchased from Indoor Ecosystems between 2019-2021 (Whitehouse,

Ohio) or Ruffing's Ranitomeya between 2021-2023 (Tiffin, Ohio, USA). One adult male and one adult female were housed together in a 30 x 30 x 45 cm terraria containing sphagnum moss substrate, driftwood, live plants, egg deposition sites, and film canisters filled with water for tadpole deposition. Terraria were automatically misted ten times daily and frogs were fed live *Drosophila* fruit flies dusted with Repashy Calcium Plus (Oceanside, CA, USA) three times per week and with springtail isopods once a week. Tadpoles used in this study were observed in the home or foster terraria as described below (all with the same dimensions and substrates). All procedures in this study were approved by the Stanford University Animal Care and Use Committee (Protocol #34242).

2.2 Offspring recognition assays

Ranitomeya imitator pairs used for this study were required to have previously successfully raised a tadpole within 31 days of the experiment. Locations of tadpole deposition were marked on the outside of the terrarium with the date and time to ensure consistency in placing tadpole canisters in the same location throughout the duration of the study. Tadpoles were randomly placed into three experimental conditions (**Fig. 1A**): same-morph cross-foster (n = 16 across 6 terraria), different-morph cross-foster (n = 12 across 5 terraria), or intra-tank displacement (n = 18 across 7 terraria). Control tadpoles (n=26 across 11 terraria) served as a measure of parental investment under normal conditions. Whenever a tadpole was moved to another terrarium, it was replaced by another tadpole such that the number of tadpoles in that terrarium was consistent between assays.

Tadpole canisters were observed daily for 14 days in each trial, specifically noting tadpole condition and trophic egg deposits as a proxy for parental investment. Tadpole stage, size, and age were randomized, with all tadpoles being less developed than Gosner stage 32 (Gosner, 1960) to avoid counting feeding patterns that resulted from changes in investments as tadpoles neared metamorphosis. Every tadpole underwent one trial as a positive control, such

that all tadpoles in the experimental groups were also observed in the control group. We did this in order to measure parental effort per tadpole under normal conditions. Sampling each terrarium several times for positive controls enabled us to account for the number of tadpoles in that terrarium (one to four) and to capture individual variations in care by the focal pair.

In cases where tadpoles were exchanged between morphs, tadpoles were assigned to the “different-morph cross-foster” category, while replacements that were consistent with the morph of the previous tadpole were assigned to the “same-morph cross-foster” category. We chose to manipulate morphs because morphs are conspecific color variations tightly linked to geographical areas where coloration divergence occurs. For our study, we focused on two morphs, *Yurimaguas* (striped) and *Rapidos* (banded), previously established to have genetic diversity (Twomey et al., 2015). Tadpoles were always transported in their respective canisters to retain any tadpole-specific olfactory cues already present in the water and to minimize stress. Tadpoles displaced within their parents’ terraria were moved at a minimum of 5 cm. Offspring remained in their original canisters and were moved either to a laterally opposite corner of the terrarium or elsewhere along the terrarium edge. Tadpoles were always moved to a location not previously occupied by a canister.

2.3 “Takeover” behavior assays

To identify changes in physiology in infanticidal versus non-infanticidal parents, we simulated a territory takeover in an assay inspired by Ringler et al. (2017). We selected paired adults that raised at least one offspring in their terrarium within 31 days of the takeover trial as a proxy for parental experience and reproductive status. Frogs from the same reproductive pair were put into separate takeover trials at the same time to mitigate any risk of mate absence influencing behavior directed towards offspring. The takeover terrarium had the same dimensions, with elements of the environment (vegetation, replacement of dead leaves and substrate, fresh water) disrupted and replaced to introduce unfamiliarity. Rearrangements and

cleaning occurred between trials. Takeover terraria always housed at least two tadpole canisters, one upright and filled with water and the second empty and sideways, to remove bias for water availability and shelter, respectively. Frogs were fed the same diet on the same schedule in their takeover tanks as their home tanks with a dish of springtails available *ad libitum.* While baseline hormone collection of the adult occurred, we placed a single, unrelated fertilized late-stage egg to hatchling (Gosner stage 19-22) on a leaf on the terrarium floor. The leaf was positioned below a Wyze Cam v3 to record behavior using previously described methods (Goolsby et al., 2023). Frogs (n=12 females, 12 males) were introduced to the terrarium and recorded for a maximum of seven days. Infanticidal behavior was characterized as either consumption of the fertilized egg or hatchling (**Video S1**) or as repetitive physical disturbances to the egg, where the snout repeatedly dug at the jelly-like casing in what we interpreted as an attempt at egg cannibalism impeded by the encasement (**Video S2**).

2.4 Steroid hormone collection and processing

Frog hormones were collected immediately before frogs were displaced to the new tank ("baseline"), 24 ± 3 hours later ("move"), and following tadpole transport or infanticidal behaviors, or on Day 7 if no such behavior occurred ("final"), with hormone collections occurring at the same time of day unless an infanticidal behavior happened earlier or later in the day (**Fig. 2A-B**). Three subjects demonstrated infanticide on the day after displacement, so hormone measurements counted for both "move" and "final" sampling events. Baseline collection always occurred between 11:30 AM and 2:30 PM to avoid confounding effects of circadian physiology on hormone concentrations. We ultimately analyzed data from 65 sampling events across 23 individuals.

As shown in previous studies with poison frogs and amphibians (Gabor et al., 2013; Baugh et al., 2018, Baugh and Gray-Gailliard, 2021; Rodríguez et al., 2022), water-borne testosterone and corticosterone were collected as a non-invasive measurement that reflects

circulating levels of both hormones. Frogs were individually moved to a petri dish containing 40 mL of distilled water treated with reverse osmosis (Josh's Frogs RO R/x, Osowo, MI, USA). The collection lasted for one hour before either transferring the frog to the takeover terrarium (on Day 0), 24 hours after takeover (on Day 1), or returning the frog to the original terrarium (dependent on behavior; if none observed, Day 7). Immediately following 60 minutes, frogs were measured for length (snout-vent length) using a digital caliper (Shahe Measuring Tools, Amazon) along with mass, using a Maxus precision pocket scale (sensitivity 200 x 0.01 g, Amazon). Individual mass was measured at each hormone sampling event, then averaged to account for variations related to body fluctuations.

