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# Chemical defenses shift with the seasonal vertical migration of a Panamanian poison frog

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## Abstract

Dendrobatid poison frogs sequester lipophilic alkaloids from their arthropod prey to use as a form of chemical defense. Some dendrobatid frogs seasonally migrate between the leaf litter of the forest floor in the dry season to the canopy in the wet season, which may yield differences in prey (arthropods) and therefore alkaloid availability over space and time. Here, we document a seasonal vertical migration of *Andinobates fulguritus* (the yellow-bellied poison frog) from ground to canopy between dry and wet seasons. We observed turnover in alkaloid composition between seasons and found that dry season frogs contained a lower relative quantity of alkaloids; however, there was no change in alkaloid richness between seasons. The 77 alkaloids of 13 structural classes identified in this population appear to be derived mostly from mites and ants, though the two most common alkaloids were mite derived. Our observed shifts in defensive profiles are consistent with well-documented turnover in mite and ant communities between seasons and vertical strata. As climate change is expected to lengthen and strengthen dry seasons in many tropical regions, our results suggest that arboreal poison frogs forced to the ground for longer periods of time may see a shift in the abundance of alkaloids, possibly decreasing their defensive potential. This study provides further predictions for the wide-reaching effects of climate change, even as nuanced as charismatic poison frogs losing their poisons.

Abstract in Spanish is available with online material.

## KEYWORDS

alkaloid, ant, arboreal, arthropod, dendrobatid, mite, Panama, seasonality, tropical forest

## 1 | INTRODUCTION

A variety of taxa have evolved forms of chemical defense to dissuade predators with harmful or unpalatable compounds (de Jong et al. 1991, Vliieger et al. 2004, Saporito, et al. 2007b, 2012, Dreon et al. 2013, Mina et al. 2015, Schulte et al. 2017, Hovey et al. 2018). Among vertebrates, five independent clades of anurans known collectively

as poison frogs have co-evolved alkaloid-based defenses, including dendrobatids from Central and South America (Saporito et al. 2012), bufonids from South America (Jeckel et al. 2015), mantellid frogs from Madagascar (Andriamaharavo et al. 2015), eleutherodactylids from Cuba (Rodríguez et al. 2011), and myobatrachids from Australia (Smith et al. 2002). Alkaloids also protect poison frogs from microorganisms, including bacteria, fungi, and parasites (Weldon et al. 2006,

Jeckel et al. 2015, Mina et al. 2015, Hovey et al. 2018). For example, pumiliotoxin 251D acts as a strong deterrent of a parasitic mosquito (Weldon et al. 2006). Alkaloids sequestered from arthropod prey are stored in specialized granular glands in the skin (Neuwirth et al. 1979) with over 1,200 alkaloids currently identified from poison frogs, belonging to over 28 structural classes (Saporito et al. 2012, Hovey et al. 2018). Most poison frog alkaloids appear to be derived from ants and mites (Daly et al. 2005, Rasputnig et al. 2011, Jones et al. 2012, Saporito et al. 2012, 2015), and consequently, many poison frogs are ant/mite dietary specialists (Gómez-Hoyos et al. 2008, Agudelo-Cantero et al. 2015, Moskowitz et al. 2018). Thus, the availability of certain alkaloid containing prey, particularly ants and mites, largely drives variation in alkaloid defenses within and among species of poison frogs (McGugan et al. 2016, Prates et al. 2019).

Shifts in frog alkaloid composition can occur rapidly over relatively small spatial and temporal scales (Saporito et al. 2006, 2007a, Andriamaharavo et al. 2015, McGugan et al. 2016, Prates et al. 2019, Moskowitz et al. 2020). For example, independent studies of *Oophaga pumilio* and *Oophaga sylvatica* populations only separated by a few hundred meters across a landscape have shown significantly different alkaloid profiles, which appear largely attributed to small-scale spatial differences in prey availability across habitats (Saporito et al. 2006, 2007a, Moskowitz et al. 2020). Similar shifts in frog alkaloid composition are also known to occur across seasons (Saporito et al. 2006, 2007a, Moskowitz et al. 2018), a process presumed to be driven by seasonality and phenology in the composition, richness, and abundance of alkaloid containing arthropods (Janzen 1973, Levings 1983, Tanaka & Tanaka 2006). There are ample data to demonstrate that differences in habitat and season can lead to large shifts in frog alkaloid composition and that these differences are perceived by potential predators (Bolton et al. 2017, Lawrence et al. 2019). However, nothing is known about shifts in alkaloid profiles for poison frogs that undergo seasonal migrations in the vertical realm—from ground to canopy microhabitats.

