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Differences in alkaloid defenses in the poison frog Oophaga pumilio between disturbed and undisturbed habitats of Bocas del Toro, Panama

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Differences in alkaloid defenses in the poison frog *Oophaga pumilio* between disturbed and undisturbed habitats of Bocas del Toro, Panama

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Abstract
Poison frogs have the ability to sequester and store alkaloids in their skin as a mechanism of defense against predation. Alkaloids in poison frogs are obtained exclusively from the consumption of alkaloid-containing arthropods. As a result, changes in the environment can potentially harm *Oophaga pumilio* by altering their source of chemical defense. To date, only two conflicting studies have examined the effects of habitat disturbance on poison frogs chemical defenses. In the present study the effects of habitat disturbance on populations of *O. pumilio* from Colon and Solarte Islands, Bocas del Toro archipelago in Panama is examined. In addition, an indirect evaluation of the effects of habitat disturbance on arthropod diversity is done. The results of this study suggest that frogs from undisturbed habitats have a higher number and amount of alkaloids than frogs from a disturbed habitats. A comparison between *O. pumilio* alkaloid profiles from Colon Island and Solarte Island, revealed that frogs from Solarte Island are less toxic than frogs from Colon Island. Overall, Solarte Island had a lower amount and number of alkaloids than Colon Island. This study is another example of the tremendous alkaloid variability within frogs from the same population and between frogs from different populations. Herein, it is shown that the number of mite derived alkaloids increased, while the number of ant derived alkaloids decreased in disturbed sites; suggesting that habitat disturbance changes the composition of alkaloid-containing arthropods and therefore alters *O. pumilio* chemical defense.

Introduction

Amphibians have contributed greatly in the discovery of biologically active compounds such as peptides, biogenic amines, steroidal bufadienolides, batrachotoxins, tetrodotoxins, and lipophilic alkaloids (Daly, 1998). In particular, alkaloids obtained from amphibian skin have been utilized as an important tool in understanding basic physiological processes in humans, such as the function of sodium channels, anesthetics, anticonvulsants, antiarrythmics, and the biological activity of certain toxins (Daly, 1998). Alkaloids are present in a wide variety of amphibians, including numerous species of salamanders and frogs, and are utilized as a chemical defense against natural predators and/or microorganisms (Saporito *et al.*, 2012). Amphibians either manufacture alkaloids or obtain them from their dietary sources (Daly, 1998).

Poison frogs are well-known for their ability to sequester and store alkaloid chemical defenses obtained from their diet (Saporito *et al*., 2010; Saporito *et al*., 2012). Poison frogs include members of the family Dendrobatidae (Central and South America), Bufonidae (South America), Mantellidae (Madagascar), Myobatrachidae (Australia), and Eleutherodactylidae (Cuba) (Saporito *et al*., 2012). More than 850 alkaloids have been identified from poison frogs worldwide (Saporito *et al*., 2012), and are broadly characterized as molecular rings that contain at least one nitrogen atom (Myers & Daly, 1993). As a response to predation, alkaloids are secreted from poison glands (i.e., storage cells) located in the skin of poison frogs (Myers & Daly, 1983; Saporito *et al*., 2010). However, small amounts of alkaloids have also been detected in liver and muscle tissue (Saporito *et al.*, 2012) as well as in eggs and tadpoles (Stynoski *et al*., unpublished). Poison frog alkaloids are sequestered via consumption of alkaloid-containing arthropods such as mites, ants, beetles, and millipedes (Darst *et al*., 2005; Saporito *et al.*, 2009). The mechanism by which alkaloids are transported from the dietary arthropod to the frog's poison glands remains unknown (Saporito *et al*., 2012).

Oophaga pumilio (previously known as *Dendrobates pumilio*) is a member of the family Dendrobatide, and represents one of the most variable species of vertebrates (Daly & Myers, 1983). This small, diurnally active, and brightly colored (Saporito *et al*., 2012) frog is usually found below 500 meters of elevation on the Caribbean side of Costa Rica, Nicaragua, and Panama (Myers & Daly, 1983). In Panama, *O. pumilio* is extremely variable in color, ranging from orange, red, blue, yellow, to black and white (Daly & Myers, 1967; Summers *et al.*, 2004). *Oophaga pumilio* also exhibits extensive parental care for its young from both male and female frogs (Myers & Daly, 1983; Stynoski *et al.*, 2009). Males generally take care of eggs by keeping them hydrated, while females actively transport recently hatched tadpoles to a water source (e.g., axil of a bromeliad) and regularly visit the free-living tadpoles to provide them with unfertilized eggs as their sole food source (Myers & Daly, 1983). Populations of *O. pumilio* in Costa Rica and Panama contain more than 200 alkaloids, representing more than 21 chemical classes (Saporito *et al*., 2007). The high diversity of alkaloid defenses in this species is unusual. An individual frog can have an average of 45 different types of alkaloids (Saporito *et al.*, 2007), some of which have not been found in any other dendrobatid frog (Myers & Daly, 1983). Alkaloid defenses have been shown to vary with geographic location, time (e.g., between seasons), and between sexes (Clark *et al*., 2006; Saporito *et al*., 2006; Saporito *et al*., 2012). In all cases, this tremendous variation in chemical defense has been attributed to differences in availability of the alkaloid-containing arthropods that make-up this species' diet.

