

Final Report

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The effect of perch height and colour intensity on escape behaviour in *Oophaga pumilio*



Photo: J. Snijders

Jeroen Snijders
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Preface

This study on the effect of perch height and colour intensity on escape behaviour in *O. pumilio* has been conducted as part of an internship at Caño Palma Biological Station. Special thanks goes out to the staff and volunteers of the station, without whom this project would not have been possible. Also I want to thank HAS University Den Bosch for providing me with the opportunity for conducting this internship.

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Abstract

Previous studies show that dorsal colour intensity in *O. pumilio* is linked to toxicity, with brighter dorsal colours indicating higher toxicity. In this study the effect of perch height and colour intensity on escape behaviour in both perching male and female *O. pumilio* was studied at Caño Palma Biological Station, located within the Barra Del Colorado Wildlife Refuge. Simulated predator approaches were used to measure the Flight Initiation Distance (FID) on a total of 125 frogs. Also measurements of perch height and colour intensity (Red Green and Blue) were taken. The results show no significant association between colour intensity and FID and flight attempt. A negative and significant association between perch height and FID was found. Also the association between perch height and flight attempt was negative and significant. Thus, frogs on a higher perch were less likely to attempt escape than frogs on a lower perch. Our results combined with previous studies show that colour intensity is not always a reliable indicator of escape behaviour for both male and female *O. pumilio*. One possible explanation is that aposematic colouration is not just the product of natural selection, but is also influenced by directional intersexual selection and intrasexual selection. It is also possible that the correlation between colour intensity and escape behaviour differs between populations. Every population lives in a different environment with different factors influencing their (escape) behaviour. Perch height seems to be a reliable indicator of escape behaviour. Returning to a higher perch costs more time and energy, which may be why the individuals that occupy higher perches allow a predator to come closer before they attempt escape.

1. Introduction

Escape is a crucial element of a prey's behavioural repertoire (Cooper, 2006). Various species of prey depend on various types of escape behaviour. For example, research on several lizard species shows that some species may run to an established refuge, while others run up a tree using different flight patterns (Cooper, 2003). However, there are also species of prey that use the color and shape of their bodies, to prevent them from having to escape. For example, young Thomson's gazelles rely on their cryptic colouration and minimal activity to avoid detection (Walther, 1968). Because these prey rely on their ability to blend in with the surroundings, they are less likely to flee because this may give away their position to unaware predators (Ydenberg & Dill, 1986).

Other species of prey use the exact opposite strategy to prevent them from having to flee. These species use conspicuous warning signals to indicate their toxicity to predators, a strategy known as aposematic signalling (Siddiqi et al., 2004). Poison dart frogs are an example of prey species that employ this strategy, using a great diversity of colours to warn their predators they are toxic (Saporito et al., 2007).

The strawberry poison dart frog (*Oophaga pumilio*) is known for its extreme variation in colour and pattern over different populations (Summers et al., 2004). In Bocas del Toro, Panama, alone there are over 20 different colour morphs to be found (Figure 1, Lawrence, n.d.). *O. pumilio* is found in low-elevation forests on the Atlantic coast of Nicaragua, Costa Rica and Panama (Graves et al., 2005). Previous studies show that dorsal colour intensity is linked to toxicity (Maan & Cummings, 2012; Summers & Clough, 2001), with brighter dorsal colours indicating higher toxicity. It is strongly suggested that birds



Figure 1. A number of different colour morphs of *Oophaga pumilio* in Bocas del Toro, Panama. Photo credit: J. P. Lawrence Photography.

are the main predators of *O. pumilio* (Dreher et al., 2015). Spectrometric measurements of body colouration in the Dreher et al. (2015) study concluded, together with results from earlier studies, that conspicuousness honestly indicates toxicity to avian predators (Maan & Cummings, 2012). This supports the claim that the colouration of *O. pumilio* is linked to toxicity.

According to the optimal escape theory, a prey should analyse the costs of staying and fleeing (Ydenberg & Dill, 1986). When the costs of staying are higher than those of fleeing, the prey should start its escape. Considering colouration is an honest indicator of toxicity, it is expected that the more conspicuous frogs will be bolder and less likely to flee from predators. However, previous study shows mixed results supporting and not supporting these expectations (Dugas et al., 2015). This suggests that there are other possible explanations, besides colour intensity, for the differences in escape behaviour.

One other possible explanation is perch location. Female *O. pumilio* prefer males that exhibit high rates of vocalization and occupy elevated perches (Graves et al., 2005). As in many frog species, territoriality of *O. pumilio* males is focused on the defense of calling sites for mate attraction and areas involved in courtship and mating, rather than the defense of feeding areas (Pröhl & Hödl, 1999). To defend a perch males use advertisement calls to deter other male competitors (Pröhl, 2005). They also may use close-range aggressive or encounter calls in interactions with competitors. This is a form of

intrasexual competition. This suggests that stronger and fitter males occupy higher quality elevated calling perches. Because these males are fitter it is expected that these frogs are less likely to flee than frogs occupying lower quality calling perches. This pattern has been observed in recent studies, where males on higher perches were less likely to attempt escape (Pröhl & Ostrowski, 2011; Dugas et al., 2015).