In sample processing, steroid hormones were pre-extracted using Sep-Pak C18 cartridges (Waters, Milford, MA, USA). The cartridges were conditioned using 2 mL of 100% ethanol, followed by 2 mL of Milli-Q water treated with reverse osmosis. Water samples were pushed through the column at a rate of approx. 10mL/minute, and then eluted with 4 mL of 100% ethanol. Ethanolic extracts were stored in glass vials at 4□ until nitrogen evaporation. One day prior to hormone quantification, the 4 mL of eluted hormone samples were divided into 2 mL new glass tubes for separate corticosterone and testosterone analyses from the same frog. The 2 mL tubes were placed in a 37□ water bath, and evaporated with gentle flow N_2 gas. After evaporation, hormone samples were resuspended in 250 µl of assay buffer specific to the corresponding ENZO kit, and stored at 4□ overnight until analysis the following day.

2.5 Steroid hormone quantification via enzyme-linked immunosorbent assays (ELISA)

After overnight incubation at 4□, samples were warmed to room temperature, and corticosterone and testosterone concentrations were determined using commercially available ELISA kits (ENZO Life Sciences, Farmingdale, NY; Corticosterone: cat. no. ADI-900-097, antibody: donkey anti-sheep IgG, sensitivity: 27.0 pg/mL; Testosterone: cat. no. ADI-900-065, antibody: goat anti-mouse IgG, sensitivity: 5.67 pg/mL) according to the manufacturer's

instructions. We chose to analyze corticosterone rather than cortisol concentrations because corticosterone is suspected to be the main adrenocorticotropic hormone (ACTH)-responsive glucocorticoid for *R. imitator* (Cockrem, 2013; Westrick et al., 2023). Samples were vortexed and 100 µL of the samples were added in duplicate in individual wells of microtiter plates. Following manufacturer instructions, plates were read at 405 nm, with correction between 570 and 590 nm, using a microplate reader (Synergy H1, BioTek Instruments, Winooski, VT, USA). Hormone concentrations were calculated using a four-parameter logistic curve in the software Gen5 (version 3.05, BioTek Instruments, Winooski, VT, USA). Samples with duplicate measurements that yielded a coefficient of variation (CV) > 30% were set aside for imputation. Before removing these duplicates, intra-assay CV values for testosterone and corticosterone were 9.7% and 13.9%. After removal, intra-assay CV values were 9.1% and 9.0%. Inter-assay CV values were 13.7% and 11.8%.

2.6 Statistics

We cleaned and analyzed all data using RStudio (R version 4.2.3, R Core Team, Boston, MA). We implemented all statistical analyses using a generalized linear mixed model (GLMM) framework. For each analysis, we used Akaike Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson, 2002) to choose between alternative model fits. We evaluated standard model diagnostics and also tested for outliers and quantile deviations using the '*DHARMa'* package (version 0.4.6, Hartig, 2022). All models in this manuscript passed all diagnostic tests. To evaluate the significance of main effects, we performed a Type III analysis of variance with Satterthwaite's method. We evaluated pairwise comparisons and accounted for multiple comparisons using the Tukey method in the *'emmeans'* package (version 1.9.0, Lenth, 2023). We accounted for effect sizes in multi-termed modeling using the '*effectsize'* package (version 0.8.6, Ben-Shachar et al., 2020) and with pairwise effect size

comparisons using '*emmeans'*. Figures were created using the package '*ggplot2*' (version 3.4.4, Wickham, 2016) and assembled in Adobe Illustrator (Adobe Illustrator 2023).

2.6.1 Offspring manipulations

We analyzed the number of eggs deposited for each tadpole among the four conditions (control, same-morph cross-foster, different-morph cross-foster, intra-tank displacement; **Fig. 1A**). To test whether egg deposition changes due to cross-fostering and intra-tank displacement, we used a GLMM with a zero inflation component implemented using the '*glmmTMB*' package (version 1.1.8, Brooks et al., 2017). We chose a Poisson distributed model based on best fit, with eggs fed as a dependent variable, experiment type and number of siblings within a terrarium as independent variables, and focal terrarium as a random effect.

2.6.2 Takeover trials

To determine how testosterone, corticosterone, and testosterone:corticosterone ratios change relative to cannibalizing behavior, we implemented a GLMM using the '*lmerTest*' package (3.1-3, Kuznetsova et al., 2017). We set the outcome ("infanticide" vs "non-infanticide"), sampling event ("baseline" vs. "move" vs. "final"), sex, and their interactions as fixed effects and individual frog identity, average individual mass, volume of collection water, and body length (snout-vent length) as random effects. We estimated average individual mass as the average body mass across each sampling event and this was necessary to account for intra-individual differences in feeding. Since mass was not collected for a single frog, we used mean imputation to estimate the single missing value as the averaged individual mass across the entire study. To improve interpretation of regression coefficients, we removed insignificant interaction terms using stepwise backward regression.

We ran separate models for testosterone, corticosterone, and ratios of testosterone to corticosterone. We selected appropriate data transformations (natural log, square root, inverse,

no transformation) for each dependent variable using AICc. Using AICc and diagnostic criteria, we selected log transformations for corticosterone and ratio modeling, and inverse transformations for testosterone modeling.

Since corticosterone, testosterone, and ratio values of samples exceeding the CV threshold of 30% are unreliable, we treated these values as missing data. We estimated these missing data using regression imputation (Ellington et al., 2015; Nakagawa and Freckleton, 2011) and the methods described above. Briefly, regression imputation estimates missing data as predictions obtained from a regression model fit using the dataset without missing data. Thus, all available information from complete and incomplete samples is used to predict missing data while simultaneously preserving all correlations found in the original dataset and without introducing new correlations. Out of 65 measurements, we imputed only 6 (9.2%) of our corticosterone values, 2 (3.1%) of our testosterone values, and 8 (12.3%) of our ratio values. All of our final models used for imputation and inference passed all diagnostic tests.

