Oecologia (2023) 201:385–396
https://doi.org/10.1007/s00442-023-05314-z

BEHAVIORAL ECOLOGY –ORIGINAL RESEARCH

Maternal chemical defenses predict offspring defenses in a dendrobatid poison frog

Olivia L. Brooks[1,3] · **Jessie J. James**[2] · **Ralph A. Saporito**[3]

Received: 9 July 2022 / Accepted: 3 January 2023 / Published online: 13 January 2023

Abstract

Within and among populations, alkaloid defenses of the strawberry poison frog (*Oophaga pumilio*) vary spatially, temporally, and with life history stage. Natural variation in defense has been implicated as a critical factor in determining the level of protection afforded against predators and pathogens. *Oophaga pumilio* tadpoles sequester alkaloids from nutritive eggs and are, thus, entirely dependent on their mothers for their defense. However, it remains unclear how tadpole alkaloid composition relates to that of its mother and how variation in maternally provisioned defenses might result in varying levels of protection against predators. Here, we demonstrate that natural variation in the alkaloid composition of a mother frog is reflected as variation in her tadpole's alkaloid composition. Tadpoles, like mother frogs, varied in their alkaloid composition but always contained the identical alkaloids found in their mother. Alkaloid quantity in tadpoles was highly correlated with alkaloid quantity in their mothers. Additionally, alkaloid quantity was the best predictor of tadpole palatability, wherein tadpoles with higher alkaloid quantities were less palatable. Mother frogs with greater quantities of alkaloids are, thus, providing better protection for their offspring by provisioning chemical defenses during one of the most vulnerable periods of life.

Keywords Alkaloids · Maternal provisioning · *Oophaga* · Palatability · Parental care · Tadpole

Introduction

The use of chemical defenses against predators, pathogens, and parasites is widespread in nature. Most chemically defended organisms are able to biosynthesize defensive compounds, whereas others must obtain them secondarily through a specialized diet of chemically defended prey items (Nishida 2002; Saporito et al. 2012). The uptake, accumulation, and storage of secondarily derived defenses from diet is generally referred to as sequestration (Mebs 2001; Savitzky et al. 2012; Jeckel et al. 2022) and is a well-studied phenomenon among phytophagous arthropods (reviewed in Opitz and Müller 2009). However, sequestration has also evolved independently in several lineages of vertebrates (reviewed in Savitzky et al. 2012) including snakes (Hutchinson et al. 2007), amphibians (Daly et al. 1994; Saporito et al. 2009), and likely birds (Dumbacher et al. 2004, 2009).

Sequestered defensive chemicals can vary widely in their chemical composition, and this variability has multiple points of origin (Speed et al. 2012). Among vertebrates, spatiotemporal variation in the abundance and availability of prey appears to directly affect a predator's own chemical defenses (Saporito et al. 2007a; Hutchinson et al. 2013). For example, macrogeographic variation in prey defense, such as variation in availability of chemically defended prey across a species' range, can result in large-scale variation in the sequestered defenses of predators (Saporito et al. 2007a; Triponez et al. 2007; Inoue et al. 2021). Furthermore, microgeographic variation in prey defenses may result in small-scale variation in the sequestered defenses of predators within and among neighboring populations (Pasteels et al. 1995; Moranz and Brower 1998). Variation in sequestered chemical defenses may also originate from seasonal or long-term heterogeneity in prey availability (Malcolm & Brower 1989; Pasteels et al. 1995).

Communicated by Donald Miles.

✉ Ralph A. Saporito
rsaporito@jcu.edu

[1] School of Biological Sciences, Illinois State University, Normal, IL 61701, USA

[2] Department of Biology, San Francisco State University, San Francisco, CA 94132, USA

[3] Department of Biology, John Carroll University, University Heights, OH 44118, USA

Chemical defenses within a species may vary with sex, age, and body size (Nishida and Fukami 1989; Alonso-Mejía and Brower 1994; Speed et al. 2012). In many species, the presence of chemical defenses is dynamic with regard to life history stage. Some organisms can sequester defenses throughout their life cycle (Nishida and Fukami 1989; Eisner et al. 2000) whereas, in other organisms, defenses are temporary (Fordyce et al. 2005). The presence of defenses may be limited to one or a few life stages where compounds are sequestered, which can be early in development (Malcolm and Rothschild 1983) or as adults (Brown 1987). In many lepidopterans, defenses sequestered during larval stages are retained through metamorphosis and into adulthood (Malcolm and Brower 1989; Bowers and Williams 1995; Nishida 2002); however, the presence or effectiveness of these defenses may decrease with age (Alonso-Mejía and Brower 1994). Some organisms are able to provision their offspring with chemical defenses prior to hatching or birth, though the effectiveness of these defenses can decrease with offspring growth and development (Hutchinson et al. 2008; Hayes et al. 2009; however, see Williams et al. 2011).

The provisioning of defensive chemicals to offspring is thought to serve as an antipredator (and possibly antimicrobial) mechanism during one of the most vulnerable periods of life (Gunzburger and Travis 2005; Stynoski and Porras-Brenes 2022). For example, female ornate bella moths (*Utethesia ornatrix*) provision egg clutches with pyrrolizidine alkaloids, which in turn act as a deterrent to their primary predator, the larvae of green lacewings (*Ceraeochrysa cubana*) (Eisner et al. 2000). In some organisms, developing embryos absorb maternally provisioned chemical defenses and newly hatched offspring retain these defenses until they begin sequestering their own. In the Asian snake (*Rhabdophis tigrinus*), bufadienolides sequestered from a diet of toads are provisioned to embryos by gestating females. Neonates retain these chemical defenses post-hatching until they begin independently feeding on toads and sequestering bufadienolides themselves (Hutchinson et al. 2008). Similar examples occur in diverse lineages, including fireflies in the genus *Photuris* (González et al. 1999), harlequin toads (*Atelopus chiriquiensis*) (Pavelka et al. 1977), and the rough-skinned newt (*Taricha granulosa*) (Hanifin et al. 2003). The chemical defenses acquired by offspring via maternal provisioning are highly variable (Eisner et al. 2000; Hanifin et al. 2003) and several studies have suggested a positive correlation between mother and offspring defense quantities (Hanifin et al. 2003; Hutchinson et al. 2008; Williams et al. 2011).

Poison frogs, a well-studied group of chemically defended vertebrates, comprise approximately 150 species with members in several families worldwide (reviewed in Saporito et al. 2012). Members of this group sequester their defensive chemicals entirely from a diet of alkaloid-containing arthropods, which is composed primarily of mites and ants (Saporito et al. 2004, 2007b, 2015). As a result, some poison frogs are reported to be unpalatable to certain predators (Hantak et al. 2016; Murray et al. 2016; Lawrence et al. 2019) and protected from microbial infection (Mina et al. 2015; Hovey et al. 2018). Alkaloid type, number, and quantity are highly variable within and among poison frog species, and populations of a single species may differ from one another across geographic space and over time (Saporito et al. 2007a; Basham et al. 2020). Within and among populations, alkaloid profiles can differ between sexes (Saporito et al. 2010), across life history stages (Stynoski et al. 2014a), and typically show an increase with age and body size (Jeckel et al. 2015). Variability in alkaloid defenses within and among poison frogs appears to play an important role in their effectiveness as deterrents against predators (Bolton et al. 2017; Lawrence et al. 2019) and pathogens (Hovey et al. 2018).

In the strawberry poison frog (Dendrobatidae: *Oophaga pumilio*), both sexes are chemically defended and invest in parental care. Males moisten terrestrial egg clutches for 7 to 10 days and upon hatching, tadpoles are singly transported by mothers to water-filled leaf axils of plants (Dugas et al. 2018). Mothers then return every 1 to 2 days for a period of 6 to 8 weeks to provision the obligatorily oophagous tadpoles with unfertilized (nutritive) eggs that contain alkaloids—providing both nutrition and defense to developing tadpoles (Stynoski et al. 2014a, b). Maternal alkaloid provisioning is particularly important, given that tadpoles are not able to feed on the alkaloid-containing arthropods normally necessary for frogs to obtain chemical defenses. Although a described phenomenon (Stynoski et al. 2014a; Fisher et al. 2019; Villanueva et al. 2022), the provisioning of alkaloid-laden, nutritive eggs to free-living tadpoles is the first known example of a vertebrate provisioning chemical defenses following birth (or hatching). Since its discovery, maternal alkaloid provisioning has been experimentally established in *O. pumilio*, and has also been described in other species of egg-feeding poison frogs (Fischer et al. 2019; Saporito et al. 2019; Villanueva et al. 2022).