Many dendrobatid frogs are partially arboreal and seasonally use canopy bromeliads and other phytotelms (water holding vegetation) to deposit tadpoles (Poelman et al. 2013, Sabagh et al. 2017, Pettitt et al. 2018), while others are fully arboreal and rarely visit the ground (Myers et al. 1984). The vertical axis of forests also produces dramatic turnover in arthropod composition over very short distances and therefore likely influences the diet of arboreal poison frog species. For example, Beaulieu, Walter, Proctor, and Kitching (2010) found that mite diversity at the ground differed significantly from mite assemblages collected in arboreal soils only 0.5 m aboveground, corroborating with the findings of other studies on mite stratification (Lindo & Winchester 2007, Karasawa & Hijii 2008). Similar patterns of vertical turnover have also been observed in ants (Bruhl et al. 1998, Hashimoto et al. 2006, Vasconcelos & Vilhena 2006, Ryder Wilkie et al. 2010, Arruda et al. 2016), and other potential prey items of poison frogs including springtails (Rodgers & Kitching 1998), beetles (Stork & Grimbacher 2006), and moths (Schulze et al. 2001, Ribeiro et al. 2016).

Here, we explore alkaloid profiles in a small neotropical poison frog *Andinobates fulguritus* (the yellow-bellied poison frog, Silverstone 1975) between the forest canopy during the wet season and the forest ground during the dry season in a Panamanian rainforest. Basham and Scheffers (2020) show that *A. fulguritus* vertically migrates 25 m annually between the wet and dry seasons. In the wet season, *A. fulguritus* inhabits arboreal epiphyte masses in the canopy, whereas in the dry season it inhabits leaf litter on the ground. Therefore, it is possible that the migration exhibited by *A. fulguritus* leads to shifts in alkaloid composition, due to changes in diet associated with the seasonal and vertical turnover in arthropod composition. Stark differences in arthropod composition by season and vertical strata are widely supported for Panamanian rainforests akin to our study system, as well as throughout the neotropics (Stork & Grimbacher 2006, Grimbacher & Stork 2009, Paniagua et al. 2009, Grøtan et al. 2014, Wardhaugh 2014, Basset et al. 2015, Kishimoto-Yamada & Itoioka 2015).

Therefore, we aim to address two main objectives:

1. Measure shifts in alkaloid profiles of *A. fulguritus* driven by a seasonal vertical migration. Such a pattern has not yet been examined, but, if present, would demonstrate the importance of seasonal vertical movement on the chemical ecology of poison frogs.
2. Provide the first description of alkaloids sequestered by *A. fulguritus*. In order to better understand the defensive ecology of arboreal poison frogs, a baseline knowledge of defensive chemicals is needed.

We found that the richness of alkaloids in *A. fulguritus* frogs was high in both wet and dry seasons. Alkaloid composition and the quantity of alkaloid differed between the seasons. Our results suggest that vertical migration allows access and accumulation of a greater quantity of alkaloids. As frogs are able to retain alkaloids over multiple seasons (Mebs et al. 2018), the arboreality of *A. fulguritus* may increase the defensive capability in these frogs. However, as climate change is expected to lengthen and strengthen dry seasons across many parts of the tropics, arboreal poison frogs such as *A. fulguritus* may be forced to the ground for longer periods (Basham and Scheffers 2020), inhibiting their activity and possibly leading to a reduction or shift in their defensive potential.

## 2 | METHODS

### 2.1 | Study area

In central Panama, we surveyed an Isthmian-Atlantic Moist Forest located within the Esteban Alphonso Lee Natural Reserve (Lat 9.358555; Lon 79.7029; 333 – 473 m a.s.l.; Figure S1), which borders the Chagres and Portobelo National Parks.