Habitat disturbance may negatively affect alkaloid defenses in poison frogs (Saporito *et al.*, 2012). Tropical deforestation rates continue to exceed the rate of forest regeneration

(Whitmore, 1998), and amphibian diversity is particularly sensitive to environmental changes (Pearman, 1998). Some invertebrate species are affected by forest fragmentation, and many are negatively affected (Didham *et al*., 1998). The tremendous variation in alkaloid composition of *O. pumilio* is directly linked to the availability of alkaloid-containing arthropods (Saporito *et al.*, 2007*;* Mebs *et al*., 2008; Saporito *et al.,* 2012), and therefore shifts in abundance of these arthropods could lead to changes in the defenses of frogs. A study examining beetle species responses to tropical forest fragmentation showed that species composition was more variable among edge sites than in undisturbed sites, and showed an increase in diversity in disturbed habitats (Didham *et al.*, 1998). Another study of forest disturbance in the Mbalmayo Forest Reserve showed that species richness was higher in the two areas that were slightly more disturbed than the undisturbed habitat (Eggleton *et al.*, 1995). The latter suggests that a small degree of disturbance can be beneficial to increase species diversity, but a high degree of disturbance will have adverse consequences. The impact of habitat disturbance in alkaloid defenses of poison frogs is not well understood.

Only two studies have examined the effect of frog alkaloid defenses between disturbed and undisturbed forests (Clark *et al*., 2006; Andrianamaharavo *et al.*, 2010). Both of these studies focused on populations of *Mantella baroni*, a poison frog in the family Mantellidae from Madagascar. Clark *et al.*, 2006 found that frogs from undisturbed habitats had a greater diversity and quantity of alkaloid defenses, whereas, Andrianamaharavo *et al.*, 2010 found that frogs from disturbed sites often had a greater number and quantity of alkaloids. The results from these two studies are conflicting, making it difficult to fully understand the effect of habitat disturbance on frog alkaloid defenses.

In the present study, alkaloid defenses in *O. pumilio* are compared between frogs from a undisturbed and disturbed habitat from Colon and Solarte island. The goals of the study are: 1) to examine the influence of habitat disturbance on the chemical defenses in *O. pumilio* and 2) to indirectly evaluate the effect of habitat disturbance on arthropod diversity, by examining differences in the dietary sources of alkaloids detected in *O. pumilio* between the two habitat types.

Methods

Frog Collections

Oophaga pumilio skin samples were collected from four sites in Panama in 2011 by Justin Yeager (graduate student) and Dr. Cori Richards-Zawacki from Tulane University (Figure 1; Figure 2). Ten males and ten females were obtained from an undisturbed and disturbed site on Solarte Island, Panama for a total of 20 frogs. In addition, ten males and ten females were obtained from an undisturbed and disturbed site on Colon Island, Panama. Frogs were captured by hand and sex was determined. All frogs were euthanized and *O. pumilio* skin samples were stored in individual vials containing 2 ml of 100% methanol (MeOH).

Chemical Analysis

From each site, three male and three female skin samples were selected for this study (using a statistical random number table) for a total of 24 samples. All alkaloid fractionations were performed following Saporito *et al*., 2010. Each vial contained approximately 2 ml of 100% MeOH. Before each extraction another 2ml of 100% MeOH was added for final MeOH volume of 4 ml. One milliliter of the 4 ml of the MeOH skin mixture was transferred to a conical vial. In each conical vial, 10 μl of a nicotine standard ((-) nicotine 99%, Sigma-Aldrich, Milwaukee, WI) was added. After the addition of nicotine to the solution, 50 µl of 1N HCl was added. The combined HCl, nicotine, and MeOH extract was then concentrated to 100 µl using N_2. The concentrated solution was diluted with 200 μl of dH_2O, and extracted four times with 300 μl of hexane. The HCl fraction was basified utilizing saturated $NaHCO_3$. This basic solution was extracted three times with 300 μl of ethyl acetate. Anhydrous Na_2SO_4 was used to dry the ethyl acetate fractions and then the volume was concentrated to 100 μl, with N_2.

All alkaloid fractions were analyzed using gas chromatography-mass spectrometry (GC-MS) on a Varian Saturn 2100T ion trap MS instrument coupled to a Varian 3900 GC with a 30m x .25 mm i.d. Varian Factor Four VF-5ms fused silica column. The procedure for the alkaloid separation and identification was based on the methods used by Grant *et al.*, 2012. Separation of alkaloids was achieved using a temperature program from 100 ºC to 280 ºC, at a rate of 10 ºC per minute. Helium was the carrier gas in this study at a flow rate of 1 ml/min. Electron impact (EI) MS and chemical ionization (CI) MS were used to analyze each alkaloid fraction. Methanol was used as the CI reagent gas.