Although provisioned alkaloids appear to provide tadpoles defense from predators, this has only been demonstrated in late-stage tadpoles (Stynoski et al. 2014a, b). Alkaloid quantity is positively associated with tadpole mass and developmental stage (Stynoski et al. 2014a; Saporito et al. 2019), suggesting that alkaloid defenses increase over the course of tadpole development. Furthermore, alkaloids vary among females within and among populations, and previous work with *O. pumilio* have found overlap in the types of alkaloids shared between females and tadpoles (Stynoski et al. 2014a). More recently, similar findings of overlap have been identified in *Oophaga sylvatica* and *Oophaga granulifera* (Fischer et al. 2019; Villanueva et al. 2022). Collectively,

these findings suggest that differences in alkaloid composition of female *O. pumilio* (and other members of *Oophaga*) may be passed on to offspring—a potentially ecologically important transgenerational effect (Saporito et al. 2007a, 2010).

In the present paper, we worked to further understand some of the fundamental ecological factors involved in maternally provisioned chemical defenses—in particular, testing the prediction that variation in a mother frog's chemical defenses is expressed as variation in her tadpole's defenses. We also tested the prediction that variation in these defenses is associated with the efficacy of offspring defense. More specifically, we tested the predictions that (1) variation in alkaloid number and type within a population of adult *O. pumilio* translates to variation in the alkaloid number and type of her tadpoles via maternal provisioning, (2) tadpole alkaloid quantity is correlated with mother alkaloid quantity, and (3) variation in tadpole alkaloid quantity results in differences in tadpole palatability to a model arthropod predator.

Materials and methods

Study site and frog collection

We conducted this study at La Selva Research Station (10°25'52.33"N, 84° 0'12.74"W)—a private reserve located in Heredia Province, Costa Rica and managed by the Organization for Tropical Studies (OTS) from March through July 2019. The majority of the reserve comprises primary rainforest, but also includes selectively logged primary forest, pasture, and abandoned cacao plantations (McDade et al. 1994; Whitfield et al. 2007).

To test the prediction that alkaloid profiles are similar between mothers and their tadpoles, we used behavioral observations to identify mother/tadpole pairs within the Huertos Plots at La Selva (10°26' N, 84° 0'46.38" W). The Huertos Plots are the site of an abandoned cacao plantation and provide an ideal location to observe parental care and egg provisioning because *O. pumilio* are abundant and reproductively active (Donnelly 1989a; Gade et al. 2016; DeMarchi et al. 2018). Mother *O. pumilio* deposit tadpoles into naturally occurring water-filled leaf axils of plants such as *Heliconia*, bromeliads, and bananas (*Musa*) (Donnelly 1989b; Haase and Pröhl 2002); however, mothers will also deposit tadpoles into cups (referred to as artificial tadpole-rearing sites), which mimic naturally occurring phytotelmata and allow for greater ease of access to tadpoles (Stynoski 2009; Stynoski et al. 2014a). We constructed tadpole-rearing cups from 30 mL plastic polypropylene beakers each affixed to a single plastic knife with a zip-tie (Fig. 1). We drilled two small holes in each cup to prevent excess rainwater from flushing tadpoles out of the top of the cup. In March 2019, we deployed a total of 786 cups along transects in the Huertos Plots with each set of cups affixed to a tree approximately 1.5 m off the ground.

We surveyed tadpole-rearing cups daily in July 2019 (30 survey days) to identify cups that contained tadpoles (presence/absence) and to record the developmental stage of each tadpole using Gosner staging (Gosner 1960). We targeted cups containing tadpoles for behavioral observations to identify mother/tadpole pairs, and observations took place daily between the hours of 0500–1130, when mother frogs provision their young (Haase and Pröhl 2002). We selected tadpoles for observation based on estimated mass and stage to ensure that tadpoles representing a wide range of development were collected. We observed targeted cups containing tadpoles from a distance of ~ 3 m until a female frog returned to provision her offspring. Mother *O. pumilio* do not recognize their own offspring and instead use spatial cues to recognize and relocate where they left their tadpole(s) (Stynoski 2009). Therefore, when a mother is provisioning a tadpole, it can be assumed that it is her offspring.

We observed mother frogs until they had climbed fully into the tadpole-containing cup and had at least partially submerged their body in the water (similar to Fisher et al. 2019). This partial submersion behavior precedes maternal feeding (Stynoski 2009; Dugas et al. 2017; Dugas et al. 2018), further reinforcing that the visiting frog was that tadpole's mother. Once a mother frog climbed fully into a tadpole-containing cup, we captured her with an aquarium net, placed her in a one-gallon Ziploc™ bag, and transported her back to an ambient laboratory. We collected tadpoles using disposable polyethylene transfer pipets and stored individuals in 20 mL glass vials with water from that tadpole's cup. We collected a total of 14 mother/tadpole pairs following this method. We collected an additional five tadpoles of varying developmental stages from cups solely for use in palatability assays (see below). All tadpoles were collected from cups except for one tadpole that was collected directly from a mother's back and was subsequently placed in a vial of rainwater collected from a nearby cup that did not contain a tadpole.

Following collection in the field, we weighed all mother frogs to the nearest 0.1 mg using a Pesola PPS200 digital pocket scale and measured for snout-to-vent length (adults; SVL 19–22 mm) (Donnelly 1989a) to the nearest 0.1 mm using Traceable® Digital Calipers. We euthanized mother frogs via freezing (Jeckel et al. 2019, 2022), following which their skins were removed and stored in separate 4 mL glass vials with Teflon-lined caps containing 2 mL of ≥ 99% methanol (GC Resolv™). We weighed tadpoles to the nearest 0.1 mg using a Pesola PPS200 digital pocket scale, euthanized them via freezing, and stored them wholly in separate

Fig. 1 **a** Tadpole rearing cups. Two cups were affixed to each tree; **b** tadpole deposited into a cup by a mother O. pumilio

4 mL glass vials with Teflon-lined caps containing 2 mL of ≥ 99% methanol (GC Resolv™).

Palatability assays

To test the prediction that variation in maternally provisioned alkaloid defenses results in varying levels of protection from predators, we conducted ant palatability assays with *Ectatomma ruidum*, following the methods of Bolton et al. (2017). We collected ants from the lab clearings and arboretum at La Selva using Jolly Ranchers™ as bait. We collected individual *E. ruidum* with pressure sensitive forceps between 1300 and 1700 h, stored ants in small plastic containers (~ 10 individuals per container) in an ambient laboratory, and food deprived them for a period of 48 h prior to trials (Bolton et al. 2017). All ants collected within a 2-meter radius of each other were assumed to be from the same nest (Lachaud 1990), and we did not sample nests more than three times throughout the study.

We performed palatability assays using the methanol extracts from tadpoles selected to represent a range of developmental stages (stages 25–43) and masses (0.6–190 mg). To ensure a range of tadpole ages and masses were represented, tadpoles used in palatability assays were sourced from mother/tadpole pairs ($n = 9$) and directly from cups ($n = 5$) for a total of 14 tadpoles. Palatability assays consisted of feeding trials wherein we placed ants individually into the center of a small, glass petri dish (~ 6 cm diameter) and ants were then allowed to choose between two sugar solutions: one with alkaloids and one without alkaloids. To create the alkaloid sucrose solution, we transferred 1 mL of the original 2 mL methanol/tadpole solution to a separate vial and evaporated to dryness. Following evaporation, we added 250 µL of a sucrose solution (50% ethanol, 20% sucrose) to the vial to create an alkaloid/ethanol/sucrose solution. Each petri dish contained two coverslips, one with 10µL of an alkaloid/ethanol/sucrose solution and one with 10 µL of a control solution (50% ethanol, 20% sucrose). We randomized the location of the solutions within the petri dish for each trial and selected ants randomly with respect to nest location. Ants were allowed a 5-min period to detect and sample either solution, but we only considered ants to have successfully fed on a solution when the ant submerged its mandibles in a solution for more than 3 s (Bolton et al. 2017). If an ant did not feed on either solution within a 5-min period, we removed the ant and replaced it with a second ant. If the second ant also did not feed on either solution within a 5-min period, we replaced the solutions, cleaned the arenas with a 10% ethanol solution, and initiated a new trial with a new ant. We conducted 15 trials per tadpole ($n = 14$ tadpoles) for a total of 210 trials. We quantified individual tadpole palatability by assigning each tadpole a palatability

score based on a palatability index that ranged from -1 to 1. The palatability index was calculated as follows: (# ants that fed on the alkaloid solution)–(# ants that fed on the control solution) ÷ total number of ants. We consider all palatability scores of 0 or greater to represent a palatable prey source, and therefore individuals scoring closer to -1 were considered more unpalatable than individuals scoring closer to 0 (Bolton et al. 2017; Dyer et al. 2003).