**FIGURE 1** a) An individual *A. fulguritus* encountered in the wet season at 22 m aboveground moving up through a patch of sodden detritus and observed eating a microscopic prey item. Three tadpoles sit on the back of the individual, to be transported to a water filled epiphyte nursery. b) The *Anacardium excelsum* (Espavé) tree at which the individual in (a) was found. Diameter at breast height = 1.5 m; tree height = 42 m. Photos © Edmund Basham

## 2.2 | Collection of *A. fulguritus*

We surveyed for *A. fulguritus* during the dry (mid-December–March) and wet seasons (April–mid-December) (Comita & Engelbrecht 2009, Basham & Scheffers 2020). Specifically, in the dry season frogs were captured between February 4, 2019 and March 9, 2019, and in the wet season between July 2, 2019 and July 27, 2019 (Figure S2). Thus, we attempted to standardize the amount of time experienced by

**TABLE 1** The number of samples from each site (tree) in each season

Tree ID #	Dry	Wet
#69	3	4
#103	3	3
#111	3	3
#114	1	0
#119	1	1
#121	2	2

individuals in both seasons before sampling, with individuals sampled between 2 and 3 months after the beginning of either season (Figure S2). Using survey methods in Scheffers et al. (2013) and Basham and Scheffers (2020), we conducted vertical, ground to canopy surveys for *A. fulguritus*, with each survey centered on a single Espavé tree, which is the principal habitat for *A. fulguritus* in this area (EB; unpublished data, Figure 1). A second researcher surveyed the ground in a 15 m radius around the tree for 1 hour searching through leaf litter, logs, and other microhabitats (Heyer et al. 1994). During arboreal surveys, we searched for *A. fulguritus* in tree holes, moss, epiphytes, and other microhabitat structures. For the purpose of this project, we selected 13 adult individuals from each season collected from five sites (Table 1; Figure S1), for a total of 26 individual samples. Sites were located in contiguous forest at a range of distances from one another (closest sites, Tree IDs #114 to #111 = 19 m; furthest sites, Tree IDs #111 to #121 = 701 m, Figure S1). Samples were chosen to maximize sample size while representing sites equally across seasons, but due to the limitations of sampling and the need to avoid using the same individuals across seasons (checked by markings), there was a slight discrepancy in sampling across sites (Table 1). However, though tree #114 was represented by a single frog in the dry season, the trees #114 and #111 were closely located (19 m), thus reducing the potential for a significant difference in arthropod prey between selected sites.

Frogs were collected solely from the leaf litter beneath Espavé trees in the dry season and from the canopy of Espavé trees in the wet season. Espavé trees are host to a wide range of epiphytes, but were dominated by a small number of highly abundant moss, orchid, bromeliad, and *ludovia* spp epiphytes. Thus, the variation in arthropod prey availability across sites was reduced through the relative homogenization of vegetation structures and resources present across all sites.

## 2.3 | Alkaloid sampling

Captured frogs were weighed, measured (mean weight = 0.28 g; mean snout-vent length = 14.3 mm), brought to the research station, and allowed to rest overnight with ample moisture to maintain full hydration. Alkaloids were collected from each frog using a transcutaneous amphibian stimulator (TAS; Grant & Land, 2002), which applies a weak electric current to the skin, causing the secretion of the

contents of their granular glands. Following the methods of Bolton et al. (2017), the TAS treatment (Frequency: 50 Hz; Pulse width: 2 ms; Amplitude: 9 V) was standardized among frogs. The frogs were then wiped with disks of absorbent bibulous paper to collect the secretions, which were deposited in 1 ml of 100% methanol in glass vials with Teflon-lined caps. Frogs were fully recovered within 5 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 other small dendrobatids (*Oophaga pumilio* and *Oophaga granulifera*), the quantity of alkaloids extracted using the TAS is only proportional to the total quantity present in whole-skin extractions of alkaloids (Saporito, unpublished data). Therefore, the alkaloid quantities reported here (see Table S1) do not represent the total quantity of skin alkaloids present in *A. fulguritus*, but are instead proportional to the total quantity contained in each frog and should be compared based on their relative differences. We report quantity as micrograms of alkaloid per gram of frog.

## 2.4 | Alkaloid extraction, identification, and quantification

For each sample, an internal standard of nicotine ((-)-nicotine 99%, Sigma-Aldrich, Milwaukee, Wisconsin) was added to 0.5 ml of the original MeOH extract. This extract was evaporated to dryness with  $N_2$  and then resuspended in 100  $\mu$ l of 100% methanol. Gas chromatography/mass spectrometry (GC/MS) was performed for each sample on a Varian Saturn 2,100 T ion trap MS instrument coupled to a Varian 3,900 GC with a 30 m  $\times$  0.25 mm i.d. Varian Factor Four VF-5 ms 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 He as the carrier gas (1 ml/min). Each sample was analyzed in triplicate using electron impact MS, and once using chemical ionization MS with methanol as the CI reagent.