In order to identify each alkaloid, a comparison based on mass spectral properties and GC retention times was performed using previously described alkaloid data in Daly *et al.*, 2005. In 1978, a coding system for frog alkaloids was implemented (Daly, 1998). The codes for the alkaloids consist of a boldface number for the molecular mass, and a bold face letter to differentiate between alkaloids with the same molecular mass (Daly, 1998). In order to determine the amount of each alkaloid, the peak area of each alkaloid was compared to the peak area of the nicotine internal standard, using a Varian MS Workstation v.6.9 SPI. Nicotine was chosen as an internal standard because it has a retention time outside the chromatographic range of most dendrobatid alkaloids.

Arthropod Analysis

To perform an indirect assessment of the arthropod composition for each site, chemical profiles were analyzed by assigning a type of arthropod to each of the alkaloid classes detected in *O. pumilio*. All arthropod assignments are based on the results of Saporito *et al*., 2012 and verbal communication from Dr. Ralph Saporito. A proportion of each arthropod type was calculated by dividing the total amount of alkaloids from each frog by the amount of alkaloids present in classes with a specific dietary source. Dietary sources for alkaloids include mites, ants, beetles, and millipedes.

Statistical Analysis

To determine whether the diversity and quantity of alkaloids differed among frogs from disturbed and undisturbed sites (Colon and Solarte Islands), independent sample t-tests were used. All data analyses were performed using SPSS v. 14.0 for Windows.

Results

Colon Island

A total of 381 alkaloids, representing 17 different structural classes of alkaloids were identified in *O. pumilio* skin extracts from Colon Island. The average number of alkaloids present in frogs from disturbed and undisturbed populations was 34 and 32, respectively. There was no difference in the number of alkaloids between sites ($T_{10} = 0.029$, $p = 0.977$) (Figure 3A). The average amount of alkaloids per frog from disturbed and undisturbed sites was 302 µg and 410 µg, respectively. There was no difference in the amount of alkaloids between disturbed and undisturbed sites (T_{10} = 0.793, p = 0.446) (Figure 3B)

Comparison of males and females. There was no difference in the number of alkaloids between females and males from the disturbed site (T_4 = 2.589, p = 0.060), however there was a difference in the amount of alkaloids between females and males (T_4 = 3.043, p = .038), in which females had more alkaloids than males. There was no difference in the number (T_4 = 0.937, p = 0.402) of alkaloids or amount of alkaloids (T_4 = 0.456, p = 0.672) between females and males from the undisturbed site.

Females from the disturbed site had an average of 25 alkaloids and 201 µg of alkaloid per frog. Females from the undisturbed site had an average of 36 alkaloids and 471 µg of alkaloid per frog (Table 1). There was no difference in the number of alkaloids (T_4 = 1.107, p= 0.330) between disturbed and undisturbed sites.

There was an average of 38 alkaloids identified in males from the disturbed site and an average of 28 alkaloids from the undisturbed site (Table 1). There was no difference in the number of alkaloids for males between the disturbed and undisturbed sites (T_4 = 2.062, p = 0.108). Male frogs from the disturbed site had an average of 403 µg of alkaloids per frog and male frogs from the undisturbed site had an average of 348 µg of alkaloid per frog. There was no difference in the amount of alkaloids between male frogs from the disturbed and undisturbed sites (T_4 = 0.413, p = 0.701).

Arthropod analysis. On the basis of the alkaloids present in frog skins, the diet of frogs from the undisturbed site was composed of mites, ants, and beetles, whereas the diet of frogs from disturbed sites was composed of only mites and ants. The proportion of mites in the diet of frogs from disturbed site was 0.84 and in the undisturbed site was 0.57. The proportion of ants in the diet of frogs from the disturbed site was 0.16 and in the undisturbed site was 0.39 (Table 3).

<u>Solarte Island</u>

A total of 23 alkaloids, representing 10 different structural classes of alkaloids were identified in *O. pumilio* skin extracts from Solarte Island. The average number of alkaloids present in frogs from disturbed and undisturbed populations was 10 and 13, respectively. There was no difference in the number of alkaloids between sites (T_{10} = 1.202, p = 0.257) (Figure 4A). The average amount of alkaloids per frog from disturbed and undisturbed sites was 221 µg and 164 µg, respectively. There was no difference in the amount of alkaloids between disturbed and undisturbed sites (T_{10} = 1.007, p = 0.338) (Figure 4B).