Alkaloid fractionation

We extracted alkaloids from each of the 14 mother frog skins and 19 whole tadpoles (14 used in mother/tadpole pairs; 5 used in the palatability assays) using an acid–base technique (Saporito et al. 2010; Jeckel et al. 2015; Hovey et al. 2018). For each sample, we added 50µL of 1 N HCL and an internal standard of nicotine to 1 mL of the original methanol extract. This solution was then concentrated to 100µL using nitrogen gas, followed by the addition of 200µL of deionized water. We then performed four extractions, each time using 300µL of hexane and discarded the hexane layer each time. We added $NaHCO_3$ to the remaining solution and then extracted three times, each time using 300µL of ethyl acetate. The ethyl acetate layer was dried using anhydrous Na_2SO_4, evaporated to dryness with nitrogen, and reconstituted with 100µL of methanol.

Alkaloid analysis

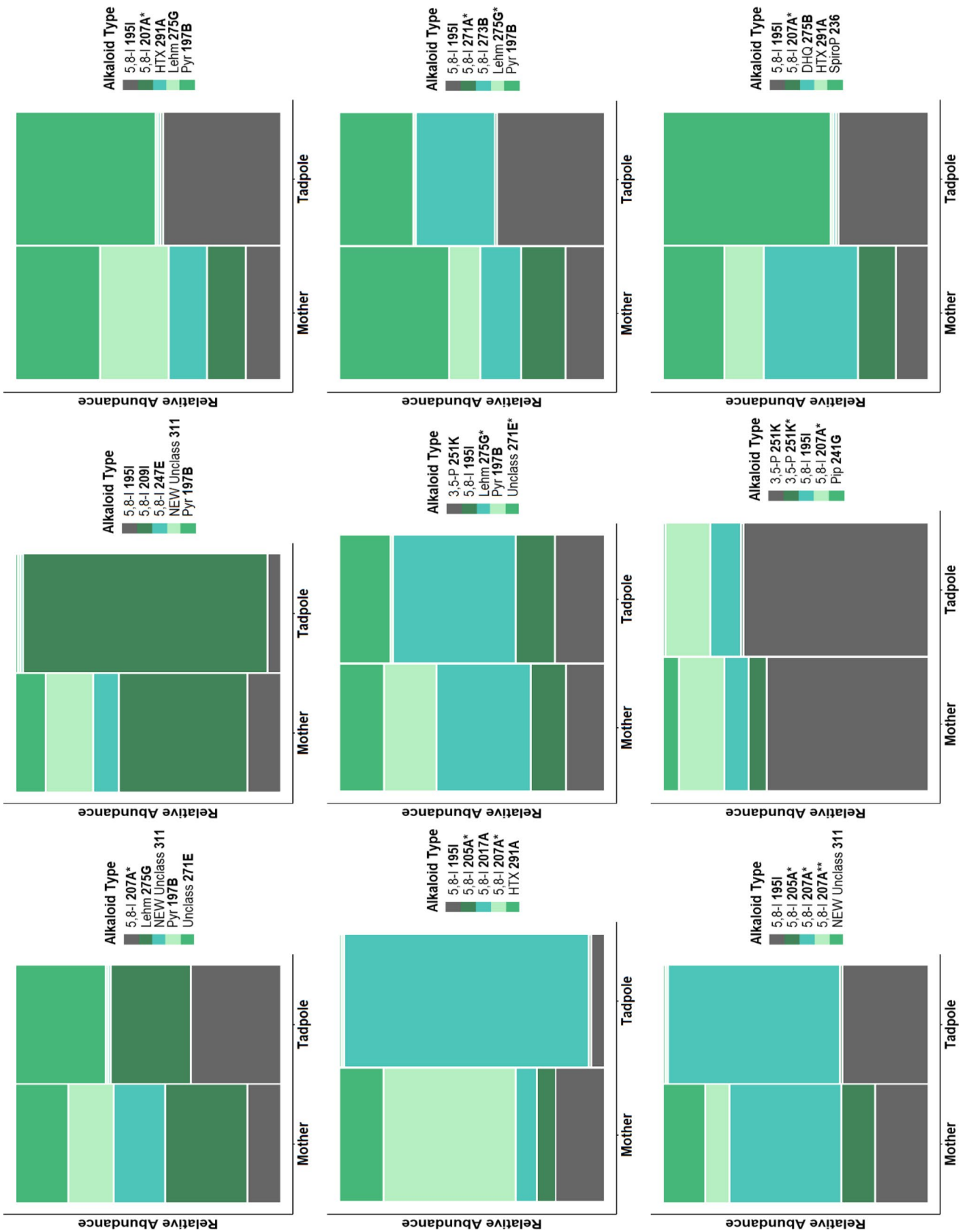
We used gas chromatography–mass spectrometry (GC–MS) to identify, characterize, and quantify alkaloids (Saporito et al. 2010; Jeckel et al. 2015; Hovey et al. 2018). The GC–MS was a Varian 3900 GC coupled with a Varian Saturn 2100 T ion trap MS using a 30 m × 0.25 mm ID Varian Factor Four VF-5 ms fused silica column. A temperature program ran from 100 to 280 °C at a rate of 10 °C per minute using helium as a carrier gas (1 mL/min). We analyzed each alkaloid fraction in triplicate using electron impact-mass spectrometry (EI-MS) and once using chemical ionization-mass spectrometry (CI-MS). We identified alkaloids by comparing GC retention times and mass spectral properties to already established dendrobatid alkaloid data (Daly et al. 2005; additional citations in Hovey et al. 2018; Saporito et al. unpub. data). We quantified alkaloids with a nicotine internal standard using a Varian MS Workstation v.6.9 SPI. For comparisons of alkaloid number and type between a mother frog and her tadpole, we excluded any alkaloids detected in quantities below 0.01 µg. For comparisons of alkaloid quantity between mother frogs and tadpoles, we excluded alkaloids detected in quantities below 0.5 µg in mother frogs and 0.01 µg in tadpoles (Lawrence et al. 2019). Quantity thresholds were selected to represent a balance between the biological relevance of the alkaloids' quantity against predators and pathogens (Weldon et al. 2006), and an attempt to encompass the full range of alkaloid types present in mothers and their tadpoles.

Statistical analyses

We began by assessing how tadpole mass and developmental stage related to both alkaloid number and quantity. We used linear regressions to test for relationship between 'tadpole mass' and 'alkaloid quantity (µg/tadpole)' as well as the relationship between 'tadpole developmental stage' and 'alkaloid quantity'. We used generalized linear models with a negative binomial distribution to test for the relationship between 'tadpole mass' and 'number of alkaloids per tadpole' as well as the relationship between 'tadpole developmental stage' and 'number of alkaloids per tadpole'.

To test the prediction that tadpoles are unpalatable to predators, we ran a binomial general linear model with the response variable of 'sugar solution consumption by ants (consumed or not consumed)' over 'total number of trials per tadpole' ($n = 15$), and the fixed effects of 'tadpole alkaloid quantity' and 'tadpole mass'. We chose to include only 'tadpole mass' in the model to avoid issues with collinearity between 'tadpole mass' and 'developmental stage' as both of these factors are highly correlated ($r = 0.93$, $n = 19$, $p < 0.001$). To further test the prediction that differences in maternally derived alkaloids provide varying levels of tadpole defense, we compared the relationship between 'palatability scores' (derived from the palatability index) and 'tadpole mass,' 'developmental stage,' 'alkaloid quantity,' and 'alkaloid number' using linear regressions.