All alkaloids from each sample were identified using a combination of gas chromatography and mass spectrometry (GC-MS). Following the methods detailed in Hovey et al. (2018), individual alkaloids were quantified by comparison to the nicotine internal standard, and identified by comparing their retention times and mass spectral data with those of known alkaloids.

## 2.5 | Data analysis

Geographic variation in alkaloid composition is common within and among populations, and close populations tend to have more similar profiles than distant ones (Saporito et al. 2006, Prates et al. 2019). Though a natural variation in arthropod prey composition is expected between our sites, the sampling of frogs from the same species and

size class of tree which host similar epiphyte species should produce a pattern of arthropod turnover relatively less than the variation expected between trees of different species. Furthermore, the trees exist within the same contiguous forest (maximum distance between trees = 701 m); thus, there should be no inherent limitation on particular arthropods accessing particular trees, further reducing the potential for geographic variation in arthropod prey. Other factors may also cause variance in alkaloid composition, such as gender, size, and age (Clark et al. 2006, Saporito et al. 2010, Jeckel et al. 2015, Hovey et al. 2018). We were unable to accurately sex the individuals in this study, but the gender ratio of individuals within each season are expected to be similar across season with a sample size of 26 total individuals. Furthermore, we only selected adult individuals, but because adults were consistently ca. 14 mm SVL, we were unable to ascertain age of individuals. We attempted to standardize for size by calculating alkaloid per gram instead of raw quantity, as larger frogs have a greater capacity to hold alkaloids (Jeckel et al. 2015). Because vertical strata and season co-occur, for simplicity we reference the strata/season relationship by season only. Frogs were photographed for their unique ventral markings which were used to compare frogs between seasons, avoiding the use of samples from the same frog in different seasons.

We employed a bootstrap simulation approach to calculate differences in the quantity and richness of alkaloids in frogs between wet and dry seasons, using 50,000 iterations. In each iteration, we randomly assigned 13 samples each to wet and dry seasons and then calculated the mean quantity (mean  $\log(\mu$ g per gram)) and richness of alkaloids in frogs for each season. The mean quantity and richness in the wet season were subtracted from the dry season means to produce a mean difference. Thus, we produced 50,000 randomly derived mean differences, which we used to generate a z-score by subtracting the mean of the distribution from the observed value and dividing by the standard deviation of the distribution. We hypothesized that a decrease in activity in the dry season (Born et al. 2010, Basham & Scheffers 2020) will cause a decline in quantity and richness of alkaloid. Thus, we calculated a *p* value from the z-score under a one-tailed hypothesis, testing an effect of a lower mean in the dry season. We also include the *p* value calculated under a two-tailed test under the hypothesis that alkaloid quantity or richness could equally increase or decrease in the dry season. We also computed differences in the quantity of individual alkaloids between seasons using the same simulation method, here using two-tailed tests due to the variation expected in individual alkaloid quantity between seasons. Tests were not run for seasonal comparisons where the alkaloid was recorded in less than five samples (39 comparisons made of 77 total alkaloids).

Although no information is currently available on the diet of *A. fulguritus*, studies of other *Andinobates* suggest that members of this genus are largely ant/mite specialists (Gómez-Hoyos et al. 2008, Agudelo-Cantero et al. 2015). Based on a growing body of research on alkaloid-bearing arthropods, most dendrobatid alkaloids can be tentatively assigned to a dietary arthropod based on their chemical structure (for information, see Saporito et al. 2012). Most

alkaloid classes—each of which is composed of several individual alkaloids, all of which are very similar in structure—possess either a straight-chain or branched-chain carbon backbone. In general, ants have been found to largely possess straight-chain alkaloids, whereas mites largely contain branched-chain alkaloids (e.g., Saporito et al. 2004, 2007b, 2012, 2015, Jones et al. 2012, and references within). Although there are some exceptions to this rule, these findings allow for the tentative or suspected assignment of certain alkaloids to an arthropod source, namely ants or mites. We used analysis of variance (ANOVA), followed by post hoc Tukey HSD pair-wise comparisons (significant at  $p = .05$ ) to test for significant differences in the quantity and richness of alkaloid provided by arthropod sources. We conducted tests across all samples, and separately between wet and dry seasons. For each model, we checked the assumptions of normality using QQ plots and checked equal variance across groups using Bartlett tests (Bartlett 1937), which tests the null hypothesis that the variances in each of the groups are the same.