Comparison of males and females. There was a difference between the number of alkaloids between females and males from the disturbed site (T_4 = 3.024, p = 0.039), in which males had more alkaloids than females. However, there was no difference in the amount of alkaloids between females and males from the disturbed site (T_4 = 2.580, p = 0.061). There was no difference in the number of alkaloids (T_4 = 1.334, p = 0.253) or the amount of alkaloids (T_4 = 1.191, p = 0.300) between females and males from the undisturbed site.

Females from the disturbed site had an average of 9 alkaloids and 163 µg of alkaloids per frog. Females from the undisturbed site had an average of 17 alkaloids and 217 µg of alkaloid per frog (Table 2). There was no difference in the number of alkaloids (T_4 = 1.786, p = 0.149) and amount of alkaloids in females (T_4 = 0.695, p = 0.525).

There was an average of 11 alkaloids identified in males from the disturbed site and an average of 10 alkaloids from the undisturbed site (Table 2). There was no difference in the number of alkaloids for males between the disturbed and undisturbed sites (T_4 = 0.521, p =

0.630). Male frogs from the disturbed site had an average of 279 µg of alkaloid per frog and male frogs from the undisturbed site had an average of 111 µg of alkaloid per frog. There was no difference in the amount of alkaloids between male frogs from the disturbed and undisturbed sites ($T_4 = 2.662$, $p = 0.056$).

Arthropod Analysis. On the basis of the arthropod present in frog skins, the diet of frogs from the undisturbed site was composed of mites, antes, beetles, and millipedes, whereas the diet of frogs from the disturbed sites was composed of only mites, ants, and beetles. The proportion of mites in the diet of frogs from the disturbed site was 0.80 and in the undisturbed site was 0.43. The proportion of ants in the diet of frogs from the disturbed site was 0.21 and in the undisturbed site was 0.54. The proportion of beetles in the diet of frogs from the disturbed site was 0.40 and in the undisturbed site was 0.30 (Table 3).

<u>Colon Island and Solarte Island</u>

Comparison of frogs from the disturbed and undisturbed sites. There was a difference in the number of alkaloids between frogs from the disturbed sites from Colon and Solarte Islands ($T_{10} = 5.852$, $p \leq 0.001$), in which frogs from Colon Island had more alkaloids than frogs from Solarte Island. There was no difference in the amount of alkaloids between frogs from the disturbed sites ($T_{10} = 1.273$, $p = 0.232$). There was a difference in number of alkaloids between frogs from the undisturbed sites ($T_{10} = 3.448$, $p = 0.006$), in which frogs from Colon Island had more alkaloids than frogs from Solarte Island. There was no difference in the amount of alkaloids between frogs from the undisturbed sites ($T_{10} = 1.851$, $p = 0.094$).

Comparison of males and females. There was a difference in the number of alkaloids between female frogs from the disturbed sites ($T_4 = 4.975$, $p = 0.119$), in which frogs from Colon Island had more alkaloids than frogs from Solarte Island. There was no difference in the amount of alkaloids between female frogs from the disturbed sites ($T_4 = 1.106$, $p = 0.094$). There was no difference in the number of alkaloids between female frogs from the undisturbed sites ($T_4 = 1.981$, $p = 0.119$) or the amount of alkaloids ($T_4 = 0.999$, $p = 0.374$).

There was a difference in the number of alkaloids between male frogs from the disturbed sites ($T_4 = 7.094$, $p = 0.002$), in which frogs from Colon Island had more alkaloids than frogs from Solarte Island. There was no difference in the amount of alkaloids between male frogs from the disturbed site ($T_4 = 1.701$, $p = 0.164$). There was a difference in the number of alkaloids between male frogs from the undisturbed sites ($T_4 = 4.098$, $p = 0.015$), in which frogs from Colon Island had more alkaloids than frogs from Solarte Island. There was no difference in the amount of alkaloids between male frogs from the undisturbed sites ($T_4 = 1.832$, $p = 0.141$).

Discussion

The results of this study show that there is no difference in the number and types of alkaloids in *O. pumilio* between disturbed and undisturbed sites from Colon and Solarte Islands. Nevertheless, there is a trend suggesting that frogs from the undisturbed site from both islands have a greater number of alkaloids, supporting Clark *et al*., 2006 results which used *Mantella boroni* frogs from Madagascar. In contrast, results from Adrianamahavaro *et al*., 2010 suggested that frogs from the disturbed site would have a greater number and amount of alkaloids than frogs from the disturbed sites, which was not supported by the results of this study.

In general, there was a higher number and amount of alkaloids present in frogs from the undisturbed sites when compared to frogs from the disturbed site on Colon Island and Solarte Island (Fig. 3 & 4). Frogs that have higher amounts of alkaloids are potentially better protected from natural predators, than frogs with less amounts of alkaloids in their skin. Frogs from disturbed habitats could be prone to higher predation rates than frogs from the undisturbed habitat, because they are less chemically defended. Therefore, the decrease in amount of alkaloids in *O. pumilio* due to habitat disturbance could negatively affect the survivorship of this species. Further studies examining habitat disturbance in *O. pumilio* need to be conducted.