To test the prediction that a tadpole's alkaloid quantity relates to that of its mother, we used two general linear models: one with 'tadpole mass' and one with 'tadpole developmental stage' as covariates. All tadpoles below stage 30 did not contain any alkaloids; therefore, we limited our analyses to only tadpoles that contained alkaloids (Fig. 2; stages 30–43). For both models, we included 'tadpole alkaloid quantity' as the response variable and 'mother frog alkaloid quantity' as our predictor. We then ran one model that included 'tadpole mass' as a covariate and one model that included 'tadpole developmental stage' as a covariate. For the model with 'tadpole developmental stage', we initially included 'mother frog alkaloid quantity', 'tadpole developmental stage', 'a quadratic effect of tadpole developmental stage', 'a mother frog alkaloid quantity' × 'developmental stage interaction', and a 'mother frog alkaloid quantity' × 'developmental stage' × 'developmental stage interaction' as predictors of 'tadpole alkaloid quantity'. We sequentially removed the non-significant interactions of 'mother frog alkaloid quantity' × 'developmental stage' × 'developmental stage' ($F_{1,9} = 0.12$, $p = 0.75$), 'mother frog alkaloid quantity' × 'developmental stage'


Alkaloid Type
5,8-I 195I
5,8-I 207A*
HTX 291A
Lehm 275G
Pyr 197B
Relative Abundance
Mother
Tadpole
Alkaloid Type
5,8-I 195I
5,8-I 271A*
5,8-I 273B
Lehm 275G*
Pyr 197B
Relative Abundance
Mother
Tadpole
Alkaloid Type
5,8-I 195I
5,8-I 207A*
DHQ 275B
HTX 291A
SpiroP 236
Relative Abundance
Mother
Tadpole
Alkaloid Type
5,8-I 195I
5,8-I 209I
5,8-I 247E
NEW Unclass 311
Pyr 197B
Relative Abundance
Mother
Tadpole
Alkaloid Type
3,5-P 251K
5,8-I 195I
Lehm 275G*
Pyr 197B
Unclass 271E*
Relative Abundance
Mother
Tadpole
Alkaloid Type
3,5-P 251K
3,5-P 251K*
5,8-I 195I
5,8-I 207A*
Pip 241G
Relative Abundance
Mother
Tadpole
Alkaloid Type
5,8-I 207A*
Lehm 275G
NEW Unclass 311
Pyr 197B
Unclass 271E
Relative Abundance
Mother
Tadpole
Alkaloid Type
5,8-I 195I
5,8-I 205A*
5,8-I 2017A
5,8-I 207A*
HTX 291A
Relative Abundance
Mother
Tadpole
Alkaloid Type
5,8-I 195I
5,8-I 205A*
5,8-I 207A*
5,8-I 207A**
NEW Unclass 311
Relative Abundance
Mother
Tadpole

◂**Fig. 2** Relative abundances of the top five major alkaloids present in mother frogs and their tadpoles. Fourteen mother/tadpole pairs were examined in the present study, but only nine alkaloid-containing tadpoles and their respective mothers are included in the figure above. Each plot represents a unique mother/tadpole pair. Alkaloids not present as one of the top five in a tadpole are marked as zero relative abundance. An asterisk indicates an isomer of that alkaloid. Abbreviations for alkaloid structural classes are as follows. 5,8-I 5,8-disubstituted indolizidines, *HTX* histrionicotoxins, 3,5-P 3,5-disubstituted pyrrolizidines, *Unclass* unclassified alkaloids and their molecular weight, *Pyr* pyrrolidines, *DHQ* decahydroquinolines, *SpiroP* spiropyrrolizidines, *Lehm* lehmizidines, *Pip* piperidines, NEW Unclass 311 is a new alkaloid with a molecular weight of 311 that could not be assigned to a structural class—additional characterization will be published in forthcoming articles

($F_{1,9}=0.01$, $p=0.93$), and 'the quadratic of tadpole developmental stage' ($F_{1,9}=0.81$, $p=0.40$).

Alkaloids are present in tadpoles as young as stage 30 (Saporito et al. 2019), which approximately coincides with the early stages of granular (poison) gland development in *O. pumilio* (stages 32–33, Stynoski and O'Connell 2017). Individuals appear to experience an increase in alkaloid quantity in the stages shortly thereafter (ca. stages 30–35, Stynoski et al. 2014a; Saporito et al. 2019), suggesting that the process of gland development observed during this developmental period influences the capacity for alkaloid uptake. For the purposes of describing tadpole alkaloid composition with respect to development of granular glands, tadpoles were placed into one of three developmental age groups: early stage (pre-gland development; 25–29), middle stage (beginning of gland development; 30–32), and late stage (fully developed glands; 41–44). We corrected all mother frog/tadpole comparative analyses with both 'mother skin mass' and 'tadpole total mass' (Stynoski et al. 2014a). We conducted analyses in R (v.4.0.4) and using the PROC GLM and PROC GLIMMIX statements in SAS (version 9.4).

Results

Mother frog and tadpole alkaloid composition

Of the 14 mother/tadpole pairs, we identified alkaloids in all 14 mother frogs and 10 tadpoles. Four tadpoles did not contain any alkaloids, all of which were below stage 30. We identified a total of 134 alkaloid types (including isomers) across all mother frogs and alkaloid-containing tadpoles. On average (mean ± SE), mother frogs contained 597 ± 125 µg per frog skin of alkaloids (range: 180–1,693 µg per frog skin) and alkaloid-containing tadpoles had on average 12 ± 3 µg per tadpole (range: 1–24 µg per tadpole). The tadpole that was collected directly from the mother's dorsum did not contain any alkaloids, providing additional evidence that tadpoles exclusively sequester alkaloids from nutritive eggs; however, see Fischer et al. (2019) for a discussion on the possibility that tadpoles absorb maternal alkaloids secreted into nursery water during feeding visits.

Relationship between mother frog and tadpole alkaloids

Alkaloid-containing tadpoles, regardless of developmental stage, contained all the same alkaloid types present in their mothers, even if only in trace amounts (< 10 ng). Individual tadpoles also tended to share the top five most abundant alkaloids (by alkaloid quantity) present in their mother frogs (Fig. 2). Major alkaloid structural classes shared between mother frogs and tadpoles included 5,8-disubstituted indolizidines, 5,6,8-trisubstituted indolizidines, piperidines, and pyrrolidines. Mother frog alkaloid quantity ($F_{1,9}=12.48$, $p<0.01$) and tadpole developmental stage ($F_{1,9}=26.16$, $p<0.01$) both predicted tadpole alkaloid quantity. Mother frog alkaloid quantity ($F_{1,9}=13.13$, $p<0.01$) and tadpole mass ($F_{1,9}=24.99$, $p<0.01$) also predicted tadpole alkaloid quantity. Tadpole alkaloid quantity increased with tadpole developmental stage and mass (Fig. 3), and mother frogs with greater alkaloid quantities also had tadpoles with greater alkaloid quantities. This was especially true for late-stage tadpoles (Fig. 4).

Tadpole alkaloid composition and palatability

Alkaloid quantity and number of alkaloids per tadpole significantly increased with tadpole mass and developmental stage ($p<0.001$ for all). Tadpole alkaloid quantity was a significant predictor of tadpole palatability ($F_{1,11}=7.90$, $p=0.02$), even after accounting for tadpole mass ($F_{1,11}=2.30$, $p=0.16$). Tadpoles with higher alkaloid quantities were less palatable than tadpoles with lower alkaloid quantities ($R^2=0.41$, $n=14$, $p=0.01$; Fig. 5), and the total number of alkaloids per tadpole, tadpole mass, and tadpole developmental stage were not significant predictors of palatability ($p>0.05$ for all).

