



Maternal Provisioning of Alkaloid Defenses are Present in Obligate but not Facultative Egg Feeding Dendrobatids

Emily D. Villanueva¹ · Olivia L. Brooks^{1,2} · Sarah K. Bolton³ · Nina Savastano¹ · Lisa M. Schulte⁴ · Ralph A. Saporito¹

Received: 3 September 2022 / Revised: 7 November 2022 / Accepted: 18 November 2022 / Published online: 23 December 2022
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Abstract

Poison frogs sequester alkaloid defenses from a diet of largely mites and ants. As a result, frogs are defended against certain predators and microbial infections. Frogs in the genus *Oophaga* exhibit complex maternal care, wherein mothers transport recently hatched tadpoles to nursery pools and return regularly to supply developing tadpoles with unfertilized (nutritive) eggs. Developing tadpoles are obligate egg feeders. Further, female *O. pumilio* and *O. sylvatica* maternally provision their nutritive eggs with alkaloid defenses, providing protection to their developing tadpoles at a vulnerable life-stage. In another genus of poison frog, *Ranitomeya*, tadpoles only receive and consume eggs facultatively, and it is currently unknown if mothers also provision these eggs (and thus their tadpoles) with alkaloid defenses. Here, we provide evidence that mother frogs of another species in the genus *Oophaga* (*Oophaga granulifera*) also provision alkaloid defenses to their tadpoles. We also provide evidence that *Ranitomeya imitator* and *R. variabilis* eggs and tadpoles do not contain alkaloids, suggesting that mother frogs in this genus do not provision alkaloid defenses to their offspring. Our findings suggest that among dendrobatid poison frogs, maternal provisioning of alkaloids may be restricted to the obligate egg-feeding members of *Oophaga*.

Keywords Facultative egg feeder · Obligate egg feeder · *Oophaga* · Parental care · *Ranitomeya* · Tadpole

Introduction

Parental care of offspring is common among animals and encompasses a variety of traits and behaviors that range in form, complexity, and length of time performed (Klug and Bonsall 2010; Westneat and Fox 2010; Balshine 2012). Although investment in parental care can be costly to the caregiver (Townsend 1986; Burris 2011; Thomson et al. 2014; Carrillo et al. 2022), it provides considerable fitness

advantages to offspring, including protection from environmental, physiological, and/or biotic stressors (Eggert et al. 1998; Langkilde et al. 2007; Dugas et al. 2016a, b). Although most well-known among mammalian and avian species (Farmer 2000; Cockburn 2006), parental care is also found in several insects (Robertson 1993; Matthews and Matthews 2009), arachnids (Evans 1998; Whitehouse and Jackson 2006), crustaceans (Baeza and Fernandez 2002; Arundell et al. 2014), fishes (Steinhart et al. 2004; Lissaker and Kvarnemo 2006), reptiles (Charruau and Henaut 2012; Alexander 2018), and amphibians (Wilkinson et al. 2008; Takahashi and Fukuda 2017; Schulte et al. 2020). Among amphibians, anurans exhibit a large diversity of parental care behaviors (Vagi et al. 2019; Schulte et al. 2020), such as egg attendance and/or hydration (Prado et al. 2002; Dalgetty and Kennedy 2010; Valetti et al. 2014), tadpole transportation (Wells 1978; Ringler et al. 2013; Pašukonis et al. 2016; Pašukonis et al. 2019), and trophic egg feeding (Brust 1993; Kok et al. 2006; Tumulty et al. 2014).

In particular, the dendrobatid poison frogs of Central and South America are a well-studied group in which many species demonstrate complex forms of parental care.

✉ Ralph A. Saporito
rsaporito@jcu.edu

¹ Department of Biology, John Carroll University, University Heights, OH 44118, USA

² School of Biological Sciences, Illinois State University, Normal, IL 61701, USA

³ Department of Biology, Notre Dame College, South Euclid, OH 44121, USA

⁴ Department of Wildlife-/Zoo-Animal-Biology and Systematics, Faculty of Biological Sciences, Goethe University Frankfurt, Frankfurt/Main, Germany

Uniparental male care appears to be the basal and most frequent form (Summers et al. 1999; Carvajal-Castro et al. 2021), in which some males are known to guard egg masses until hatching, providing protection from desiccation and predation (e.g., *Ranitomeya vanzolini* [previously *Dendrobates*] in Caldwell and de Oliveira 1999), and frequently transport terrestrially hatched tadpoles to water sources to continue development, either individually or in groups (Wells 1978; Ringler et al. 2013; Pašukonis et al. 2016; Pašukonis et al. 2019). However, uniparental female care, asymmetrical biparental care, and biparental care is also prevalent among dendrobatids, with both parents providing care at different stages of offspring development (Pröhl and Hödl 1999; Summers et al. 1999; Brown et al. 2008b; Ryan and Barry 2011; Ringler et al. 2015; Schulte and Summers 2021).