3. Results

3.1 Offspring location as an indirect recognition cue

To identify which offspring features *R. imitator* parents use when caring for young, we subjected tadpoles to either cross-fostering or displacement within their parents' terraria and observed egg deposition and tadpole mortality for 14 days. We hypothesized that in addition to offspring manipulations, like cross-fostering, the number of siblings present may also influence how many eggs can be provisioned. We manipulated offspring identity (same-morph cross-foster, different-morph cross-foster) and offspring location (intratank displacement, **Fig. 1A**). We found that offspring manipulation type ($\chi^2 = 17.74$, $p < 0.0005$; **Table S1**) was an overall better predictor of eggs provisioned than the number of siblings present in a tank at a time (CI: -0.51 – 0.22, $\chi^2 = 0.62$, $p = 0.43$; **Table S1**). Surprisingly, even within positive controls, sibling number

does not correlate with number of eggs fed (CI: -0.46 – 0.47, χ^2 = 6.00E-04, p = 0.98; **Table S1**).

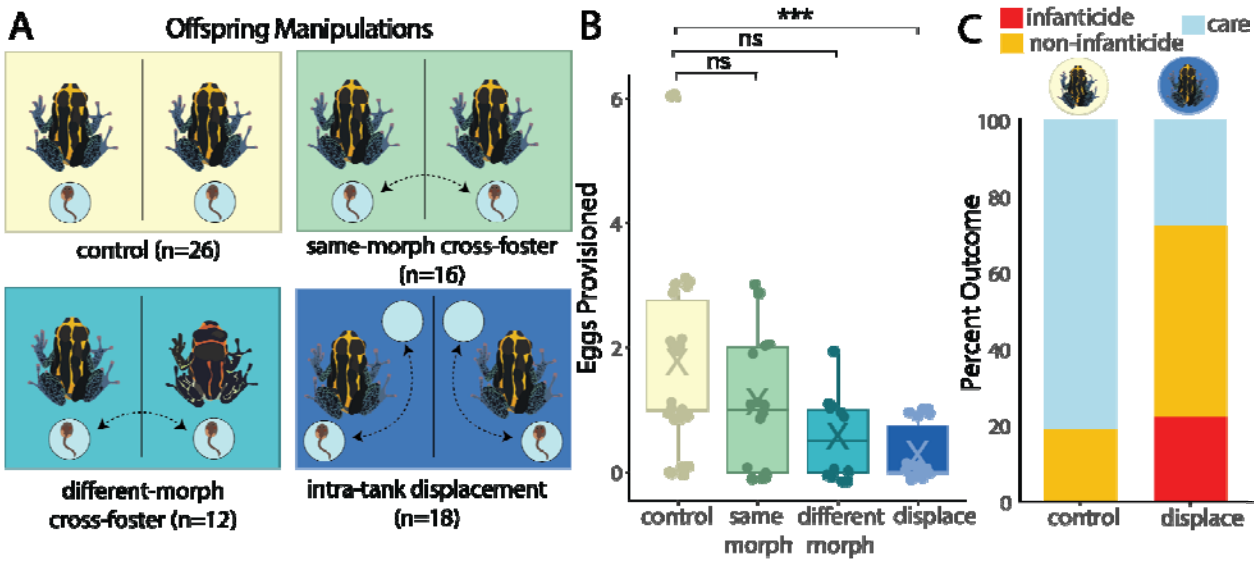

Figure 1. Disrupting offspring location impedes egg provisioning and promotes infanticide. (A) Control tadpoles were the offspring of the parents in that terrarium and were not moved from their original locations. Cross-fostered tadpoles were exchanged between terraria, such that they were unrelated to the parents in the new terrarium. They were specifically moved to the same location previously occupied by the offspring they replaced. The different-morph cross-foster manipulation followed the same procedure as same-morph cross-fosters, with the additional condition that tadpoles were exchanged between terraria housing different morphs (striped, banded), discerned by parents' coloration patterns. Finally, intra-tank displacement tadpoles were related to the parents in the terrarium but were displaced by a minimum of five centimeters to a different location in the terrarium. **(B)** Tadpoles displaced within their parents' tanks received significantly less feeding in the form of trophic eggs from parents (Tukey adjustment for a family of 4 estimates: p < 0.0008). Significance: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns < 0.1. "ns" = not significant. **(C)** Only tadpoles displaced within their parents' tanks (n=4 out of 18) were subjected to infanticide with no deposition of a second tadpole.

We next asked whether offspring displacement influenced parental provisioning of young and found a strong effect on the number of eggs provisioned (CI: -2.43 – -0.37, z = -2.66, p < 0.01, **Table 1**; model diagnostics may be found in **Fig S2** with details for analysis of deviance in

Table S1). Specifically, over 14 days, displaced tadpoles received significantly fewer trophic eggs compared to positive controls (**Fig. 1B**, p_{adj} = 0.0008, □ = 1.81). Across our experiment, control tadpoles were fed the most, at an average of 1.77 eggs every two weeks, whereas same morph and different morph cross fosters were fed less than positive controls, being fed 1.13 and 0.58 eggs on average, respectively. Displaced tadpoles were the least cared for, being fed an average of 0.27 eggs within two weeks. In other words, positive control and cross fosters averaged between 1-2 eggs being fed whereas tadpoles that were spatially moved, even with their biological parents, were fed 0-1 eggs over 2 weeks. Tadpoles cross-fostered within and outside of their morph did not experience a significant reduction in feeding compared to positive controls (**Fig. 1B**, p_{adj} = 0.71-0.15, □ = -0.910 - -0.409, respectively; **Table S2**). Tadpoles displaced within their parents' terraria were subjected to a variety of infant-directed behaviors, including "care" as exemplified by egg feeding, "non-infanticide" as exemplified by the absence of observed feeding, and "infanticide" as shown by physical injury to the tadpole without deposition of a second tadpole, which was only observed in the displacement manipulation (**Fig. 1C**).

Table 1. Final model fit of eggs fed with offspring manipulations. SD = Standard Deviation. SE = Standard Error, z = standardized Z value, Pr() = P value.