Discussion

Previous studies have demonstrated that *O. pumilio* tadpoles sequester alkaloid defenses from nutritive eggs (Stynoski et al. 2014a; Saporito et al. 2019), and that tadpoles and mothers of *O. pumilio* and other provisioning poison frogs share similar alkaloids (Fischer et al. 2019; Villanueva et al. 2022). However, it has remained unclear how tadpole alkaloid profiles directly relate to their mothers' profiles and how maternally provided alkaloids influence tadpole protection from predators. In the present study, tadpoles were found to share very similar alkaloid profiles to their mothers based

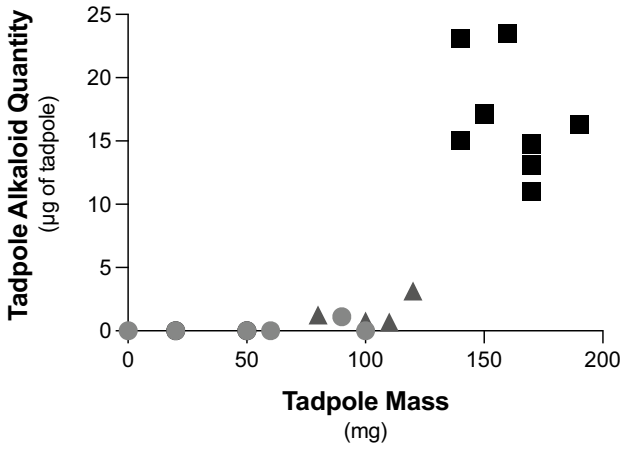


Fig. 3 The relationship between tadpole alkaloid quantity and tadpole mass ($n = 19$). *Circles* early-stage tadpoles (25–29); *triangles* middle-stage tadpoles (30–35); *squares* late-stage tadpoles (41–44). Categories were selected to represent tadpoles before the development of glands (early stage), tadpoles undergoing the development of glands (middle stage), and tadpoles with more mature glands (late stage)

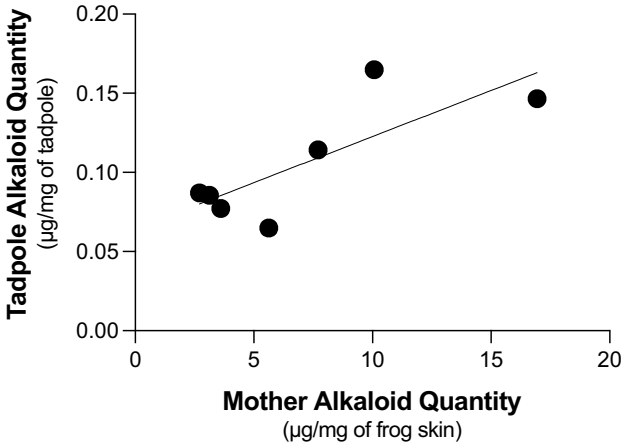


Fig. 4 The relationship between mother *O. pumilio* alkaloid quantity and her tadpole's alkaloid quantity. The mother/tadpole pairs selected for this comparison comprise seven late-stage tadpoles (stages 41–43) and their respective mothers

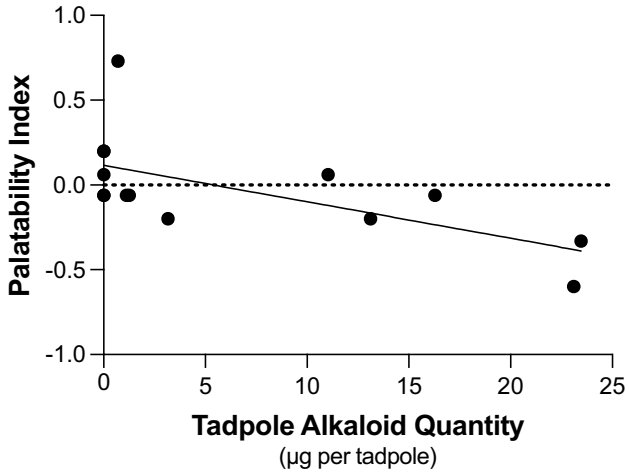


Fig. 5 The relationship between *O. pumilio* tadpole ($n = 14$) alkaloid quantity and palatability

on relative quantity, and there was 100% overlap in the type of alkaloids present in a mother and its tadpole. Together, these findings support the prediction that tadpole defenses in *O. pumilio* are largely a reflection of their mother's defenses. In a similar study with *O. sylvatica*, Fischer et al. (2019) reported only 54% overlap in alkaloid types between tadpoles and mothers, suggesting that complete overlap might not be common to all members of *Oophaga*. However, alkaloid profiles in Fischer et al. (2019) were not compared directly between tadpoles and mothers, and their analysis was restricted only to alkaloids shared by all mother frogs, limiting our ability for comparison. Finally, in the present study, mother *O. pumilio* with greater alkaloid quantities reared tadpoles with greater alkaloid quantities supporting our prediction that the quantity of alkaloid defenses in a tadpole are directly related to the amount of alkaloid in mothers. Collectively, these findings suggest that both the type and quantity of alkaloid defenses in mother *O. pumilio* are passed on directly to their offspring.

The high degree of similarity in alkaloid profiles suggests that mother frogs are passively provisioning alkaloid defenses to nutritive eggs, rather than actively modulating what is provisioned. In a similar system, mother Asian tiger snakes (*Rhabdophis tigrinus*) also appear to provision bufadienolide defenses to their offspring, although some modification of defensive compounds appears to occur (Hutchinson et al. 2008). Older and more developed *O. pumilio* tadpoles contained more alkaloids, which appears partly due to accumulation of alkaloids over time (Fig. 3). However, mother *O. pumilio* are also known to vary in their provisioning behavior (Maple 2002; Dugas et al. 2016), and are more likely to provide larger meals to their older and more developed offspring (Dugas et al. 2016, 2017), suggesting that increases in alkaloid defenses in older tadpoles may also be attributed to differences in behavioral provisioning. Finally, adult *O. pumilio* are known to vary significantly in alkaloid defenses among populations (Saporito et al. 2007a), suggesting that the alkaloid composition of tadpoles might also vary in a similar manner. Future studies should examine how natural variation in alkaloid composition as well as differences in provisioning behavior among mothers from different populations of *O. pumilio* influence the alkaloid composition of tadpoles and its implications for tadpole defense.

Provisioned alkaloid defenses are presumed to act as an effective deterrent against certain tadpole predators (Stynoski et al. 2014a, b) and possibly microbes (Hovey

et al. 2018), and variation in these defenses likely plays an important role in determining a tadpole's level of protection. In the present study, tadpole alkaloid quantity was the best predictor of palatability. Tadpoles with greater alkaloid quantities were less palatable to a model arthropod predator supporting our prediction that tadpole alkaloid profiles are important in providing protection against predators. Tadpole palatability was not solely related to tadpole mass, but was instead a reflection of the alkaloid quantity provisioned by mothers. Previous work has suggested that tadpole size and age are the most important factors driving protection against predators (Stynoski et al. 2014a, b). For example, experimental studies have demonstrated that late-stage *O. pumilio* tadpoles are chemically defended against bullet ants (*Paraponera clavata*) and ctenid spiders (*Cupiennius* spp.) (Stynoski et al. 2014a, b). By decoupling the effects of alkaloid quantity and tadpole size/age, our work demonstrates that variation in maternally derived defenses is critical for determining how well protected a tadpole is from predators.

Relatively little is known about natural poison frog tadpole predators in the wild (reviewed in Santos and Cannatella 2011). Anecdotal and experimental records of *O. pumilio* tadpole predation events suggest that tadpoles of all sizes and developmental stages are preyed upon by snakes and spiders (Maple 2002; Stynoski et al. 2014a, b; Sellmeijer and van den Burg 2020), but none of these reports include a measure of tadpole alkaloid quantity. In the present study, 11% (8 of 70) of the tadpoles being reared by mothers in cups—ranging from approximately stage 25–43—were preyed upon. Previous work at the same study site found that 26% of *O. pumilio* tadpoles reared in cups were preyed upon (Stynoski 2009). While a specific predator was not identified, these findings suggest that predation risk is not based solely on the presence or absence of alkaloids. Although tadpoles with greater alkaloid quantities appear to be less palatable to an arthropod predator, the likelihood of a potential predator attacking and consuming an *O. pumilio* tadpole is also dependent on the specific predator and its physiology. Certainly, there is evidence of snakes that are immune to the alkaloid defenses of adult *O. pumilio* (and other alkaloid-defended poison frogs), which likely provides them similar immunity from defended tadpoles (Saporito et al. 2007c; Jovanovic et al. 2009; Lenger et al. 2014; Solano et al. 2017). Indeed, snakes have been reported consuming *O. pumilio* tadpoles with no apparent ill side effects and appear to be the most common predators of *O. pumilio* tadpoles within our study area (Stynoski et al. 2014b). Future research should explore predators of *O. pumilio* tadpoles in the wild, and the role maternally provisioned chemical defenses play in protecting tadpoles from a variety of predators. Additional work should address how and if predators are able to determine if tadpoles contain alkaloids (e.g., size, chemical cues, etc.), similar to research with juveniles and adults (Murray et al. 2016; Stuckert and Summers 2022) and if predators target young tadpoles that have not yet begun sequestering alkaloids.