Maternal provisioning represents a highly specialized form of parental care present in several genera of dendrobatids, wherein females periodically return to tadpole nurseries to feed their offspring trophic eggs that provide nutrients for developing tadpoles (Brust 1993; Kok et al. 2006; Tumulty et al. 2014). In members of the genus *Oophaga*, egg-feeding is obligate and acts as the sole source of nutrients for tadpoles; if tadpoles do not receive unfertilized trophic eggs, they fail to develop (Brust 1993; Maple 2002). However, in members of the genus *Ranitomeya*, tadpoles obtain some nutrients from food available within their nursery, including small aquatic insects, detritus, and occasionally other tadpoles, but also facultatively consume maternally derived eggs (Brown et al. 2008b; Brown et al. 2009). More specifically, *Ranitomeya* species with biparental care (e.g., *Ranitomeya imitator*) use very small phytotelmata as nurseries, which do not contain sufficient nutrients for tadpole growth and survival (Caldwell and de Oliveira 1999; Brown et al. 2008a, b). Therefore, *R. imitator* tadpoles are regularly provisioned by mothers with unfertilized trophic eggs, which act as a supplementary food source and are necessary for their survival (Brown et al. 2008a, b). However, in *Ranitomeya* species with uniparental care by males (e.g., *Ranitomeya variabilis*), females do not provide unfertilized trophic eggs. Instead, fertilized egg clutches are occasionally deposited into nurseries, which are consumed by tadpoles and also act as a supplementary food source, representing a different form of facultative food provisioning (Poelman and Dicke 2007; Schulte 2014).

In species of *Oophaga* that have been examined, maternal provisioning is taken a step further; mothers provision offspring with trophic eggs that contain alkaloid defenses, thus providing offspring with both a source of nutrients and chemical defenses at a vulnerable life-stage (Stynoski et al. 2014a, b; Saporito et al. 2019; Fischer et al. 2019; Brooks et al. 2022). Alkaloid defenses in dendrobatid frogs

are the result of sequestration from a diet rich in alkaloid-containing arthropods, largely mites and ants (Saporito et al. 2007, 2012, 2015), and thus mothers of *Oophaga* provision trophic eggs with dietary alkaloids. Unlike adult frogs, *Oophaga* tadpoles do not contain the mouthparts or ability to consume alkaloid-containing arthropods (Weygoldt 1980; Van Wijngaarden and Bolaños 1992; Stynoski et al. 2014a) and are therefore entirely dependent on alkaloid provisioning by mothers for their defense. Provisioned alkaloid defenses have an important antipredator (and possibly antimicrobial) function, particularly for older tadpoles (Stynoski et al. 2014b; Murray et al. 2016; Brooks et al. 2022), and may provide a considerable fitness advantage. Currently, maternal provisioning of alkaloid defenses has only been reported in two of the 12 species of *Oophaga*: *O. pumilio* (Stynoski et al. 2014a; Saporito et al. 2019; Brooks et al. 2022) and *O. sylvatica* (Fischer et al. 2019). Maternal alkaloid provisioning has been experimentally demonstrated in *O. pumilio* (Saporito et al. 2019), and recent studies have explored the relationship between mother and offspring alkaloid composition in both species (Fischer et al. 2019; Brooks et al. 2022). However, further understanding of the ecology and evolution of this adaptation is limited by a lack of knowledge on its presence among other members of the genus *Oophaga* as well as other dendrobatid lineages with egg-eating tadpoles, such as members of *Ranitomeya*.

Therefore, the purpose of our research was to determine if maternal alkaloid provisioning is present in a previously unexamined member of the genus *Oophaga*, as well as two species in the genus *Ranitomeya*. In particular, we examined mothers and tadpoles of *Oophaga granulifera* for evidence of maternal alkaloid provisioning. Similar to *O. pumilio*, females of this species transport newly hatched tadpoles from the forest floor to individual phytotelmata, where they return periodically to provision the tadpole with trophic eggs (Van Wijngaarden and Bolaños 1992; Savage 2002). Like other members of *Oophaga*, the tadpoles of *O. granulifera* are obligate egg-eaters and have mouthparts specialized for a diet of trophic eggs (Weygoldt 1980; Van Wijngaarden and Bolaños 1992). Furthermore, to examine whether or not alkaloid provisioning is restricted to obligate egg feeders in the genus *Oophaga*, or if this adaptation is also present in facultative egg feeders, we examined mothers, tadpoles, and eggs of *R. imitator* (a species that provisions trophic eggs), and *R. variabilis* (a species that provisions fertilized eggs), for evidence of maternal alkaloid provisioning.