All frogs utilized in this study, six frogs per site, are part of a larger project that is currently studying the effects of habitat disturbance on *O. pumilio* chemical defense and carotenoid concentration. This larger sample size will potentially detect statistical significant differences in alkaloid defenses of frogs from disturbed and undisturbed habitats.

The extensive parental care that *O. pumilio* exhibits is unique and important for this organism. A study by Saporito *et al*., 2010 showed that there are sex-related differences in the chemical defenses in *O. pumilio*, in which females have a greater number of alkaloids than males. Therefore, it is important to examine the possibility that such differences may be affected by habitat disturbance. In the present study, there was a difference in the amount of alkaloids present in female and male frogs from the disturbed site from Colon Island, in which females had a higher amount of alkaloids than males. In addition, there was a difference in the number of alkaloids present in female and male frogs from the disturbed site from Solarte Island, in which females had a higher number of alkaloids than males. The differences among males and females were not always significant, however on average females had a higher number and amount of alkaloids than males. Studies by Donnelly (1991) and Saporito *et al*., 2010 suggest that the sex-related differences in alkaloid composition in *O. pumilio* are due to their behavior. Male *O. pumilio* are territorial and have small home ranges, and as a result may have less alkaloid-containing arthropods available to them when compared to females that have larger home ranges. In addition, Stynoski *et al*., unpublished demonstrated that female *O. pumilio* provide alkaloid defenses to their tadpoles. The latter suggest that females may need higher amounts of alkaloids than males, in order to provision alkaloids to their tadpoles. As a result, changes in alkaloid composition of female frogs due to habitat disturbance could have a great impact on the survivorship of *O. pumilio*. Future studies should further examine the potential impacts of habitat disturbance between sexes of *O. pumilio*, and the effects this may have on offspring survival.

The results of the present study are another example of the tremendous alkaloid variation present within and among frog populations. The number of alkaloids in frogs from the disturbed site from Colon Island ranged from 19 to 33 alkaloids per frog, whereas it ranged from 20 to 47 alkaloids per frog from the undisturbed site. The amount of alkaloids in frogs from the disturbed site from Colon Island ranged from 137 µg to 510 µg alkaloids per frog, whereas it ranged from 105 µg to 900 µg alkaloids per frogs from the undisturbed site. The number of alkaloids in frogs from the disturbed site from Solarte Island ranged from 9 to 13 alkaloids per frog, whereas it ranged from 7 to 26 alkaloids per frog from the undisturbed site. The amount of alkaloids in frogs from the disturbed site from Colon Island ranged from 156 µg alkaloids per frog to 347 µg of alkaloids per frog, whereas it ranged from 49 µg to 324 µg alkaloids per frog in the undisturbed site. The data from each site also reflects the variability between frogs from different sites. The variability between frogs within the same population and among populations is likely due to seasonal, geographical, and dietary differences (Clark *et al*., 2006; Saporito *et al*., 2006; Saporito *et al*., 2012).

There was a difference in the number of alkaloids in frogs from the disturbed and undisturbed sites from Colon and Solarte Islands, in which frogs from Colon Island had a higher number of alkaloids than frogs from Solarte Island. Although, there are no differences in the amount of alkaloids in frogs between disturbed and undisturbed sites from Colon and Solarte Islands, frogs from Colon Island had a higher amount of alkaloids than frogs from Solarte Island. A study by Maan & Cummings (2012) suggested that the bright coloration of *O. pumilio* was a signal of toxicity, in which brighter colored frogs are more toxic. The results of Maan & Cummings (2012) suggested that frogs from Solarte Island were significantly more toxic than frogs from Colon Island, which is contrary to the results of this study. The assessment of the frog's toxicity in Maan & Cummings (2012) was not based on the chemical analysis of the frog's skin; rather it was based on the analysis of their brightness and the response of the toxins on sleeping mice. On the basis of number of quantity of alkaloids, the results of the present study suggest that frogs from Colon Island are likely more toxic than frogs from Solarte Island, despite being less brightly colored (Figure 2). Frogs from the undisturbed site from Colon Island had an average of 31 alkaloids per frog, whereas frogs from Solarte Island had an average of 14 alkaloids per frog. Frogs from the undisturbed site from Colon Island had an average of 410 µg of alkaloids per frog, whereas frogs from Solarte Island had an average of 164 µg of alkaloids per frog. The differences in number and amount of alkaloids among sites is substantial, and suggests that further studies analyzing the chemical composition and relationship between morphology and toxicity among populations should be performed.