In the present study, very small alkaloid quantities were detected in tadpoles as young as stage 28 (20 mg), yet tadpoles did not consistently demonstrate a capacity for sequestering alkaloids until reaching stages 30–32 (ca. 80 mg). Poison glands in *O. pumilio* tadpoles begin their development around stages 32–33, which approximately coincides with the detection of maternally provisioned alkaloids in tadpoles (Stynoski et al. 2014a; Stynoski and O'Connell 2017; Saporito et al. 2019). The variable presence of alkaloids in early-stage tadpoles (< stage 30) suggests that gland development largely controls when tadpoles are physiologically able to sequester (i.e., store) maternally derived alkaloid defenses. Therefore, the detection of alkaloids in tadpoles that have presumably not yet developed glands, in the present study and Saporito et al. (2019), may not be the result of sequestration by those individuals, but may instead represent the presence of alkaloid-laden nutritive eggs passing through the digestive tract of these tadpoles. Further implicating the importance of gland development on the sequestration of alkaloids in tadpoles is the observation that tadpoles just beginning to develop glands only possessed minute quantities of alkaloids, whereas late-stage tadpoles (stages 41–44) possessed much larger quantities. Although tadpoles begin to develop glands as young as stage 32, glands do not begin to mature until much later in development (ca. stage 40), suggesting that tadpoles are not physiologically capable of fully sequestering alkaloids until glands are more fully developed (Stynoski and O'Connell 2017). Furthermore, nothing is known about the location of alkaloids in nutritive eggs, which could also influence when tadpoles are able to begin accumulating alkaloids. Young tadpoles are known only to eat the inner yolk of nutritive eggs, and do not consume the entire nutritive eggs (yolk and outer jelly capsule) until later in development (Dugas et al. 2016). It is possible that maternally provided alkaloids are present entirely (or partly) in the jelly capsule of nutritive eggs, which could prevent (or reduce) access of alkaloids to younger tadpoles until they are able to consume eggs in their entirety; however, poison frog alkaloids are largely lipophilic, and it is more likely they are found in the lipid-filled yolk tissue of eggs. Further, other organisms that maternally provision are known to deposit defensive chemicals primarily into egg yolks (Hanifin et al. 2003; Hutchinson et al. 2008). Additional research is needed to identify the location of alkaloid defenses in provisioned eggs and to determine if mother frogs deposit alkaloids equally into clutches throughout the provisioning period. Independent of alkaloid location within eggs, the development and maturation of poison glands in *O. pumilio*

tadpoles appear to be particularly important to alkaloid sequestration, which likely has consequences for how well protected a tadpole is from predators (and possibly microbes) throughout the course of its development.

Maternal provisioning of nutritive eggs is not unique to *Oophaga pumilio.* All members of the genus *Oophaga* are obligate egg eaters, and maternal alkaloid provisioning has recently been described in *Oophaga sylvatica* (Fischer et al. 2019) and *Oophaga granulifera* (Villanueva et al. 2022). Furthermore, the mantellid poison frog, *Mantella laevigata*, also provision their offspring with alkaloid-laden eggs suggesting the convergent evolution of maternal alkaloid provisioning within poison frogs (Fischer et al. 2019). However, not all poison frogs that provision nutritive eggs also provision alkaloids. Tadpoles in the dendrobatid poison frog genus *Ranitomeya* are facultative egg eaters, and mother frogs only provide nutritive eggs when food resources within a nursery are low (Brown et al. 2010). Villanueva et al. 2022 recently described the absence of alkaloids in natural eggs and tadpoles of *Ranitomeya variabilis* and *Ranitomeya imitator*. Future research should explore the extent to which maternal provisioning of alkaloids is present among egg-eating poison frogs, and further examine how natural alkaloid variation within and among species contributes to tadpole protection from local predators and pathogens.

In summary, *Oophaga pumilio* is the first known organism to maternally provision chemical defenses to offspring post-hatching or birth. Tadpoles sequester maternally derived alkaloid defenses from nutritive eggs and as a result, share a similar alkaloid profile to their mother. All alkaloid types found in a mother frog were also found in her tadpole. Mother frogs with high alkaloid quantities also had tadpoles with high alkaloid quantities—a quality in tadpoles that was associated with a decrease in palatability. Individual mother frogs varied in their alkaloid composition and because tadpoles depend solely on their mother for their alkaloid defenses, tadpoles also varied in their alkaloid composition. Variation in adult alkaloid composition has important implications not only for adult protection against predators and pathogens, but also how well-protected tadpoles are from similar threats.

Acknowledgements We thank the John Carroll University Tropical Biology class of 2019 for their help in deploying tadpole-rearing cups, especially A. Jones, M. Hatlovic, and K. Waters, as well as O. Medina-Baez, A. Perrino, J. Ryan, L. Phillip, and C. Thomas. We thank M.B. Dugas for assistance with statistical analyses. We thank M. Nichols for assistance in maintaining the GC–MS and J. Your for help with building tadpole cups. We thank the La Selva Research Station and Costa Rican government for allowing us to conduct this research, and Enrique Alonso Castro Fonseca and Orlando Vargas Ramírez for logistical support.

Author contribution statement OLB and RAS conceived of the project and designed the experiments. OLB, RAS, and JJJ contributed to data collection. OLB and RAS analyzed the data. OLB and RAS wrote the manuscript.

Funding Funding support was provided by John Carroll University.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed. Research protocols were approved by the John Carroll University Institutional Animal Care and Use Committee (IACUC approval 1700). Costa Rican research permits "SINAC-ACC-PI-R-068–2019" and "R-037–2019-OT-CONAGEBIO" were granted by the Sistema Nacional de Áreas de Conservacion (SINAC) and the Comision Nacional para la Gestion de la Biodiversidad (CONAGEBIO), Ministerio de Ambiente y Energía, respectively. The Convention on International Trade in Endangered Species of Wild Flora and Flora (CITES) export permits "2019-CR4841/SJ#5863" and "2019-CR4636/SJ#5650" were granted by the Sistema Nacional de Áreas de Conservacion (SINAC) of the Costa Rican government.

References

Alonso-Mejía A, Brower LP (1994) From model to mimic: age-dependent unpalatability in monarch butterflies. Experientia 50:176–181. https://doi.org/10.1007/BF01984960

Basham EW, Saporito RA, González-Pinzón M, Romero-Marcucci A, Scheffers BR (2020) Chemical defenses shift with the seasonal vertical migration of a Panamanian poison frog. Biotropica 53:28–37. https://doi.org/10.1111/btp.12842

Bolton SK, Dickerson K, Saporito RA (2017) Variable alkaloid defenses in the dendrobatid poison frog *Oophaga pumilio* are perceived as differences in palatability to arthropods. J Chem Ecol 43:273–289. https://doi.org/10.1007/s10886-017-0827-y

Bowers MD, Williams EH (1995) Variable chemical defence in the checkerspot butterfly *Euphydryas gillettii* (Lepidoptera: NymphaIidae). Ecolog Entomol 20:208–212

Brown KS (1987) Chemistry at the Solanaceae/Ithomiinae interface. Ann Mo Bot Gard 74:359–397. https://doi.org/10.2307/2399406

Brown JL, Morales V, Summers K (2010) A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. Am Nat 175:436–446. https://doi.org/10.1086/650727

Daly JW, Garraffo HM, Spande TF, Jaramillo C, Rand AS (1994) Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? J Chem Ecol 20:943–955. https://doi.org/10.1007/BF02059589

Daly JW, Spande TF, Garraffo HM (2005) Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. J Nat Prod 68:1556–1575. https://doi.org/10.1021/np0580560

DeMarchi JA, Britton A, O'Donnell K, Saporito RA (2018) Behavioural preference for low levels of UV-B radiation in two neotropical frog species from Costa Rica. J Trop Ecol 34:336–340. https://doi.org/10.1017/S0266467418000287