Methods and Materials

***Oophaga granulifera* tadpole and frog collection.** An age-series of wild-caught *O. granulifera* tadpoles were collected and examined for alkaloids from Firestone Center for Restoration Ecology (FCRE), Costa Rica in July 2018. Tadpoles ($n = 11$; Gosner stages ranging from 25 to 41, Gosner 1960) were collected directly from *Musa acuminata* (banana) petioles. Whole tadpoles were sacrificed and stored individually in glass vials with Teflon-lined caps containing 2 mL of 99.9% methanol, hereafter referred to as the tadpole methanol extracts.

To compare tadpole alkaloid defenses with adult frogs from the same location, three male and three female frogs were collected from the leaf-litter surrounding the *Musa acuminata*. Following the methods of Bolton et al. (2017) and Basham et al. (2020), alkaloids were extracted from each frog using a Transcutaneous Amphibian Stimulator (TAS; Grant & Land 2002). The TAS is an electrical device that delivers a weak current to the skin of a frog, causing it to release stored defensive chemicals. The TAS treatment (Frequency: 50 Hz; Pulse width: 2 ms; Amplitude: 9 V) was standardized between frogs. Following application of the TAS, discs of absorbent bibulous paper were used to collect the alkaloid secretions, which were deposited in 2 mL of 99.9% methanol in glass vials with Teflon-lined caps, hereafter referred to as the frog TAS methanol extracts. Frogs were fully recovered within five minutes and were later released at the same point of capture.

The TAS is a nonlethal method of collecting frog skin alkaloids, and previous studies have found no difference in the number and types of alkaloids collected using the TAS method when compared to the more traditional, yet lethal, whole-skin extraction method (Clark et al. 2006, Hantak et al. 2013, Bolton et al. 2017, Schulte et al. 2017). However, based on studies of small dendrobatids (*O. pumilio* and *O. granulifera*), the quantity of alkaloids extracted using the TAS are only proportional to the total quantity present in whole-skin extractions of alkaloids (Saporito, unpublished data). Therefore, the alkaloid quantities reported here do not represent the total quantity of skin alkaloids present in *O. granulifera*, but are instead proportional to the total quantity contained in each frog and should be compared based on their relative differences.

***Ranitomeya imitator* and *Ranitomeya variabilis* tadpole, egg, and frog collection.** An age-series of wild-caught *R. imitator* and *R. variabilis* tadpoles were collected and examined for alkaloids from the upper Cainarachi Valley, between Tarapoto and Yurimaguas (km 32), San Martín, Peru in April and May 2013. Tadpoles ($n = 12$ for *R. imitator* and $n = 12$ for *R. variabilis*; Gosner stages ranging from 25 to 42) were collected directly from natural or artificial

phytotelmata. Whole tadpoles were sacrificed and stored individually in glass vials with Teflon-lined caps containing 2 mL of 99.9% methanol, hereafter referred to as the tadpole methanol extracts. To determine if alkaloids are present in eggs provided by mother frogs, ten unfertilized trophic eggs and six fertilized eggs of *R. imitator* and four fertilized eggs of *R. variabilis* were collected from natural or artificial phytotelmata and examined for alkaloids. Whole eggs were stored individually in glass vials with Teflon-lined caps containing 200 μ L of 99.9% methanol, hereafter referred to as the egg methanol extracts. To examine alkaloid defenses in adults from the same location, three *R. imitator* and two *R. variabilis* frogs were collected from the leaf-litter at the same location. Frogs were euthanized by freezing and then skins were removed (Shine et al. 2015; Lillywhite et al. 2017). Whole frog skins were stored in 4 mL of 99.9% methanol in glass vials with Teflon-lined caps, hereafter referred to as the frog skin methanol extracts.