As previously stated, alkaloid variability within frogs and among populations is influenced to some degree by diet. Therefore, in this study an indirect assessment of the frog's diet was performed based on their alkaloid profiles. The indirect assessment of the frog's diet would potentially illustrate changes in the alkaloid-containing arthropod composition due to habitat disturbance. On Colon Island, the alkaloids in frogs from both undisturbed and disturbed sites were primarily derived from mites and ants. The amount of mite derived alkaloids increased in frogs from the disturbed site, while the amount of ant derived alkaloids decreased. There were also no alkaloids derived from beetles in frogs from the undisturbed site, while there were a small proportion of such alkaloids in frogs from the disturbed site. On Solarte Island the amount of alkaloids derived from mites increased in frogs from the disturbed sites, while the amount of ant derived alkaloids decreased. The amount of beetle derived alkaloids was not affected by habitat disturbance in frogs from Colon Island. The changes in the composition of alkaloids derived from ants and mites may or may not affect *O. pumilio*'s chemical defense. In order to determine if the increase in mite derived alkaloids and a decrease in ant derived alkaloids causes a change in their chemical defense, predation trials to determine whether a predator rejects or prefers frogs from disturbed or undisturbed habitat should be performed. The latter study could provide information in order to determine if the differences found among sites are biologically important. Lastly, changes in the composition of the arthropod derived alkaloids do not imply that alkaloid-containing arthropods and non-alkaloid-containing arthropods are affected in the same way by habitat disturbance. Studies examining the arthropod fauna in disturbed and undisturbed habitats should be performed, since it will provide additional information on how habitat disturbance will affect other organisms that rely on them.

Summary

The results of this study suggest that even though there was no statistical difference between disturbed and undisturbed sites, undisturbed sites had a higher number and amount of alkaloids; supporting this Clark *et al*., 2006 study. Frogs with higher amount of alkaloids are potentially better chemically defended than frogs with less alkaloids, therefore habitat disturbance may negatively affect *O. pumilio* by increasing predation rates in frogs from disturbed sites. The number and amount of alkaloids from Solarte Island was unexpected, since Maan & Cummings (2012) suggested that Solarte Island was significantly more toxic than frogs from Colon Island. Herein, I found that frogs from Colon Island, regardless of their habitat, had a higher number and amount of alkaloids than frogs from Solarte Island. Lastly, the results from the arthropod analysis revealed that the number of mite derived alkaloids increased, whereas the number of ant derived alkaloids decreased in frogs from the disturbed sites. Predation trials assessing if predators have a preference between frogs from a disturbed or undisturbed site are needed in order to determine the biological relevance of the results of this study. In addition, this study examined habitat disturbance on *O. pumilio*, whereas the Clark *et al*., 2006 and Adrianamahavaro *et al*., 2010 studied *M. boroni*. Further studies should be done to determine if both species are affected in a similar way. Since the results from this study supports Clark *et al*., 2006 it may be an indication that both species might be affected similarly; however, further studies need to be done to determine if this is true.

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Figures and Tables

Figure 1. Map of Bocas del Toro Archipelago, Panama. Research sites were located on Isla Colon and Isla Solarte.

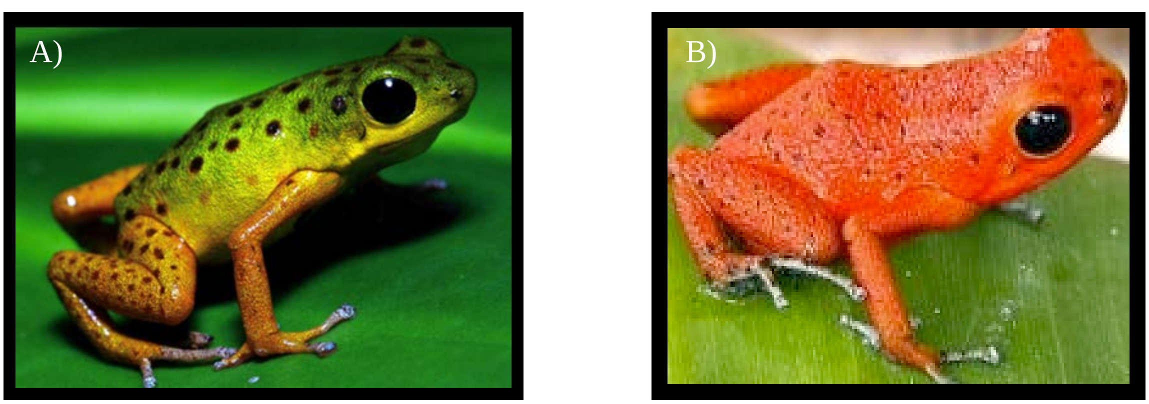
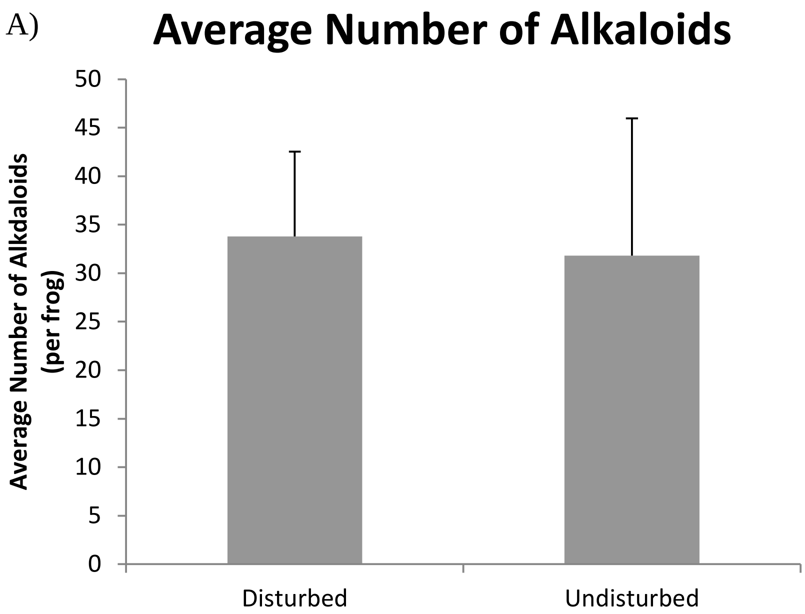