Donnelly MA (1989a) Reproductive phenology and age structure of *Dendrobates pumilio* in northeastern Costa Rica. J Herpetol 23:362–367. https://doi.org/10.2307/1564047

Donnelly MA (1989b) Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. Oecologia 81:212–218. https://doi.org/10.1007/BF00379808

Dugas MB (2018) Simple observations with complex implications: what we have learned and can learn about parental care from a frog that feeds its young. Zool Anz 273:192–202. https://doi.org/10.1016/j.jcz.2017.11.012

Dugas MB, Wamelink CN, Killius AM, Richards-Zawacki CL (2016) Parental care is beneficial for offspring, costly for mothers, and limited by family size in an egg-feeding frog. Behav Ecol 27:476–483. https://doi.org/10.1093/beheco/arv173

Dugas MB, Strickler SA, Stynoski JL (2017) Tadpole begging reveals high quality. J Evol Biol 30:1024–1033. https://doi.org/10.5061/dryad.sf1s3

Dumbacher JP, Wako A, Derrickson SR, Samuelson A, Spande TF, Daly JW (2004) Melyrid beetles (Choresine): a putative source for the batrachotoxin alkaloids found in poison-dart frogs and toxic passerine birds. Proc Natl Acad Sci 101:15857–15860. https://doi.org/10.1073/pnas.0407197101

Dumbacher JP, Menon GK, Daly JW (2009) Skin as a toxin storage organ in the endemic New Guinean genus *Pitohui*. Auk 126:520–530. https://doi.org/10.1525/auk.2009.08230

Dyer LA, Dodson CD, Gentry G (2003) A bioassay for insect deterrent compounds found in plant and animal tissues. Phytochem Anal 14:381–388. https://doi.org/10.1002/pca.734

Eisner T, Eisner M, Rossini C, Iyengar VK, Roach BL, Benedikt E, Meinwald J (2000) Chemical defense against predation in an insect egg. Proc Natl Acad Sci 97:1634–1639. https://doi.org/10.1073/pnas.030532797

Fischer EK, Roland AB, Moskowitz NA, Vidoudez C, Ranaivorazo N, Tapia EE, Trauger SA, Vences M, Coloma LA, O'Connell LA (2019) Mechanisms of convergent egg provisioning in poison frogs. Curr Biol 29:4145–4151. https://doi.org/10.1016/j.cub.2019.10.032

Fordyce JA, Marion ZH, Shapiro AM (2005) Phenological variation in chemical defense of the pipevine swallowtail, *Battus philenor*. J Chem Ecol 31:2835–2846. https://doi.org/10.1007/s10886-005-8397-9

Gade MR, Hill M, Saporito RA (2016) Color assortative mating in a mainland population of the poison frog *Oophaga pumilio*. Ethology 122:851–858. https://doi.org/10.1111/eth.12533

González A, Hare JF, Eisner T (1999) Chemical egg defense in *Photuris* firefly 'femmes fatales.' Chemoecology 9:177–185. https://doi.org/10.1007/s000490050051

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Gunzburger MS, Travis J (2005) Critical literature review of the evidence for unpalatability of amphibian eggs and larvae. J Herpetol 39:547–571. https://doi.org/10.1670/1-05A.1

Haase A, Pröhl H (2002) Female activity patterns and aggressiveness in the strawberry poison frog *Dendrobates pumilio* (Anura: Dendrobatidae). Amphibia-Reptilia 23:129–140. https://doi.org/10.1163/156853802760061778

Hanifin CT, Brodie ED III, Brodie ED Jr (2003) Tetrodotoxin levels in eggs of the rough-skin newt, *Taricha granulosa,* are correlated with female toxicity. J Chem Ecol 29:1729–1739. https://doi.org/10.1023/A:1024885824823

Hantak MM, Paluh DJ, Saporito RA (2016) Bufadienolide and alkaloid-based chemical defences in two different species of neotropical anurans are equally effective against the same arthropod predators. J Trop Ecol 32:165–169. https://doi.org/10.1017/S0266467416000055

Hayes RA, Crossland MR, Hagman M, Capon RJ, Shine R (2009) Ontogenetic variation in the chemical defenses of cane toads (*Bufo marinus*): toxin profiles and effects on predators. J Chem Ecol 35:391–399. https://doi.org/10.1007/s10886-009-9608-6

Hovey KJ, Seiter EM, Johnson EE, Saporito RA (2018) Sequestered alkaloid defenses in the dendrobatid poison frog *Oophaga pumilio* provide variable protection from microbial pathogens. J Chem Ecol 44:312–325. https://doi.org/10.1007/s10886-018-0930-8

Hutchinson DA, Mori A, Savitzky AH, Burghardt GM, Wu X, Meinwald J, Schroeder FC (2007) Dietary sequestration of defensive steroids in nuchal glands of the Asian snake *Rhabdophis tigrinus*. Proc Natl Acad Sci 104:2265–2270. https://doi.org/10.1073/pnas.0610785104

Hutchinson DA, Savitzky AH, Mori A, Meinwald J, Schroeder FC (2008) Maternal provisioning of sequestered defensive steroids by the Asian snake *Rhabdophis tigrinus*. Chemoecology 18:181–190. https://doi.org/10.1007/s00049-008-0404-5

Hutchinson DA, Savitzky AH, Burghardt GM, Nguyen C, Meinwald J, Schroeder FC, Mori A (2013) Chemical defense of an Asian snake reflects local availability of toxic prey and hatchling diet: variation in chemical defense of *Rhabdophis tigrinus*. J Zool 289:270–278. https://doi.org/10.1111/jzo.12004

Inoue T, Nakata R, Savitzky AH, Yoshinaga N, Mori A, Mori N (2021) New insights into dietary toxin metabolism: diversity in the ability of the natricine snake *Rhabdophis tigrinus* to convert toad-derived bufadienolides. J Chem Ecol 47:915–925. https://doi.org/10.1007/s10886-021-01287-6

Jeckel AM, Saporito RA, Grant T (2015) The relationship between poison frog chemical defenses and age, body size, and sex. Front Zool 12:27. https://doi.org/10.1186/s12983-015-0120-2

Jeckel AM, Kocheff S, Saporito RA, Grant T (2019) Geographically separated orange and blue populations of the Amazonian poison frog *Adelphobates galactonotus* (Anura, Dendrobatidae) do not differ in alkaloid composition or palatability. Chemoecology 29:225–234. https://doi.org/10.1007/s00049-019-00291-3

Jeckel AM, Bolton SK, Waters KR, Antoniazzi MM, Jared C, Matsumura K, Nishikawa K, Morimoto Y, Grant T, Saporito RA (2022) Dose-dependent alkaloid sequestration and N-methylation of decahydroquinoline in poison frogs. J Exp Zool 2022:1–10. https://doi.org/10.1002/jez.2587

Jovanovic O, Vences M, Safarek G, Rabemananjara FCE, Dolch R (2009) Predation upon *Mantella aurantiaca* in the Torotorofotsy wetlands, central-eastern Madagascar. Herpetology Notes 2:95–97

Lachaud J (1990) Foraging activity and diet in some neotropical ponerine ants. *Ectatomma ruidum roger* (Hymenoptera, Formicidae). Folia Entomológica Mexicana 78:241–256

Lawrence JP, Rojas B, Fouquet A, Mappes J, Blanchette A, Saporito RA, Bosque RJ, Courtois EA, Noonan B (2019) Weak warning signals can exist in the absence of gene flow. Proc Natl Acad Sci 116:19037–19045. https://doi.org/10.1073/pnas.1901872116

Lenger DR, Berkey JK, Dugas MB (2014) Predation on the toxic *Oophaga pumilio* (Anura: Dendrobatidae) by *Rhadinaea decorata* (Squamata: Collubridae). Herpetology Notes 7:83–84

Malcolm SB, Brower LP (1989) Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. Experientia 45:284–295. https://doi.org/10.1007/BF01951814

Malcolm S, Rothschild M (1983) A danaid Mullerian mimic, *Euploea core amymone* (Cramer) lacking cardenolides in the pupal and adult stages. Biol J Lin Soc 19:27–33. https://doi.org/10.1111/j.1095-8312.1983.tb00774.x

Maple M (2002) Maternal effects on offspring fitness in *Dendrobates Pumilio,* the strawberry poison frog. In: Din M (ed) PhD Dissertation, University of Kentucky, Lexington. Kentucky, USA