Sampling completeness of alkaloid richness was assessed by calculating the mean of four commonly employed abundance-based estimators of species richness (CHAO1, JACK1, Abundance-based Coverage Estimator and Bootstrap; Colwell et al. 2004, Basham et al. 2016), for each season, and for both seasons combined (R package “wigid”; Meredith 2019).

We calculated the effect of site and season on the similarity of alkaloid composition among the samples (i.e., do frogs sampled from the same site/season have more similar alkaloid composition than frogs from other sites/seasons?). We transformed data to presence/absence and used the “metaMDS” function from the R package “vegan” (Oksanen et al., 2019), in which we set the number of axes ( $k = 3$ ) and the number of random starts (trymax = 100) with the “Jaccard” distance metric. The Jaccard index provides values of similarity ranging from 0 to 1 (Legendre & Legendre 2012) and is robust in handling presence/absence data (Qian & Ricklefs 2007, Beck et al. 2013). We then applied permutational multivariate analysis of variance tests with 1,000 permutations (ANOSIM function in “vegan”) to examine the effects of season and site on alkaloid composition. For the effects of site, we ran separate tests for each season, and a test for both seasons combined. To test the effect of geographic distance on composition, we employed mantel tests (R package “vegan”) which uses a spearman correlation with 9,999 permutations. Here, we used mantel tests to correlate between the “Jaccard” distance matrix of alkaloid composition between frogs and the complimentary matrix of geographic distance between those frogs. These analyses were repeated separately for each season and combined.

### 3 | RESULTS

A total of 77 alkaloids (including isomers) were identified, belonging to 13 known structural classes (Table S1). 1,4-disubstituted quinolizidines (1,4-Q), 5,8-disubstituted indolizidines (5,8-I), and histrionicotoxins (HTX) alkaloids were present in the greatest number and quantity, though a significant number were of unknown classes (Table 3).

Accumulation curves of alkaloid richness show that our sample size was sufficient in capturing the majority of alkaloids present in the population (77 observed/ 101 estimated  $\times 100 = 77\%$  captured; Figure S3) and suggests that overall estimated richness of alkaloids is greater in the wet season (87) compared to the dry season (78).

The most abundant alkaloids in terms of presence across samples (i.e., represented in over 80% of samples) were as follows: 1,4-Q 231A (isomer 2), 233A (isomer 1), and 233A (isomer 2); 3-5 pyrrolizidine (3,5-P) 195 (isomer 1); 5,8-I 203A (isomer 1), 217B (isomer 2), 219F (isomer 1); HTX 285A (isomer 1), 285C, 287D (isomer 2); Izidine 233B; Unclass 235P; and tentatively new alkaloids “247(Rt13.99)”, “249(Rt13.92)” (Table S1).

Of the 39 tested alkaloids, seven alkaloids were present in significantly lower concentrations during the dry season, and a single alkaloid had higher concentrations in the dry season (Table S1). Alkaloids 1,4-Q 231A (isomer 1) and 5,8-I 203A (isomer 1) were present in high relative quantities in all samples and made up the core of the defensive profile of *A. fulguritus* (Table S1), both of which are presumed to be mite derived (Saporito et al. 2007b). Based on their chemical structure, the majority of alkaloids in the present study appear to be derived from mites and ants.