Alkaloid Analysis. From each methanol extract, alkaloid fractions were prepared, identified, and quantified following methods in Bolton et al. 2017, Saporito et al. 2019, and Basham et al. 2020, using GC-MS on a Varian Saturn 2100T ion trap MS instrument coupled to a Varian 3900 GC with a 30 m x 0.25 mm i.d. Varian Factor Four VF-5ms fused silica column. GC separation of alkaloids was achieved using a temperature program from 100 to 280 °C at a rate of 10 °C per minute with helium as the carrier gas (1 mL/min). Each alkaloid sample was analyzed with electron impact and chemical ionization MS. Alkaloids were identified by comparing the observed mass spectral (MS) properties and gas-chromatography (GC) retention times (Rt) with those of previously reported anuran alkaloids in Daly et al. (2005) and Saporito unpublished data. Most anuran alkaloids have been assigned code names that consist of a bold-face number corresponding to the nominal mass and a bold-face letter to distinguish alkaloids of the same nominal mass (Daly et al. 2005). Isomers of previously characterized alkaloids were tentatively identified on the basis of their electron impact (EI) and chemical ionization (CI) mass spectral data and GC retention times. Following the methods of Garraffo et al. (2012), we considered alkaloids to be new isomers if they shared identical EI-MS data with a previously identified alkaloid but differed in Rt by at least 0.15 min. Each individual egg, tadpole, and adult frog extract was analyzed in three chromatographic replicates and the average quantity of alkaloids was calculated by comparing the observed alkaloid peak areas to the peak area of a nicotine internal standard, using Varian MS Workstation v.6.9 SPI.

Data Analysis. Linear regression was used to examine the relationship between tadpole developmental stage (Gosner stage) and tadpole mass with alkaloid quantity.

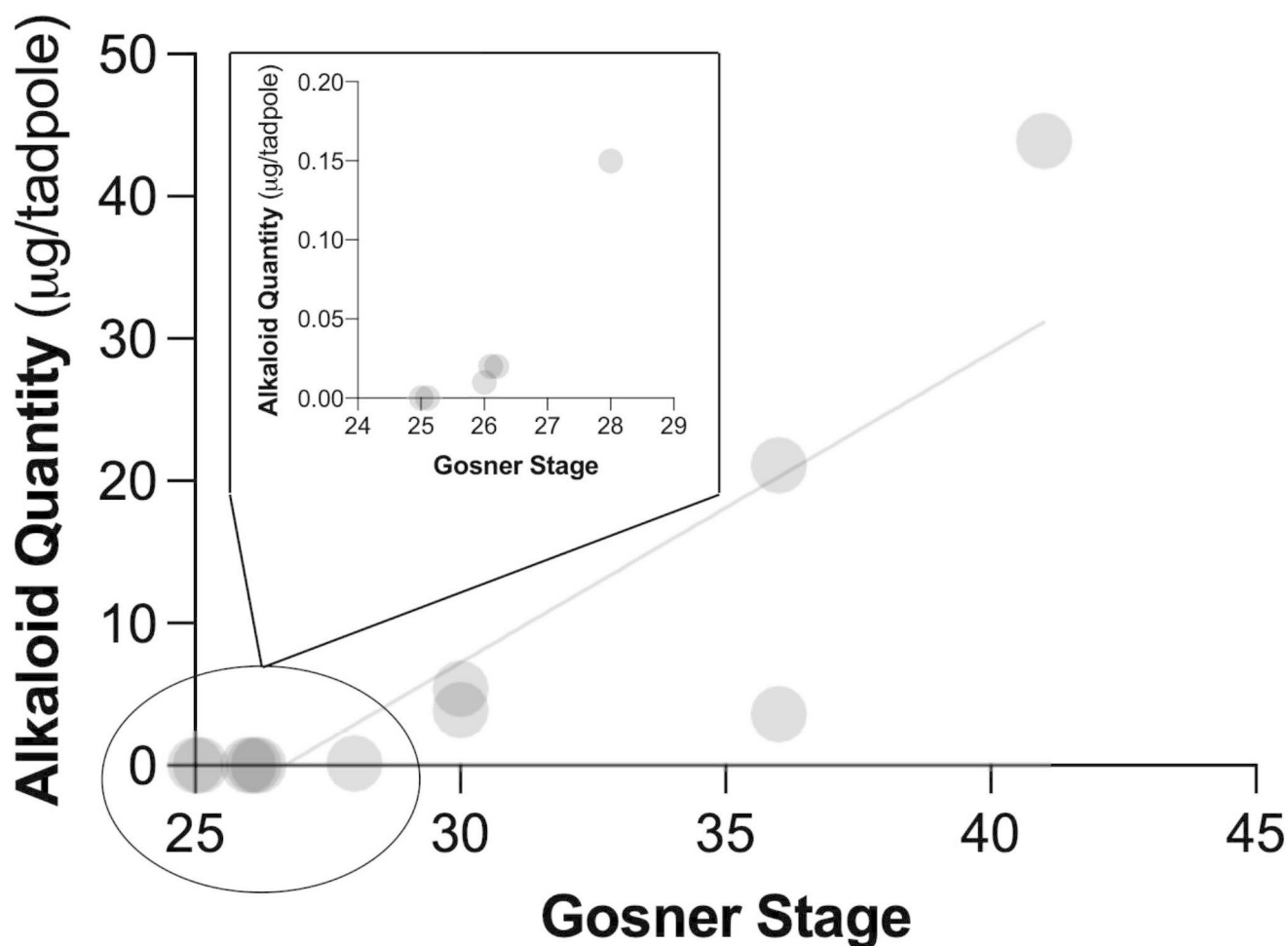


Fig. 1 Relationship between *O. granulifera* tadpole alkaloid quantity and tadpole developmental stage