Random Effects	Variance	*SD*		
Tank	0.23	0.48		
Fixed Effects	Estimate	*SE*	*z*	Pr (>|z|)
Intercept	0.40	0.64	0.63	0.53
Different Morph	-0.50	0.50	-1.01	0.31
Control	0.41	0.31	1.32	0.19
Displacement	-1.40	0.53	-2.66	**7.80E-03**

Siblings	-0.15	0.19	-0.78	0.43

3.2 Infanticide in new territory

After concluding that offspring location is an important factor in parental decisions of care versus infanticide, we performed a "takeover" behavioral assay to test for hormonal correlates of infanticide. We analyzed corticosterone concentration, testosterone concentration, and ratios of testosterone/corticosterone with respect to outcome ("infanticide" versus "non-infanticide"), sampling event ("baseline", "move", and "final"), and interactions between outcome and event themselves **(Fig. 2A-B**).

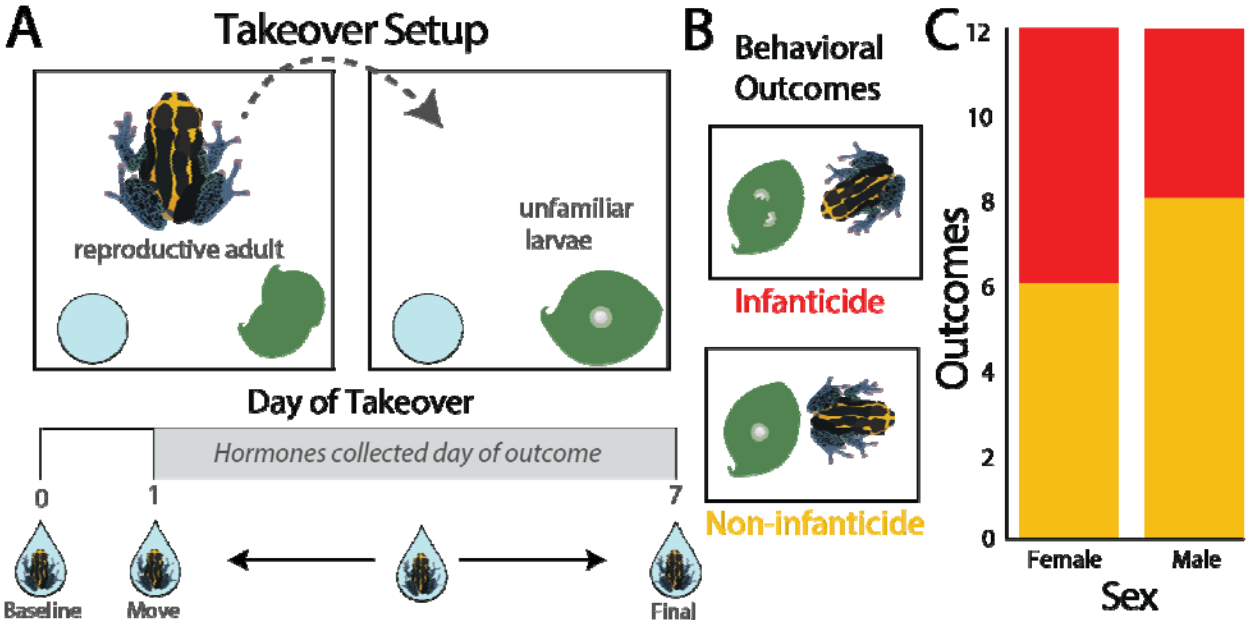

Figure 2. Takeover setup and behavioral outcomes. (A) Individual adult frogs were displaced from their home terraria to novel terraria along with a single, unrelated, fertilized egg or hatchling placed on a leaf. Trials lasted for a maximum of seven days. Hormones were collected via water-borne hormone sampling on the day of the move (Day 0), one day afterward (Day 1), and upon observation of infanticidal behavior, or on Day 7 if no such behavior occurred. **(B)** Behavioral outcomes towards eggs or recently hatched tadpoles as observed on motion-trigger Wyze v3 cameras included "infanticide" in the form of cannibalism or physical disturbance to the egg, or "non-infanticide" where these behaviors were not

observed, including one observation of transport by a female. **(C)** Females exhibited both outcomes (non-infanticide: n = 6, infanticide: n = 6), as did males (non-infanticide: n = 8, infanticide: n = 4).

Of the twelve trials performed with females, six of the twelve females fell within the "infanticide" outcome, while the other five were categorized as ignoring the conspecific young. One individual demonstrated parental care, performing tadpole transport (**Video S3**). As we only observed transport by one individual, we did not have enough statistical power to analyze this result distinct from other non-infanticidal females, and therefore we placed them in the "non-infanticide" category. We further rationalized this choice with the observation that concentrations of circulating androgens and glucocorticoids do not significantly differ between females that do or do not perform transport in other poison frogs (Fischer and O'Connell, 2020). Of the twelve trials performed with males, eight showed no infanticidal behavior towards conspecific young while four showed infanticidal behavior (**Fig. 2C**). Latency to infanticide decisions were variable, with the behavior occurring on a range of days, from the day after movement to the new terrarium to the full seven days (**Fig. S1**).

3.3 Infanticide and corticosterone

Log corticosterone (corticosterone hereafter) concentrations did not vary significantly between infanticidal versus non-infanticidal adults (**Table 2**, $\eta_p^2 = 0.01$, t = -0.47, p = 0.65; **Fig. 3A, Table S7**) nor did they vary significantly between males and females (**Table 2,** $\eta_p^2 = 0.17$, t = 1.99, p = 0.06; **Fig. 3A, Table S7**). On average, corticosterone levels increased during takeover of novel territory ($\eta_p^2 = 0.17$, t = 2.45, p = 0.02, **Table 2**). Model diagnostics may be found in **Fig S3** with analysis of deviance details in **Table S6-7.** In pairwise analyses across sampling events, corticosterone levels significantly decreased from "move" to "final" sampling days (Cohen's *d* = 0.83, t = 2.46, $p_{adj} = 0.047$; **Table S3**). No other pairwise comparisons between behavioral outcomes and sampling events were significant (**Table S3**). In summary,

corticosterone levels changed the most between 24 hours into a takeover and subsequent sampling periods; this change was consistent between males and females, as well as between infanticidal and non-infanticidal adults.