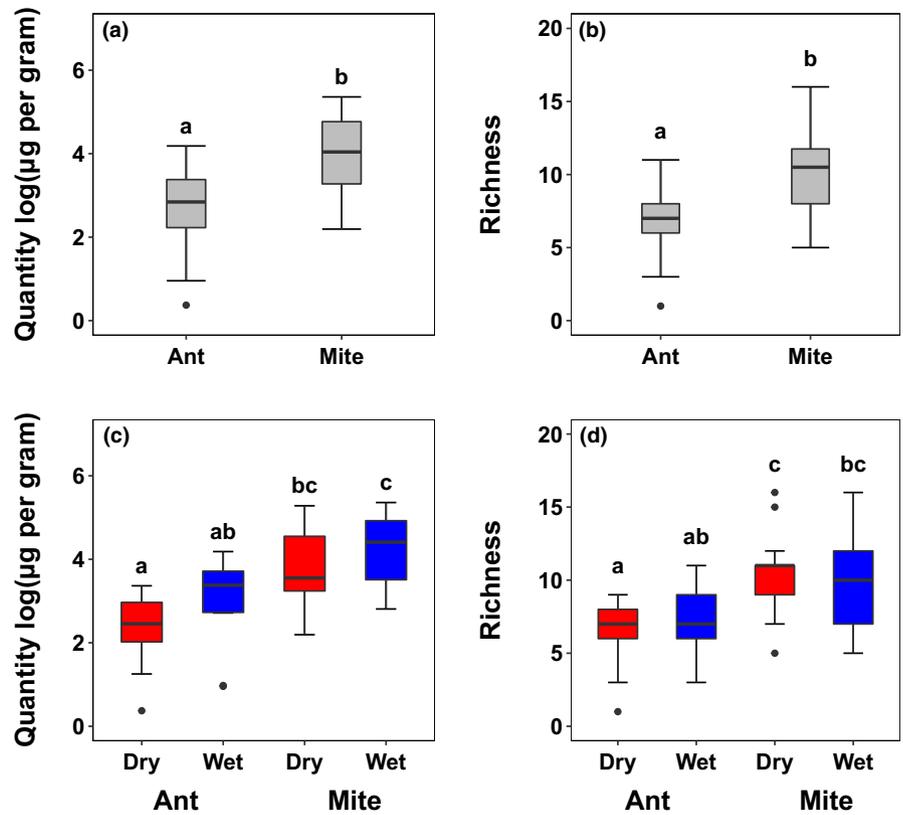
ANOVA with post hoc Tukey pair-wise comparisons showed that the quantity and richness of mite-derived alkaloids were greater than those derived from ants (Figure 2a,b). However, there were no significant shifts in the quantity or richness of sources across season (Figure 2 b,c). In all models, QQ plots were acceptable for data normality and Bartlett tests were insignificant, confirming the model assumptions of equal variance across groups ( $p$  values  $> .05$ ).

Frogs sampled from the leaf litter in the dry season had lower relative alkaloid quantities compared to those in the canopy during the wet season (one-tailed test; Table 2), and alkaloid richness did not differ from wet to dry season (Table 2). Though the two-tailed test  $p$  value for a difference in quantity between seasons was not significant, the evidence from the distinctly lower dry season mean quantity of alkaloid as well as the lower quantity of 7 of 8 individual alkaloids (the 8 alkaloids that shifted significantly; Table S1) in the dry season strongly suggests a seasonal shift.

The composition of alkaloids in frogs differed significantly between seasons (Table 3; Figure 3a). Interestingly, when data from both seasons were combined, alkaloid composition was influenced by site (tree), with frogs from the same site having more similar alkaloid compositions than to frogs from other sites (Table 3). However, these relationships were not significant within each season, possibly due to the reduced sample size (Table 3). Furthermore, mantel correlations of composition with geographic distance were not significant for either season or combined (Table 3), suggesting that the turnover in alkaloid composition, and by extension prey composition, was equivalent between sites irrespective of distance.

Of the 77 alkaloids, a subset of 18 alkaloids were considered tentatively new, all of which possessed clear mass spectral characteristics common to frog/arthropod alkaloids, but could not be identified as a previously known alkaloid. Unfortunately, these tentatively new alkaloids were present in quantities too small for further chemical

**FIGURE 2** Plots (a) and (c) show the quantity ( $\log(\mu\text{g per gram})$ ) and plots (b) and (d) show the richness of alkaloids likely derived from ants and mites, either across all samples (a,b), or between seasons (c, d). Boxplots with letters place above the maximum value that are not shared are significantly different by the ANOVA test at the 5% level of significance. Red shading denotes dry season, blue shading denotes wet season, and gray shading as combined seasons



characterization and therefore were not named; however, they were assigned unique identifiers that include their molecular weight, followed by GC retention time in parenthesis, all surrounded by quotes (Table S1). Future studies will be necessary to identify and characterize these new alkaloids in *A. fulguritus*.