Another study shows that female *O. pumilio* also exhibit territorial behaviour, mainly to defend their feeding grounds (Meuche et al., 2011). However, previous studies on the relationship between perch height and escape behaviour did not focus on female behaviour (Dugas et al., 2015; Pröhl & Ostrowski, 2011). As such, we will look at the effects of sex on two previously investigated topics: the correlation between colour intensity and perch height related to escape behaviour in *O. pumilio* for the population near Caño Palma Biological Station, located in Barra del Colorado National Wildlife Refuge, Costa Rica. This will show whether *O. pumilio* occupying higher perches are less likely to flee than the individuals occupying lower perches, whether more conspicuously coloured individuals will exhibit bolder escape behaviour, and the effects that sex may have on either of these two relationships.

2. Materials & Methods

2.1. Study site and species

The study was conducted around the Caño Palma Biological Station (N 10°35'36.1"; W 83°31'39.4"), which is located within the Barra Del Colorado Wildlife Refuge (Figure 2). The research area is an ancient flood plain covered by lowland Atlantic tropical rain forest (COTERC, n.d.). The average daily temperature is about 26 °C and rainfall may exceed 6,000 mm per year. The tropical wet forest around the station houses a large population of *O. pumilio* (Belderok, 2015; Cappello & Dekker, 2015). The area used for this study was based on existing transects around the station (red area figure 2). These existing transects are approximately 1,5km long in the north, south and west direction of the Caño Palma Biological Station. On the east side of the station is the canal Caño Palma and the Caribbean Sea.

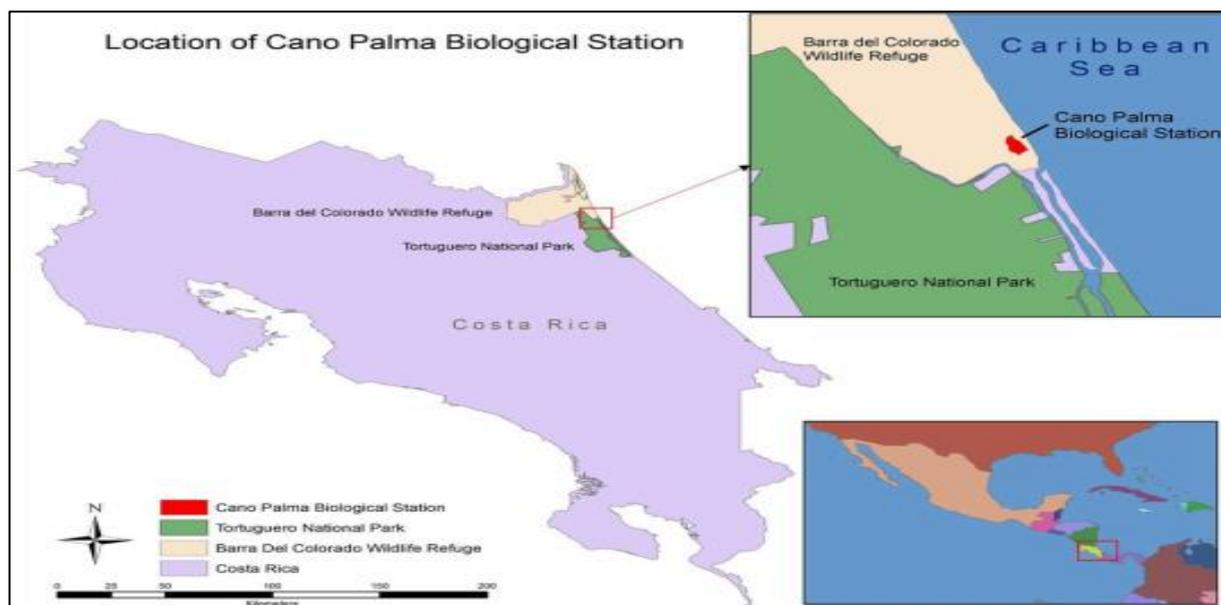


Figure 2. Location of the research area. This study will be conducted close to the Caño Palma Biological Station, located within the Barra del Colorado Wildlife Refuge. Figure derived from: COTERC, n.d..

The target species of this study was the Strawberry poison dart frog, *Oophaga pumilio*. Within these species there is a great phenotypical variety between populations. However, during this study only one population was studied. The *O. pumilio* of this population are red and usually have small dark spots on their back (*Photo 1*). Most of them also have blue hind-legs, which is why they are also called the “blue jeans frogs”.