Results

Alkaloids were detected in both tadpoles and adults of *O. granulifera*. Alkaloid quantity increased with tadpole developmental stage ($p \leq 0.001$; $R^2 = 0.74$; Fig. 1) and tadpole mass ($p = 0.007$; $R^2 = 0.58$; Fig. 2). Alkaloids were not detected in tadpoles, unfertilized trophic eggs, or fertilized eggs of *R. imitator* or *R. variabilis*; however, adult *R. imitator* and *R. variabilis* contained alkaloids.

A total of 78 alkaloids (including isomers) were identified across all adults and tadpoles of *O. granulifera*. On average (mean \pm SE), adults contained $1,115 \pm 321$ μg of alkaloids (range: 667–2,696 μg) and tadpoles contained 9 ± 5 μg of alkaloids (range: 0.008–44 μg). The most common and abundant alkaloids present across *O. granulifera* were the 5,8-disubstituted indolizidine **205A**, 3,5-disubstituted pyrrolizidine **223H**, and decahydroquinoline **219A**, which were present in all adults and the nine tadpoles that contained alkaloids. Two tadpoles of developmental stage 25 did not contain alkaloids. See Table 1 for list of all

alkaloids present in *O. granulifera* tadpoles and frogs. In both species of *Ranitomeya*, a total of 64 alkaloids (including isomers) were identified in adult frogs, with an average of 240 ± 98 μg alkaloids per frog (range: 61–598 μg). See Table 2 for a list of all alkaloids detected in *R. imitator* and *R. variabilis* frogs.

Discussion

The presence of alkaloids in female *O. granulifera*, coupled with the positive association between alkaloid quantity and tadpole mass and developmental stage, provide evidence that females provision their tadpoles with alkaloid defenses. These findings are similar to those previously described in *O. pumilio* (Stynoski et al. 2014a; Saporito et al. 2019; Brooks et al. 2022) and *O. sylvatica* (Fischer et al. 2019), and further suggest that maternal alkaloid provisioning may be a parental care adaptation common to all members of the genus *Oophaga*. Interestingly, there is no evidence of maternal alkaloid provisioning in *R. imitator* or