## 4 | DISCUSSION

Our findings illustrate a marked shift in alkaloid defense profiles of *A. fulguritus* between dry season microhabitats on the ground and wet

season microhabitats in the canopy. This shift is likely due to differences in the arthropod communities that live in the leaf litter compared to arboreal habitats (Charles et al. 2005, Yanoviak et al. 2007, Karasawa & Hijii 2008, Basset et al. 2015), which may provide a new palette of alkaloids to *A. fulguritus* as they shift vertically across seasons. Additionally, the lower alkaloid quantity observed in *A. fulguritus* during the dry season may be attributed to a decrease in frog activity and feeding behavior. In a study of the dendrobatid poison frog *Dendrobates tinctorius*, it was found that its diet shifted in concert with a retreat to palm bracts and tree holes during the dry season, wherein frogs were less active and consumed fewer prey (Born et al. 2010). Indeed, Basham and Scheffers (2020) found *A. fulguritus* in lower abundance during the dry season (Figure 3b), which corroborates the

**TABLE 2** Mean alkaloid quantity and richness between seasons

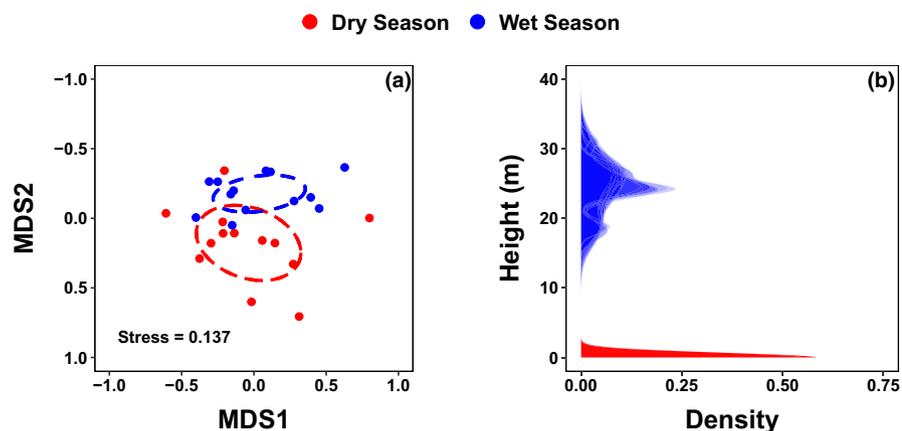
	Dry	Wet	One-tailed <i>p</i> value	Two-tailed <i>p</i> value
Alkaloid quantity ( $\mu\text{g per gram}$ )	84.30	139.00	<b>.039</b>	.078
Richness	24.08	25.38	.323	.646

Note: Bold values denote significant *p* values ( $p < .05$ ). *p* values derived using a bootstrap simulation with 50,000 iterations. Data for *p* value estimation were log transformed. In each iteration, we randomly assigned 13 samples each to wet and dry seasons and then calculated a mean difference between randomized seasonal means. We generated a z-score by subtracting the mean of the distribution of mean differences from the observed value and dividing by the standard deviation of the distribution. *p* values were then calculated from the z-score under a one-tailed hypothesis testing a decline in the dry season, and a two-tailed hypothesis testing for general differences among seasons.

**TABLE 3** Breakdown of R statistics and *p* values derived from the “anosim” function (R Package “vegan”) comparing alkaloid composition across sample groups

Variables	ANOSIM R	ANOSIM P	Mantel R	Mantel P
Season	0.148	<b>0.007</b>	NA	NA
Tree - Wet	0.219	0.076	0.082	0.260
Tree - Dry	0.209	0.116	0.090	0.255
Tree - Combined	0.187	<b>0.013</b>	0.080	0.175

Note: Bold values denote significant *p* values ( $p < .05$ ). Tests were run separately for the effects of season and site. For the effects of site, we ran separate tests for each season, and one with all data combined. Also included are Mantel R statistics and *p* values of the effect of geographic distance on composition.