Figure 2. *Oophaga pumilio* from (A) Colon Island and (B) Solarte Island.

A)



B)

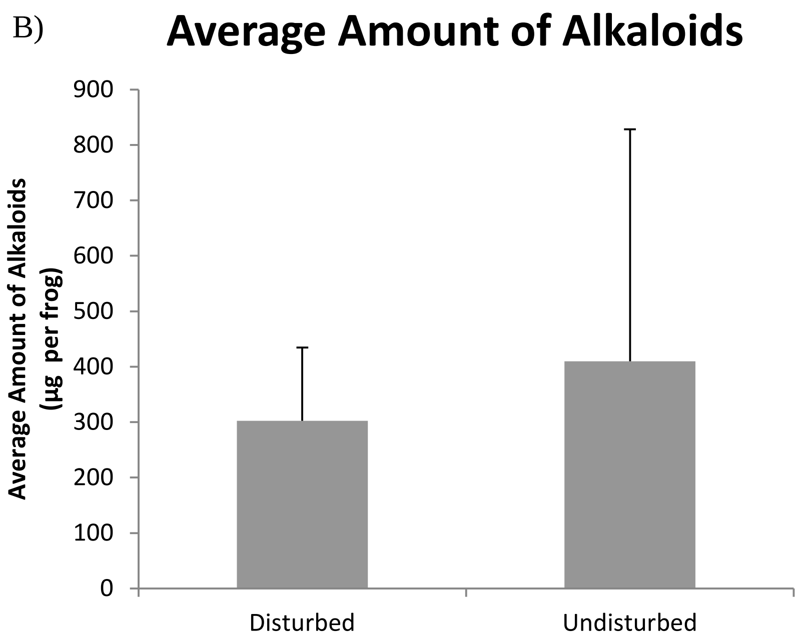
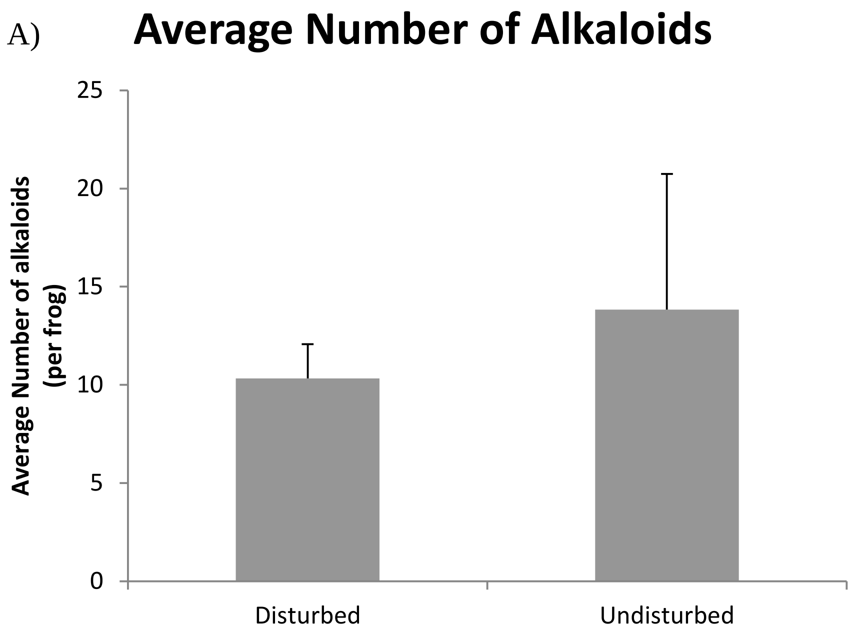


Figure 3. *Oophaga pumilio* (A) average number of alkaloids (+/- 1 S.E.) and (B) average amount of alkaloids (+/- 1 S.E.) from Colon Island.