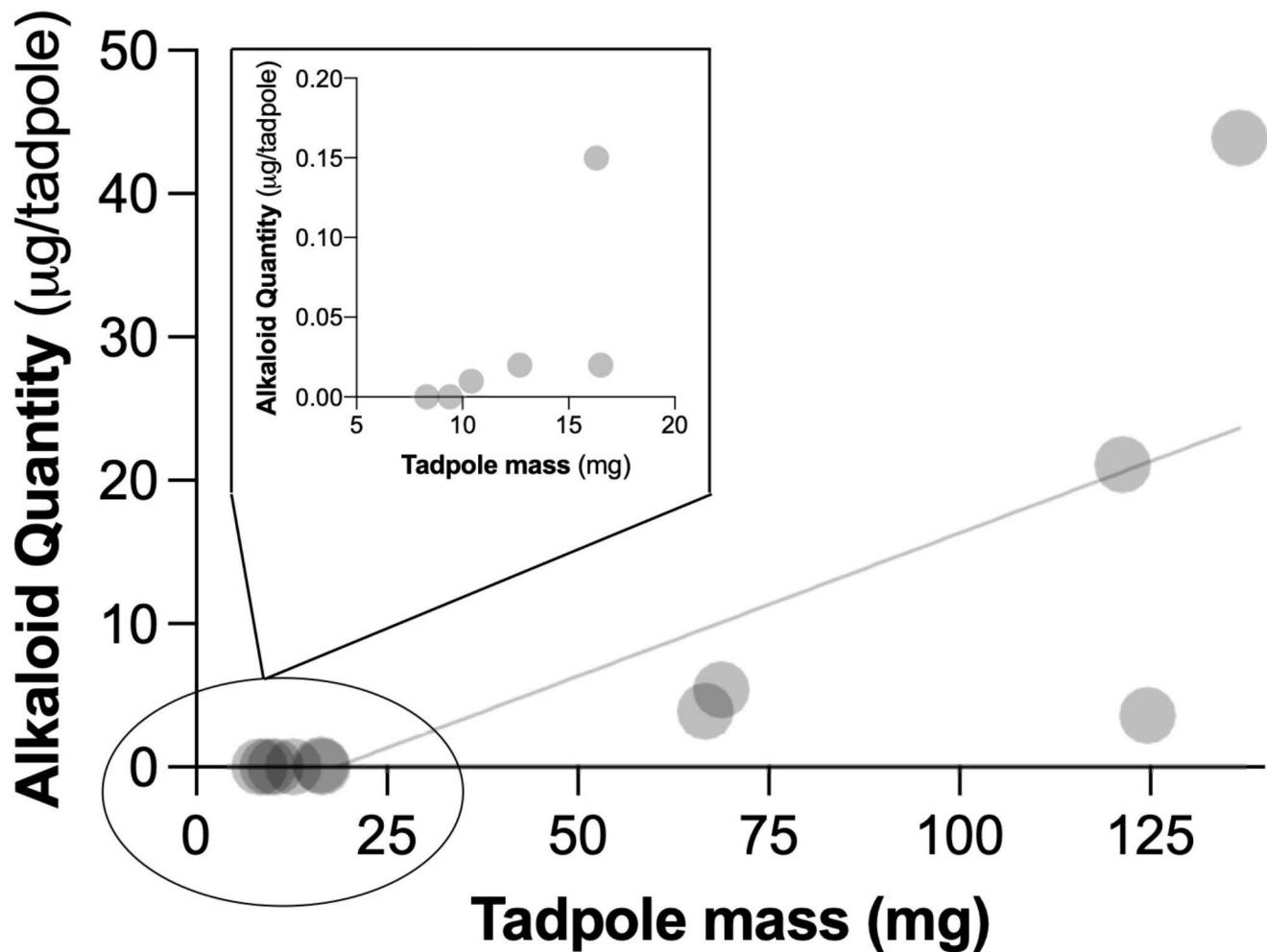


Fig. 2 Relationship between *O. granulifera* tadpole alkaloid quantity and tadpole mass

R. variabilis, two species of dendrobatids that facultatively feed eggs to developing tadpoles. Although female *R. imitator* provide unfertilized trophic eggs to developing tadpoles as a supplemental but necessary food source (Brown et al. 2008a, b), surprisingly no alkaloids were detected in eggs or tadpoles. Likewise, no alkaloids were detected in fertilized eggs or tadpoles of *R. variabilis*. The lack of evidence for maternal alkaloid provisioning in two facultatively oophagous *Ranitomeya* species suggests that, at least among dendrobatids, this form of parental care may be unique to *Oophaga*, possibly in relation to their obligate egg-feeding behaviors. Among other dendrobatids, trace quantities of batrachotoxin have been identified in eggs of *Phyllobates terribilis*, a non-egg-feeding species, though no alkaloids were detected in tadpoles (Myers et al. 1978). Additionally, alkaloids have not been detected in wild-caught *Dendrobates auratus* tadpoles (Saporito, unpublished data), another non-egg-feeding species. However, in a possible case of convergent evolution, maternal provisioning is also present in at least one species of the poison frog genus *Mantella*,

in which tadpoles are presumably facultatively oophagous (Glaw et al. 2000; Heying 2001). Fischer et al. (2019) found that both trophic eggs and tadpoles of *M. laevigata* contained alkaloids. Alkaloids have also been identified in the oocytes of the poison frog *Melanophryniscus simplex* (Grant et al. 2012), though another study demonstrated that the post-ovipositional eggs of *M. devincenzii* lacked alkaloids (Mebs et al. 2007), suggesting alkaloids may be lost from eggs following oviposition. Future research should explore the possibility of maternal provisioning of alkaloids in other lineages of dendrobatids, as well as other poison frogs more broadly.