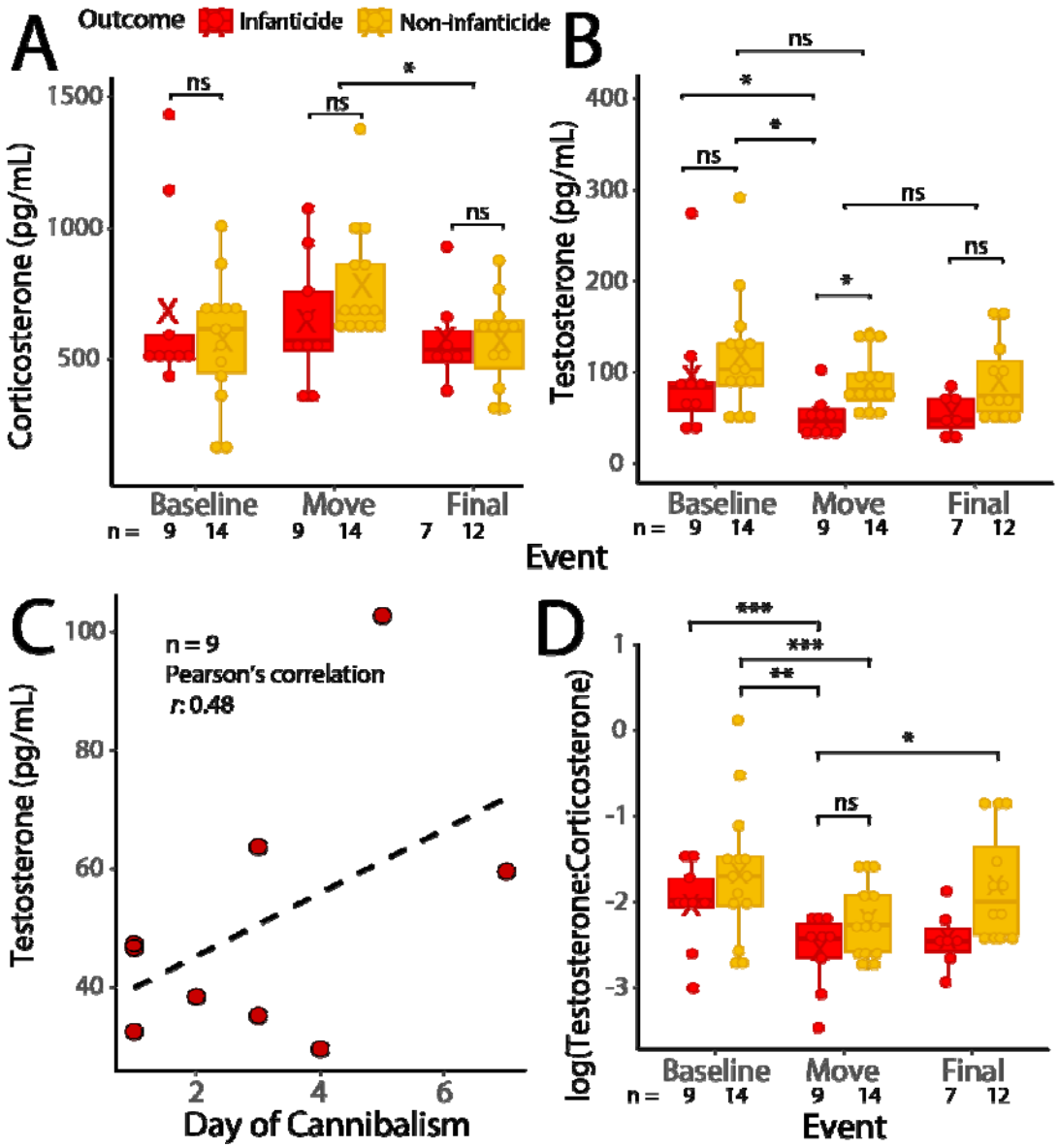

Figure 3. Hormone concentrations by event and behavioral outcome. (A) Corticosterone and **(B)** testosterone concentrations by sampling events and behavioral outcomes. **(C)** Testosterone concentrations of infanticidal individuals 24 hours after being moved into novel territory by day of infanticide. There is a moderate, but not significant, correlation between testosterone and day of infanticide (Pearson's product-moment correlation r = 0.48, p = 0.18). **(D)** Natural log of

testosterone:corticosterone concentration ratios by sampling events and behavioral outcomes. The “X” in each box plot represents the average. The horizontal lines represent the medians of each group. All results are shown in raw units. Signif. codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < . < 0.1 < ns < 1.

Table 2. Final corticosterone model fit of log(corticosterone) by sampling event, behavioral outcome, and sex. Frog identity, frog length, and average mass are random effects. SD = Standard Deviation. SE = Standard Error, t = t-statistic, Pr() = P value.

Random Effects	Variance	*SD*			
Body length	0.02	0.13			
Frog identity	0.03	0.17			
Average mass across trial	0.01	0.12			
Residual	0.08	0.29			
Fixed Effects	Estimate	*SE*	df	*t*	Pr (>|t|)
Intercept	6.25	0.12	31.14	52.52	**<2.00E-16**
Outcome	-0.06	0.13	18.56	-0.47	0.65
Move	0.22	0.09	40.02	2.45	**0.02**
Final	-0.03	0.09	43.24	-0.28	0.78
Sex	0.25	0.13	19.86	1.99	0.06

3.4 Infanticide and testosterone

Across all samples, the inverse transformation of testosterone (testosterone hereafter) concentration varied significantly both with sampling events (η_p^2 = 0.28, p=0.01, F=5.02, **Fig. 3B, Table S7**). Testosterone concentrations also changed between behavioral outcomes (η^2 = 0.27, p=0.02, F = 6.43, **Fig. 3B, Table S7**), but were not sex-specific (η_p^2 = 0.02, p=0.49, F=0.50, **Fig. 3B, Table S7**). Infanticidal individuals, across their trial, averaged about ⅔ of the

testosterone levels (67.94 pg/mL) of non-infanticidal individuals (99.44 pg/mL). In other words, testosterone decreased, on average, during potentially stressful events (baseline compared to move), and during infanticidal behaviors. Most notably, testosterone levels significantly decreased 24 hours after takeover onset ($t = 3.45$, $p < 0.01$), but the extent of this decrease was significantly dependent on whether or not the individual performed infanticide ($\eta_p^2 = 0.24$, $t = -2.45$, $p = 0.02$, **Table 3**). Model diagnostics may be found in **Fig S4** with full analysis of deviance details in **Table S9.**

Table 3. Final testosterone model fit of 1/(testosterone) by sampling event, behavioral outcome, and sex. Frog body length, identity, and water collection volume are random effects. SD = Standard Deviation. SE = Standard Error, t = t-statistic, Pr (>|t|) = P value.