A)



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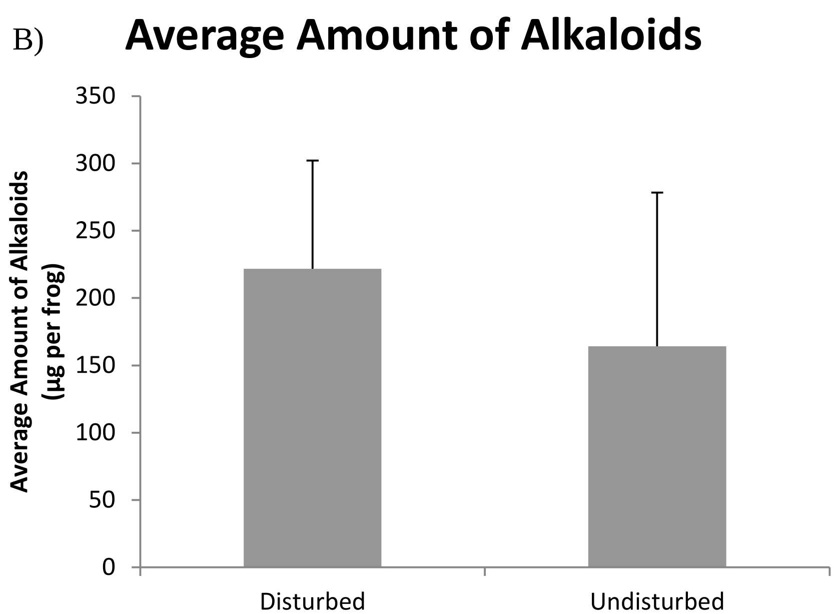

Figure 4. *Oophaga pumilio* (A) average number of alkaloids (+/- 1 S.E.) and (B) average amount of alkaloids (+/- 1 S.E.) from Solarte Island.

Table 1. The average number and amount of alkaloids for frogs on Isla Colon.

Colon Island

	Frogs	Number of Alkaloids	Amount of Alkaloids
Disturbed	**All**	34	302 µg
	Females	25	201 µg
	Males	38	403 µg
Undisturbed	**All**	31	410 µg
	Females	36	471 µg
	Males	27	347 µg

Table 2. The average number and amount of alkaloids, for frogs on Isla Solarte.

Solarte Island

	Frogs	Number of Alkaloids	Amount of Alkaloids
Disturbed	**All**	10	221 µg
	Females	9	163 µg
	Males	11	279 µg
Undisturbed	**All**	13	164 µg
	Females	17	217 µg
	Males	10	111 µg

Table 3. Proportion of alkaloids derived from a specific dietary source from frogs on Colon Island and Solarte Island.

Colon Island

	Frog	Sex	Mites	Ants	Millipedes	Beetles	Unknown
Undisturbed	1	F	0.23	0.61	0.00	0.09	0.02
	2	F	0.86	0.12	0.00	0.00	0.03
	3	F	0.69	0.30	0.00	0.01	0.01
	4	M	0.34	0.67	0.00	0.00	0.00
	5	M	0.66	0.34	0.00	0.00	0.02
	6	M	0.64	0.34	0.00	0.00	0.02
Total			**0.57**	**0.39**	**0.00**	**0.02**	**0.02**
Disturbed	1	F	0.97	0.04	0.00	0.00	0.00
	2	F	0.62	0.38	0.00	0.00	0.00
	3	F	0.98	0.02	0.00	0.00	0.00
	4	M	0.89	0.11	0.00	0.00	0.00
	5	M	0.85	0.15	0.00	0.00	0.00
	6	M	0.73	0.26	0.00	0.00	0.00
Total			**0.84**	**0.16**	**0.00**	**0.00**	**0.00**

Solarte Island

	Frog	Sex	Mites	Ants	Millipedes	Beetles	Unknown
Undisturbed	1	F	0.88	0.10	0.01	0.01	0.00
	2	F	0.07	0.93	0.00	0.00	0.00
	3	F	0.60	0.38	0.01	0.01	0.00
	4	M	0.27	0.69	0.00	0.04	0.00
	5	M	0.09	0.91	0.00	0.00	0.00
	6	M	0.65	0.20	0.00	0.14	0.00
Total			**0.43**	**0.54**	**0.00**	**0.03**	**0.00**
Disturbed	1	F	0.51	0.47	0.00	0.02	0.00
	2	F	0.71	0.23	0.00	0.06	0.00
	3	F	0.85	0.00	0.00	0.16	0.00
	4	M	0.82	0.17	0.00	0.01	0.00
	5	M	0.92	0.08	0.00	0.00	0.00
	6	M	1.00	0.31	0.00	0.00	0.00
Total			**0.80**	**0.21**	**0.00**	**0.04**	**0.00**