McDade LA, Bawa KS, Hespenheide HA, Hartshorn GS (1994) La Selva: ecology and natural history of a neotropical rainforest, 1st edn. The University of Chicago Press, Chicago and London

Mebs D (2001) Toxicity in animals. Trends in evolution? Toxicon 39:87–96. https://doi.org/10.1016/S0041-0101(00)00155-0

Mina AE, Ponti AK, Woodcraft NL, Johnson EE, Saporito RA (2015) Variation in alkaloid-based microbial defenses of the dendrobatid

poison frog *Oophaga pumilio*. Chemoecology 25:169–178. https://doi.org/10.1007/s00049-015-0186-5
Moranz R, Brower LP (1998) Geographic and temporal variation of cardenolide-based chemical defenses of queen butterfly (*Danaus gilippus*) in northern Florida. J Chem Ecol 24:905–932. https://doi.org/10.1023/A:1022329702632
Murray EM, Bolton SK, Berg T, Saporito RA (2016) Arthropod predation in a dendrobatid poison frog: does frog life stage matter? Zoology 119:169–174. https://doi.org/10.1016/j.zool.2016.01.002
Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. Annu Rev Entomol 47:57–92. https://doi.org/10.1146/annurev.ento.47.091201.145121
Nishida R, Fukami H (1989) Ecological adaptation of an Aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*, to aristolochic acids. J Chem Ecol 15:2549–2563. https://doi.org/10.1007/BF01014731
Opitz SEW, Müller C (2009) Plant chemistry and insect sequestration. Chemoecology 19:117–154. https://doi.org/10.1007/s00049-009-0018-6
Pasteels JM, Dobler S, Rowell-Rahier M, Ehmke A, Hartmann T (1995) Distribution of autogenous and host-derived chemical defenses in *Oreina* leaf beetles (Coleoptera: Chrysomelidae). J Chem Ecol 21:1163–1179. https://doi.org/10.1007/BF02228318
Pavelka LA, Kim YH, Mosher HS (1977) Tetrodotoxin and tetrodotoxin-like compounds from the eggs of the Costa Rican frog *Atelopus chiriquiensis*. Toxicon 15:135–139. https://doi.org/10.1016/0041-0101(77)90032-0
R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Santos JC, Cannatella DC (2011) Phenotypic integration emerges from aposematism and scale in poison frogs. Proc Natl Acad Sci 108:6175–6180. https://doi.org/10.1073/pnas.1010952108
Saporito RA, Garraffo HM, Donnelly MA, Edwards AL, Longino JT, Daly JW (2004) Formicine ants: an arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. Proc Natl Acad Sci 101:8045–8050. https://doi.org/10.1073/pnas.0402365101
Saporito RA, Donnelly MA, Jain P, Garraffo HM, Spande TF, Daly JW (2007a) Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. Toxicon 50:757–778. https://doi.org/10.1016/j.toxicon.2007.06.022
Saporito RA, Donnelly MA, Norton RA, Garraffo HM, Spande TF, Daly JW (2007b) Oribatid mites as a major dietary source for alkaloids in poison frogs. Proc Natl Acad Sci 104:8885–8890. https://doi.org/10.1073/pnas.0702851104
Saporito RA, Zuercher R, Roberts M, Gerrow KG, Donnelly MA (2007c) Experimental evidence for aposematism in the poison frog *Oophaga pumilio*. Copeia 4:1006–1011
Saporito RA, Spande TF, Garraffo HM, Donnelly MA (2009) Arthropod alkaloids in poison frogs: a review of the 'dietary hypothesis.' Heterocycles 79:277–297
Saporito RA, Donnelly MA, Madden AA, Garraffo HM, Spande TF (2010) Sex-related differences in alkaloid chemical defenses of the dendrobatid frog *Oophaga pumilio* from Cayo Nancy, Bocas Del Toro, Panama. J Nat Prod 73:317–321. https://doi.org/10.1021/np900702d
Saporito RA, Donnelly MA, Spande TF, Garraffo HM (2012) A review of chemical ecology in poison frogs. Chemoecology 22:159–168. https://doi.org/10.1007/s00049-011-0088-0
Saporito RA, Norton RA, Garraffo HM, Spande TF (2015) Taxonomic distribution of defensive alkaloids in Nearctic oribatid mites (Acari, Oribatida). Exp Appl Acarol 67:317–333. https://doi.org/10.1007/s10493-015-9962-8
Saporito RA, Russell MW, Richards-Zawacki CL, Dugas MB (2019) Experimental evidence for maternal provisioning of alkaloid defenses in a dendrobatid frog. Toxicon 161:40–43. https://doi.org/10.1016/j.toxicon.2019.02.008
Savitzky AH, Mori A, Hutchinson DA, Saporito RA, Burghardt GM, Lillywhite HB, Meinwald J (2012) Sequestered defensive toxins in tetrapod vertebrates: principles, patterns, and prospects for future studies. Chemoecology 22:141–158. https://doi.org/10.1007/s00049-012-0112-z
Sellmeigher B, van den Burg MP (2020) Tadpole predation in the chemically defended *Oophaga pumilio* (Anura: Dendrobatidae) by *Oxybelis aeneus* (Squamata: Colubridae). Herpetol Not 13:301–303
Solano M, Vega A, Saporito RA (2017) Phyllobates lugubris (Lovely poison frog) Predation by Coniophanes fissidens (Yellowbelly snake). Herpetol Review. 48:831
Speed MP, Ruxton GD, Mappes J, Sherratt TN (2012) Why are defensive toxins so variable? An evolutionary perspective. Biol Rev 87:874–884. https://doi.org/10.1111/j.1469-185X.2012.00228.x
Stuckert AMM, Summers K (2022) Investigating signal modalities of aposematism in a poison frog. J Evol Biol 2022:1–7. https://doi.org/10.1111/jeb.14111
Stynoski JL (2009) Discrimination of offspring by indirect recognition in an egg-feeding dendrobatid frog, *Oophaga pumilio*. Anim Behav 78:1351–1356. https://doi.org/10.1016/j.anbehav.2009.09.002
Stynoski JL, O'Connell LA (2017) Developmental morphology of granular skin glands in pre-metamorphic egg-eating poison frogs. Zoomorphology 136:219–224. https://doi.org/10.1007/s00435-017-0344-0
Stynoski JL, Porras-Brenes K (2022) Meta-analysis of tadpole taste tests: consumption of anuran prey across development and predator strategies. Oecologia 199:845–857. https://doi.org/10.1007/s00442-022-05221-9
Stynoski JL, Torres-Mendoza Y, Sasa-Marin M, Saporito RA (2014a) Evidence of maternal provisioning of alkaloid-based chemical defenses in the strawberry poison frog *Oophaga pumilio*. Ecology 95:587–593
Stynoski JL, Shelton G, Stynoski P (2014b) Maternally derived chemical defenses are an effective deterrent against some predators of poison frog tadpoles (*Oophaga pumilio*). Biol Let 10:20140187
Triponez Y, Naisbit RE, Jean-Denis JB, Rahier M, Alvarez N (2007) Genetic and environmental sources of variation in the autogenous chemical defense of a leaf beetle. J Chem Ecol 33:2011–2024. https://doi.org/10.1007/s10886-007-9351-9
Villanueva E, Brooks OL, Bolton SK, Savastano N, Schulte L, Saporito RA (2022) Maternal provisioning of alkaloid defenses in dendrobatid poison frogs. J Chem Ecol 23:1–10
Weldon PJ, Kramer M, Gordon S, Spande TF, Daly JW (2006) A common pumiliotoxin from poison frogs exhibits enantioselective toxicity against mosquitoes. Proc Natl Acad Sci 103:17818–17821. https://doi.org/10.1073/pnas.0608646103
Whitfield SM, Bell KE, Philippi T, Sasa M, Bolanos F, Chaves G, Savage JM, Donnelly MA (2007) Amphibian and reptile declines over 35 years at la selva, costa rica. Proc Natl Acad Sci 104:8352–8356. https://doi.org/10.1073/pnas.0611256104
Williams BL, Hanifin CT, Brodie ED, Caldwell RL (2011) Ontogeny of tetrodotoxin levels in blue-ringed octopuses: maternal investment and apparent independent production in offspring of Hapalochlaena lunulata. J Chem Ecol 37:10–17