Random Effects	Variance	*SD*			
Body length	6.44E-06	2.53E-03			
Frog Identity	2.03E-05	4.50E-03			
Water volume	2.74E-05	5.24E-03			
Residual	8.24E-06	2.87E-03			
Fixed Effects	Estimate	*SE*	df	*t*	**Pr (>|t|)**
Intercept	1.60E-02	2.58E-03	29.17	6.18	**9.6E-07**
Outcome	-3.96E-03	2.58E-03	27.36	-1.54	0.14
Move	6.03E-03	1.75E-03	23.50	3.45	**2.16E-03**
Final	3.31E-03	1.88E-03	22.83	1.76	0.09
Sex	-1.64E-03	2.32E-03	19.64	-0.71	0.49
Move : Outcome	-5.06E-03	2.07E-03	19.40	-2.45	**0.02**
Final : Outcome	-6.14E-04	2.32E-03	23.03	-0.27	0.79

In pairwise comparisons, we examined testosterone levels relating to interactions between sampling events and outcomes. We found that, within infanticidal adults, levels of testosterone between baseline hormone sampling and moving to a new territory were significantly different (Cohen's *d* = -2.08, t = -3.21, p = 0.03, Tukey's method for comparing family of 6 estimates, **Fig. 3B; Table S4**). Similarly, baseline testosterone levels in adults who did not perform infanticide were significantly greater than testosterone concentrations in infanticidal individuals moving into a new territory (Cohen's *d* = 3.48, t ratio = 3.57, p = 0.01, Tukey's method for comparing family of 6 estimates, **Fig. 3B; Table S4**). Finally, infanticidal and non-infanticidal individuals have different testosterone levels when moved into a new territory (average of 50.62 pg/mL and 88.26 pg/mL, respectively; Cohen's *d* = 3.15, t ratio = 3.32, p = 0.026, Tukey's method for comparing family of 6 estimates, **Fig. 3B; Table S4**). Within infanticidal individuals, we found a moderately positive (r = 0.48), albeit statistically insignificant, correlation between testosterone levels 24 hours into the territory takeover assay and latency to infanticide (p = 0.19; **Fig 3C**), which further suggests a relationship between lower testosterone and infanticide.

3.5 Infanticide and testosterone:corticosterone ratios

Finally, we examined whether the (natural) log ratios of testosterone to corticosterone (ratios hereafter) changed across the takeover sampling events or between infanticidal and non-infanticidal individuals. The ratio decreased from baseline to move (η_p^2 = 0.38, t = -5.04, p < 1.22E-05, **Table 4**) and between outcomes (η_p^2 = 0.24, t = 2.31, p < 0.03, **Table 4**). Similar to corticosterone and testosterone, ratios were not sex-specific in their changes (η_p^2 = 0.07, t = -1.22, p < 0.24, **Table 4**). Model diagnostics may be found in **Fig S5** with analysis of deviance details in **Table S11.** In pairwise comparisons, we examined testosterone:corticosterone ratios relating to interactions between sampling events and outcomes. We found that, regardless of behavioral outcome, ratio levels changed from baseline to 24 hours into the territory takeover

significantly changed (Cohen's *d* = 1.56, t ratio = 4.91, p < 0.001, **Fig. 3D**, **Table S5**). Likewise, ratio levels of infanticidal individuals moving to a new territory were significantly different from baseline ratios and final ratios of individuals that did not perform infanticide (Cohen's *d* = -2.87 and -2.25, t ratio = -4.31 and -3.31, p < 0.01 and 0.05, respectively, **Fig 3D, Table S5**).

Table 4. Final ratio model fit of log(ratio) by sampling event, behavioral outcome, and sex. Frog body length and identity are random effects.

Random Effects	Variance	*SD*			
Body length	0.06	0.24			
Frog Identity	0.14	0.36			
Residual	0.12	0.35			
Fixed Effects	Estimate	*SE*	df	*t*	Pr (>|t|)
Intercept	-1.95	0.18	27.36	-10.80	**2.29E-11**
Outcome	0.45	0.20	16.81	2.30	**0.03**
Move	-0.54	0.11	38.90	-5.04	**1.10E-05**
Final	-0.22	0.12	42.23	-1.88	0.07
Sex	-0.24	0.20	20.67	-1.20	0.24

4. Discussion

In this investigation, we first delineated tadpole-based features for parental care versus infanticide, identifying location as a salient kin recognition cue. Then, we documented adult infanticidal behavior towards eggs and tadpoles for the first time in *Ranitomeya* frogs. By analyzing concentrations of water-borne steroid hormones corticosterone and testosterone, we demonstrated that lower testosterone concentrations and testosterone:corticosterone ratios precede infanticide.

4.1 Parental decision-making in familiar and unfamiliar territories

We observed that displaced tadpoles experienced significant decreases in trophic egg deposition compared to control tadpoles, unlike tadpoles cross-fostered within or between morphs **(Fig. 1B)**. Similar to other poison frogs (Stynoski, 2009; Ringler et al., 2016; Ringler et al., 2017), *R. imitator* parents likely discriminate between young on the basis of location. For example, in the brilliant-thighed poison frog (*Allobates femoralis*), males care for any clutch in their home terraria but cannibalize clutches upon establishing new territories, and females perform care based on the exact location of offspring (Ringler et al., 2016; Ringler et al., 2017). Offspring location therefore appears to be a critical cue driving parental care across poison frogs despite diversity in care systems and ecological constraints. Parental care triggered by cues of relatedness has also been observed beyond amphibians (Klug and Bonsall, 2007). Critical decisions between care or cannibalism are especially abundant in anamniotes and squamates (Ray and Maruska, 2023). For example, spawning appears to impede cannibalism in parenting African *Neolamprologus caudopunctatus* cichlids, resulting in the care of foreign young (Cunha-Saraiva et al., 2018), whereas South American Midas cichlids (*Amphilophus citrinellus*) require olfactory recognition of offspring and will adopt unrelated fry with the same odor (McKaye and Barlow, 1976). Similarly, brooding children's pythons (*Antaresia children*) will provide care when eggs are cross-fostered or even replaced with stones (Brashears and DeNardo, 2012).