In the present study, the most common and abundant alkaloids identified in adult *O. granulifera* were also the major alkaloids present in tadpoles, a finding similar to that reported in *O. pumilio* (Stynoski et al. 2014a; Brooks et al. 2022) and *O. sylvatica* (Fischer et al. 2019). Collectively, these data provide additional evidence that alkaloid provisioning is passive rather than active, wherein mother frogs transfer alkaloids to their offspring (via trophic eggs) in

Table 1 Alkaloids identified in tadpoles and adults of *Oophaga granulifera* arranged by structural class

5,8-I	Branched Alkaloids										Unbranched Alkaloids					Unclassified
	5,6,8-I	Dehydro-5,8-I	PTX	DeoxyPTX	aPTX	Tri	3,5-P	3,5-I	Pyr	DHQ	HTX	Izidine	Lehm	Pip	Unclass	
<u>193E</u>	<u>223A</u>	<u>201A</u>	<u>251D</u>	<u>193H</u>	<u>225E</u>	<u>207J</u>	<u>195F</u>	<u>167E</u>	<u>183B</u>	<u>195J</u>	<u>235A</u>	<u>225J</u>	<u>275A</u>	<u>183A</u>	<u>193A</u>	
<u>205A</u>	<u>259C</u>	<u>207E</u>	<u>281A</u>		<u>267A</u>	<u>219A</u>	<u>223H</u>	<u>195B</u>	<u>197B</u>	<u>219A</u>	<u>259A</u>		<u>289A</u>	<u>211I</u>	<u>197D</u>	
<u>207Q</u>						<u>235AA</u>	<u>249I</u>	<u>249A</u>	<u>225H</u>	<u>221D</u>	<u>261A</u>		<u>293I</u>	<u>213B</u>	<u>209G</u>	
<u>209I</u>							<u>251K</u>			<u>223F</u>				<u>223DD</u>	<u>271E</u>	
<u>217B</u>										<u>243A</u>						
<u>223D</u>										<u>245E</u>						
<u>225M</u>										<u>249D</u>						
<u>245D</u>										<u>253D</u>						
<u>247E</u>										<u>253E</u>						
										<u>267L</u>						

^a Underlined alkaloids indicate those that appear in tadpoles and adults.

^b Branched alkaloids are those with branch-points in their carbon skeleton and appear to be mostly derived from oribatid mites, whereas unbranched alkaloids are those without branch-points and are mostly derived from ants (Saporito et al. 2007, 2012, 2015).

^c Abbreviations for alkaloid structural classes are as followed: 5,8-I (5,8-disubstituted indolizidine); 5,6,8-I (5,6,8-trisubstituted indolizidine); dehydro-5,8-I (dehydro-5,8-disubstituted indolizidine); PTX (pumiliotoxin); deoxyPTX (deoxypumiliotoxin); aPTX (allopumiliotoxin); Tri (tricyclic); 3,5-P (3,5-disubstituted pyrrolizidine); 3,5-I (3,5-disubstituted indolizidine); DHQ (decahydroquinoline); HTX (histrionicotoxin); Lehm (lehmiizidine); Pip (2,6-disubstituted piperidine); Unclass (unclassified alkaloids).

types and quantities proportional to their own alkaloid composition. Although *O. granulifera* tadpoles and their specific mothers were not examined in the present study, Brooks et al. (2022) did examine *O. pumilio* tadpoles and their mothers and found tadpoles to contain the identical types of alkaloids as their mothers. Further, alkaloid quantities in older tadpoles were positively associated with alkaloid quantities in mother frogs. Based on these findings, natural variation in alkaloid profiles of mothers is directly responsible for the defensive profiles, and therefore level of protection from predators and/or pathogens, of their offspring. Additional research will be necessary to determine the extent of this relationship in *O. granulifera* and if it is common to other members of *Oophaga*.

The present study found an increase in the quantity of alkaloids with developmental stage and tadpole mass, wherein older, larger tadpoles contained more alkaloids, which is consistent with previous studies (e.g., Stynoski et al. 2014a, Brooks et al. 2022). Older, more developed tadpoles will have consumed more trophic eggs and thus accumulated larger quantities of alkaloids. Additionally, Dugas et al. (2016b) demonstrated that mother *O. pumilio* also provision larger meals to their oldest tadpoles, favoring their offspring that are closer to reaching metamorphosis. Therefore, older and larger tadpoles are likely exposed to provisioned alkaloids for both a longer period of time and in greater quantity than younger and smaller tadpoles. Given the similarities in parental care behavior between females of *O. pumilio* and *O. granulifera*, it is possible that shifts in provisioning based on tadpole age also occur in *O. granulifera*, which may also explain increases in alkaloid quantity with tadpole development. Granular gland development also plays a role in the accumulation of alkaloids throughout tadpole development. Histological studies of *O. pumilio* tadpoles have reported the absence of glandular structures between stages 25–27, the presence of rudimentary granular glands at stage 32–37, and mature glands that begin to migrate to the dermis at stage 40 (Stynoski and O'Connell 2017). Although no data are available on granular glands in *O. granulifera* tadpoles, it is likely that glands are more developed in older tadpoles, which would result in their possession of larger quantities of alkaloids compared to younger tadpoles. Interestingly, in the present study, alkaloids were detected in tadpoles of stage 26, which is younger than that detected in *O. pumilio* (stage 30; Saporito et al. 2019), perhaps suggesting slightly earlier development of these glands in *O. granulifera*. Alternatively, alkaloids may be stored (even temporarily) in tissues other than granular glands in tadpoles; for example, alkaloids have been identified throughout the digestive tract and in the liver, kidney, and muscle tissue of dendrobatid and bufonid poison frogs (Grant et al. 2012; Jeckel et al. 2020, 2022). Future research