**FIGURE 3** a) NMS ordination of alkaloid composition in frogs collected in the wet season (blue) and dry season (red). Each point represents the alkaloid composition of a frog. b) Figure adapted from Basham and Scheffers (2020) demonstrating the relative density of *A. fulguritus* with vertical height between the wet (blue) and dry (red) seasons

findings of Born et al. (2010) and supports the hypothesis of lower activity and feeding during the dry season. However, poison frogs are able to retain alkaloids over multiple years (Jeckel et al. 2015, Mebs et al. 2018), and this was suggested to explain the relatively small shift in alkaloid profile between seasons in the mantellid poison frog *Mantella laevis*, despite an observed shift in diet (Moskowitz et al. 2018). Thus, it would appear that not all poison frogs (and/or populations) exhibit strong seasonal variation in alkaloid profiles, even in the presence of a dietary shift, which could be explained by the fact that frogs (and/or populations), which have lived through multiple seasons and/or have experienced less predation (and thus reduced loss of their alkaloid defenses via secretion), may see a homogenization of their defensive profiles. Questions still remain regarding the rate of alkaloid gain and loss in the different seasons, and more rigorous sampling following clear temporal sampling procedures may elucidate these patterns in the future.

We find that the majority of alkaloids in *A. fulguritus* are likely mite and ant derived, though mite-derived alkaloids were greater in quantity and richness. Thus, we can infer that *A. fulguritus* is likely an ant/mite specialist, which aligns with other species of the *Andinobates* genus. In terms of relative quantity, mites appear to provide the greatest quantity and richness of alkaloids present in *A. fulguritus*, of which the two most abundant presumably mite-derived alkaloids were 1,4-Q 231A (isomer 1) and 5,8-I 203A (isomer 1). Ant-derived alkaloids also represented a significant portion of overall composition, of which the two most abundant alkaloids were HTX 287D (isomer 2) and HTX 285A (isomer 1). These four alkaloids were also found in other species of frog such as *Mantella baroni* and *O. pumilio*, of Madagascar and Costa Rica, respectively (Saporito, et al. 2007a, Andriamiharavo et al. 2010), highlighting the convergence in evolution that has occurred to result in the utilization of the same chemical compounds for defense across geographically disparate areas.

Basham and Scheffers (2020) showed that arboreal amphibians often descend from the canopy toward the ground during dry seasons. The forest canopy is exposed to greater levels of sunlight and wind, and has less water holding capacity than the ground (Parker et al. 1995, Lowman & Rinker 2004, Scheffers et al. 2013, Oliveira & Scheffers 2019). During the dry season, the soil and leaf litter on

the ground buffer climate and provide the necessary moisture and cover necessary for many forest amphibians to avoid desiccation. Some amphibians estivate (enter a state of dormancy) in order to avoid seasonally harmful conditions (Storey 2002), but *A. fulguritus* maintains some activity throughout the year at our study location by shifting its population from the canopy to the ground (Basham & Scheffers 2020). However, anthropogenic climate change is adding heat to the global climate system, resulting in longer and more intense droughts (IPCC 2014, Trenberth et al. 2014, Corlett 2016), which may force many arboreal species to seek buffered habitats on the ground (Basham & Scheffers 2020). For *A. fulguritus*, we hypothesize that more time on the ground due to lengthened dry seasons could result in a relative decline in the annual quantity of alkaloid defenses, possibly due to a decrease in activity or activity specific to a single microhabitat. We further hypothesize that a decline in alkaloid defenses could cause a subsequent shift in fitness, especially if changes in alkaloid profiles lead to differences in the ability of these frogs to defend themselves against predators and harmful microorganisms.

The hypothesis that longer dry seasons will reduce activity and prey consumption, causing a decrease in alkaloid sequestration, may be applied to other species which do not undertake any form of vertical migration but are likewise negatively affected by seasonal climate stress. However, it is likely that there will be great variation in climate change effects on alkaloid defenses between species and populations due to the context of habitat type, seasonality, local climate, prey availability, frog behavior, physiological climate tolerance, and other factors. This study provides further predictions for the wide-reaching effects of climate change, even as nuanced as influencing a charismatic poison frog's defense. We also highlight the need for further exploration in this field in order to understand the mechanisms and ecological consequences of these changes across the poison frog taxa.

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## AUTHOR CONTRIBUTIONS

E.W.B, R.A.S, and B.R.S conceived, designed, and funded the study. E.W.B., M.G-P, and A. R-M conducted fieldwork. E.W.B, R.A.S, and B.R.S analyzed the data and wrote and edited drafts of the manuscript.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.rv15dv45s> (Basham et al. 2020).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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