A proportion of displaced tadpoles were found dead shortly after being moved to a new location in their home terrarium, which we interpreted to be caused by infanticide (**Fig. 1C**). These offspring did not encounter any other tadpoles, making intra-sibling aggression unlikely, despite being well documented in *Ranitomeya* (Brown et al., 2009; Schulte and Mayer, 2017; McKinney et al., 2022). Offspring cannibalism as a behavioral consequence of intraspecific competition has been previously suggested in other poison frogs (Summers, 1989), where adults might commit infanticide of unrecognized offspring to prevent misdirected care, to

promote survival of their own offspring by parasitizing occupied deposition sites, or to prevent offspring cannibalism by unrelated tadpoles. Other species of *Ranitomeya* have been found to parasitize the care of other parents, another indicator that brood parasitism is a weapon between conspecific competitors (Poelman and Dicke, 2007; Brown et al., 2009, respectively). To understand why tadpoles were killed rather than eaten by adults, we theorize that dead tadpoles may yield a precious protein source for cannibalistic *R. imitator* tadpoles that adults may deposit. Protein can otherwise be difficult to obtain in low resource environments and may minimize future costs of females having to provision unfertilized egg meals to nutritionally needy tadpoles (Yoshioka et al., 2016). Thus, we hypothesize that infanticide by *R. imitator* adults evolved for parents to maximize their individual reproductive success and opportunities while minimizing those of potential competitors, following evaluation of their environments.

We quantified the behavioral responses of reproductive adults to unrelated, conspecific young in an unfamiliar environment and found that both males and females cannibalized the stimulus. This marks the first documented report of adults cannibalizing young in the *Ranitomeya* genus and supports previous reports of non-filial cannibalism in other poison frogs (Townsend et al., 1984; Summers, 1989; Ringler et al., 2017; Spring et al., 2019; Dugas et al., 2023). We considered multiple adaptive rationales for non-filial cannibalism in *R. imitator.* First, cannibalism could be a behavioral decision relevant to a trade-off between acquiring caloric content (which risks eating offspring) and providing care (which risks caring for unrelated young), an idea which has been tested extensively in fish (Bose, 2022). Fertilized conspecific eggs constitute part of other frog diets (Beard, 2007). Furthermore, feeding behavior appears mechanistically linked to parental status across taxa including mammals, birds, and fish (O'Rourke and Renn, 2015; Fischer and O'Connell, 2017). Thus, we reasoned that some frogs might be more likely to cannibalize the stimulus egg or hatchling based on differences in how recently they parented, although all subjects produced a tadpole within the previous 30 days. To account for various degrees of hunger and potential effects of parental status, frogs were

supplied with food *ad libitum*, so it is unlikely that the frogs in the present study cannibalize to compensate for nutritional deficits. Therefore, it appears more likely that cannibalism occurred as a response to intraspecific competition rather than as a means of acquiring food.

In our experimental paradigm, we disrupted pair bonds; therefore, cannibalism of conspecific, unrelated young might occur to procure access to new mates. Sexually selected infanticide is well-documented in mammals, birds, and insects (Palombit, 2015), and some evidence exists for this rationale exists in other poison frogs (Summers, 1989; Spring et al., 2019). For instance, *A. femoralis* females are less likely to cannibalize unrelated young in the presence of a male with whom they produced a clutch (Spring et al., 2019). While *R. imitator* are usually monogamous, mate poaching is nonetheless a proposed rationale for male aggression in this species (Brown et al., 2009). Another area for future exploration is clarifying the importance of territoriality, as *A. femoralis* males demonstrate a nearly universal switch to clutch cannibalism in new territories, while caring for those in their home environment (Ringler et al., 2017). As infanticide was still performed by a minority of frogs in the takeover assay, it is worth clarifying the importance of territoriality relative to other potential decision-making cues in this species, namely nutritional need, mate access, parental experience, and offspring size or age.

4.2 Hormonal correlates of infanticide

To clarify whether physiological stress correlates with infanticide, we analyzed cannibalistic behavior and sampling events in relation to corticosterone levels. Parental glucocorticoid responses to social instability have mostly been studied in mammals, which presents a logistical challenge for comparison to amphibians, as glucocorticoids are also involved in lactation (Engh et al., 2006). Still, we expected to find that cannibalistic adults would exhibit greater corticosterone concentrations upon displacement to new territory, as glucocorticoids increase when encountering stressful and challenging environments (Sapolsky et al., 2000). Additionally, we sought to test the idea that adults which underwent this

physiological stress would be more likely to perform infanticide. Instead, corticosterone concentrations were not significantly associated with infanticide at any sampling event, but significantly changed over the course of the trial regardless of behavioral outcomes (**Fig. 3A**). Elevated glucocorticoids have been proposed to promote both avoidant and approaching behaviors (Terburg et al., 2009), which may relate to decisions to cannibalize and ignore unrelated offspring, respectively. It is also possible that the stress of displacement masks any corticosterone-specific effects associated with cannibalism. Based on our observations, we conclude that although physiological stress responses in *R. imitator* do change with time in a novel territory, the behavioral reactions to this stress likely depend more so on non-corticosteroid pathways, potentially involving other steroids or neuromodulators.