Table 2 Alkaloids identified in adults of *Ranitomeya imitator* and *Ranitomeya variabilis* arranged by structural class

Species	Branched Alkaloids										Unbranched			Unclassified		
	5,8-I	5,6,8-I	PTX	hPTX	aPTX	Tri	4,6-Q	3,5-I	Pyr	DHQ	N-methyl-DHQ	HTX	Izidine	Unclassified	Unclassified	
<i>R. imitator</i>	263K	221P				207J		223AB	225C	195A	237U	235A		195M		
		249C								219A		237F		223DD		
		253P								223F		239H		231I		
		263A								223Q		259A		30I		
										243A		261A				
										249E		283A				
										269AB		284A				
												285A				
<i>R. variabilis</i>	207A	221P	237A	239M	253A		237I	195B		249E	237U	235A	191D			
	219J	231B	251D		267A			223AB				259A	231H			
	235B	249C										283A				
	263K	273A										285A				
		279F														

^a Branched alkaloids are those with branch-points in their carbon skeleton and appear to be mostly derived from oribatid mites, whereas unbranched alkaloids are those without branch-points and are mostly derived from ants (Saporito et al. 2007, 2012, 2015).

^b Abbreviations for alkaloid structural classes are as followed: 5,8-I (5,8-disubstituted indolizidine); 5,6,8-I (5,6,8-trisubstituted indolizidine); PTX (pumiliotoxin); hPTX (homopumiliotoxin); aPTX (allopumiliotoxin); Tri (tricyclic); 4,6-Q (4,6-disubstituted quinolizidine); 3,5-I (3,5-disubstituted indolizidine); Pyr (2,5-disubstituted pyrrolidine); DHQ (decalhydroquinoline); N-methyl-DHQ (N-methyl-decalhydroquinoline); HTX (histriocotoxin); Izidine (other izidines); Unclassified (unclassified alkaloids).

should focus on determining the ontogeny of granular gland development in *O. granulifera* tadpoles, as well as examine alternative tissues for the presence of alkaloids.

In poison frogs of the genus *Oophaga*, tadpoles contain alkaloids sequestered from a diet of trophic eggs, which they obligately consume. Older tadpoles have greater quantities of alkaloids than younger tadpoles, and these defenses have been shown to provide variable protection against potential predators (Stynoski et al. 2014b; Brooks et al. 2022). Here, we demonstrated that maternal provisioning of alkaloids is present in an additional species of *Oophaga*, *O. granulifera*, and that this form of parental care is absent in other dendrobatid poison frogs, the facultative egg feeding species *R. imitator* and *R. variabilis*. Our findings suggest that, among dendrobatids, maternal provisioning may be restricted to members of *Oophaga*. Future research should continue to explore the occurrence of maternal provisioning of alkaloids within this genus and examine how variation within and among species and populations contributes to differences in offspring alkaloid composition, particularly as it pertains to defense efficacy.

Acknowledgements We thank the Firestone Center for Restoration Ecology (FCRE), and J. Araya, K. Bacik, M. Ballagh, D. MacFarlane, Greedy, and A. Vega for their support in carrying out this research. Specimen collection permits were issued by Sistema Nacional de Áreas de Conservación (SINAC), Área de Conservación Osa (SINAC-ACOSA-PI-PC-65-17) and Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) export permits were issued by Ministerio del Ambiente y Energía (MINAE), República de Costa Rica (2018-CR3961/AL#S4983). The John Carroll University Institutional Animal Care and Use Committee (IACUC protocol #1700) approved all methods used in the study.

Author Contributions Sarah K. Bolton, Ralph A. Saporito, and Lisa M. Schulte conceived, designed, and coordinated the study, as well as conducted all of the field work. Olivia L. Brooks, Nina Savastano, Ralph A. Saporito, and Emily D. Villanueva extracted, collected, and analyzed all of the chemical samples. Ralph A. Saporito performed the data analysis. The first draft of the manuscript was written by Ralph A. Saporito and Emily D. Villanueva, to which all authors provided edits and revisions. All authors read and approved the final manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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