Previous investigations have shown conflicting results regarding the relationship between testosterone levels and infanticide (Rosenberg and Sherman, 1975; Martínez et al., 2015). However, circulating androgens are classically associated with territorial aggression in mammals, birds, and recently, other poison frogs (Wingfield et al., 1990; Duque-Wilckens et al., 2019; Rodriguez et al., 2022). Therefore, we expected to find that frogs which performed infanticide upon displacement to a new territory would exhibit greater levels of testosterone. Contrary to what we expected, circulating testosterone appears to be negatively associated with infanticide in this species (**Fig. 3B**); in fact, latency to cannibalism showed a positive relationship to testosterone levels the day after territory takeover begins (**Fig. 3C**). Our finding aligns with investigations in some fish, where plasma 11-ketotestosterone concentrations were lower in cannibals and comparable to those typical of the parental phase (Takegaki et al., 2023). Interestingly, low testosterone is also associated with parental care in male poison frogs (Townsend and Moger, 1987); therefore, it seems plausible that decreased androgens can be associated with both care and infanticide in amphibian parents.

Moreover, local aromatization of testosterone to estradiol has been linked to aggression in several mammals, fish, and birds (Trainor et al., 2006; Huffman et al., 2013). The conversion

of testosterone to estradiol by aromatase is a highly conserved process in vertebrates, including amphibians (Coumailleau et al., 2015), and the co-option of estrogens rather than androgens to facilitate aggression can potentially avoid the costs of elevated testosterone, which can be especially detrimental in parents (Wingifeld et al., 2001). Investigations in mice have suggested that estrogen receptor α expressing (*ESR1+)* neurons are necessary and sufficient for infanticide (Hashikawa et al., 2017; Liu et al., 2022; Mei et al., 2023). Within birds, estrogens are thought to increase aggression more broadly, as male aromatase activity is positively associated with aggressive pecking responses, aggressive song production, and chasing behavior (Schlinger and Callard, 1989; 1990; Soma et al., 2000). However, estrogen signaling has yet to be adequately explored in any form of amphibian aggression.

Similar to testosterone, testosterone:corticosterone ratios changed significantly from baseline to move in the overall sample, where lower ratios were associated with cannibalism. Therefore, testosterone:corticosterone ratios may be similarly reflective of environmental perturbations and the behavioral niches that poison frogs occupy to cope with a perceived social challenge. However, the interpretation of our result is impeded by the dearth of exploration of androgen:glucocorticoid ratios in amphibians. Our result may hint at a currently uncharted avenue for investigating the importance of steroidal ratios in infanticidal behavior, or it might only reflect the association of cannibalism with low testosterone. Because testosterone and cortisol/corticosterone canonically regulate each other's biological functions as terminals of the HPG and HPA axes, respectively, interactions between the two may inform behavioral outcomes, as has been suggested previously for even non-mammals, such as fish (Agues-Barbosa et al., 2022). However, the trend observed here is opposite of that reported in the largely mammalian literature, where higher ratios are proposed to represent proneness to social aggression in humans (Terburg et al., 2009). Reasons for this distinction may include those previously listed with respect to testosterone and corticosterone individually, and the lack of studies in this field which address non-mammalian taxa or aggression directed towards young.

A promising future avenue for dissecting the physiology of parental care versus infanticide in poison frogs lies in the concerted analysis of steroid hormones and neural activity. Oxytocin and vasopressin are well-known for their roles in mammalian parenting (Feldman and Bakermans-Kranenberg, 2017), but their homologs do not appear to mediate parental care in *R. imitator* (Schulte and Summers, 2017). Emerging research on the neural underpinnings of parental behavior have implicated galanin and *Urocortin-3* in infanticide within cichlids and mice respectively (Butler et al., 2020; Autry et al., 2021), while galanin is also associated with parental care in both cichlids and mice, in addition to poison frogs (Wu et al. 2014; Fischer et al., 2019; Butler et al., 2020). This apparent overlap in the neural circuitry responsible for seemingly antagonistic parental behaviors merits greater consideration of peripheral mediators. An emerging area of research centers the principle that steroid hormones can alter sensory representations and information coding by directly acting on neural circuits (Remage-Healey, 2014). Therefore, biological underpinnings of parental behavior in this species might be more aptly investigated in terms of interactions between steroids and neuropeptides, rather than studying either in isolation. This seems especially plausible for infanticide, a behavior which appears to be guided by a striking interplay between physiological and environmental inputs.

5. Conclusions

In this study, we aimed to clarify environmental and hormonal cues for parental care and infanticide in mimic poison frogs. Our results suggest that, consistent with other poison frogs, care and infanticide in this species are antagonistically linked on the basis of a simple external cue of offspring location. Although infanticide has been observed in other poison frogs, this is the only such report in a monogamous or biparental amphibian, which indicates that infanticide by both sexes occurs regardless of mating or parenting systems in this clade. We also showed that mimic poison frogs perform infanticide in both familair and unfamiliar territories, wherein they targeted related and unrelated young, respectively. Based on the ecological history of *R.*

imitator, we posit that infanticide in this species serves to prevent misdirected care and eliminate intraspecific competition for limited resources. Moreover, our hormonal analyses indicate that low concentrations of circulating androgens can precede infanticide following social and environmental perturbations, which contributes a unique perspective to the broader aggression literature. Overall, these findings offer fresh insights into how adults adjust their behavior and physiology to make life-or-death decisions towards offspring in the face of social instability. In the future, a concerted analysis of endocrine and neural activities compared between individuals performing infanticide, neglect, and care is a promising next direction to uncover evolutionary innovations in physiology underlying offspring-directed behaviors.

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CRediT authorship contribution statement

Amaris R. Lewis: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review and Editing, Visualization, Project Administration, Funding Acquisition. **Billie C. Goolsby:** Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review and Editing, Visualization, Project Administration. **Bryan H. Juarez:** Methodology, Formal Analysis, Resources, Writing - Original Draft, Writing - Review and Editing, Visualization, Supervision.

Madison P. Lacey: Investigation, Resources, Writing - Review and Editing. **Lauren A. O'Connell**: Resources, Writing- Review & Editing, Funding Acquisition, Supervision**.**

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Data availability

Data and code used in this study can be found on DataDryad at https://doi.org/10.5061/dryad.1c59zw43m. The link is currently private but can be accessed for peer review at https://datadryad.org/stash/share/6hlmFvb9nW-bq0gXHuDj0G230tCxjFfd3LolYfr9I0M.

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