2.2. Data collection

Data was collected from February 2016 until April 2016 for two or three days per week between 5 and 12 AM, when *O. pumilio* activity is highest (Graves, 1999; Cappello & Dekker, 2015). Within the research area *O. pumilio* were located and their escape behaviour, perch height and colour intensity were observed. Frogs were searched for by slowly and systematically walking through the forest while listening and looking for them. The goal was to find at least 100 individuals in order to conduct reliable statistical analysis. When an individual was located it was approached slowly to a position approximately 3 m away (Dugas et al., 2015). Next, the frog was approached at a normal walking pace, which was kept constant between different frogs. At the moment the frog left its perch a marker was dropped to the ground, at the position of the observer at that time. After that, the frog was caught. Whenever handling the frogs, biodegradable gloves were worn. New gloves were used for every individual frog, to prevent the transfer of any diseases within the population.

Once the frog was caught flight initiation distance, perch height and sex were determined (*Attachment 1*). The sex was determined by the absence or presence of a darkened gular region that indicates the loose skin of the male vocal sac (Donnelly, 1989). The distance between the dropped marker and the perch, the flight initiation distance (FID), was measured. Next, the perch height was measured, from the position where the frog was sitting all the way to the ground. Then the frog was photographed against a 18% grey card colour standard using a Nikon D3100 DSLR camera. Four high definition (14.2 megapixel) photographs were taken of every frog: one of the dorsum (without flash), one of the ventral side and one of both lateral sides. With these photos colour intensity was measured and recaptures were identified. This non-invasive method of recapture identification has been proven successful in a previous study on *Dendrobates auratus*, a species closely related to *O. pumilio* (Cove & Spínola, 2013). Finally, the frog was released on the perch where it was found.



Photo 1. Dorsal side of “blue jeans” O. pumilio individual found at Cano Palma Biological Station. Photo by: J. Snijders

2.3. Photo analysis

To obtain accurate colour intensity, the white balance of the dorsal photographs was corrected using the program View NX2 and the 18% grey scale card. Once the photos had the correct white balance, analyses were made using the computer software ImageJ 1.49. First, the colour intensity of the frog was measured using the “RGB Measure” plug-in tool. RGB (Red Green Blue) measurements were made of five different 20x20 pixel areas on the frog’s dorsum (*Photo 2*). RGB measurements of the Grey Scale card in every picture were also made. These measurements give the red, green and blue intensity of the selected area. In a previous comparable study this number and size of RGB- spots were considered a reliable reflection of the overall colour intensity of the frog (Dugas et al., 2015). Areas with dirt, glare, shadow or dark markings have been avoided for the RGB measurements.

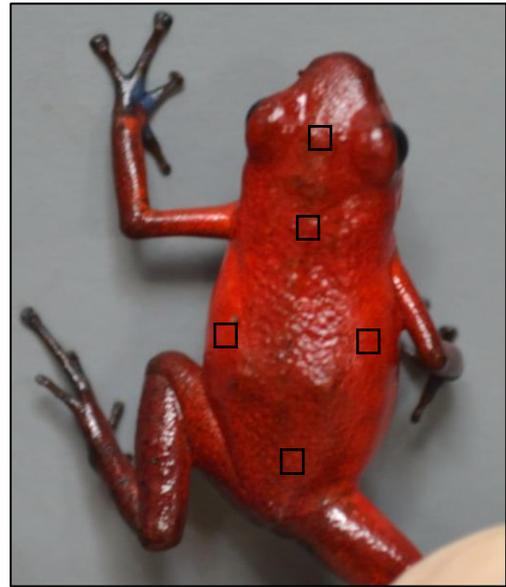


Photo 2. RGB-Measurement spots. The five different areas on the frogs dorsum which are used for the RGB-measurements. 20x20 pixel areas are selected and analyzed with the “RGB Measure” plug-in tool in ImageJ software. Photo: J. Snijders

2.4. Data analysis

Statistical analyses were done to examine whether the results are significant or not, using IBM SPSS v.20. Different models were created in this process, every time the best fitting model was used. In all analysis the confidence interval was 95%.

Because the frogs were photographed in non-standard conditions in the rainforest, the mean R, G and B colour scores were corrected. This was done by taking residuals of the mean R, G or B colour scores of the frog, regressed on R, G or B scores of the grey scale card (Louiseau et al., 2008). Next a Principal Component Analysis (PCA) was conducted to reduce the number of colour parameters in further analysis. This way colour could be treated as a continuous variable.

The association between colour intensity and FID was analysed by a Generalized Linear Model (GZLM). In order to use this model the variable FID had to be transformed into categorical data, creating the variable FID_category. A total of 115 categories were created, to minimize the loss of data. A GZLM with a negative binomial distribution and log link function was used. The dependent variable in this model was FID_category, explained by the two covariates PC1 and Perch Height. Next a binary logistic regression was used to analyse the association between PC1 and Flight Attempt.

The association between Perch Height and escape behaviour was also analysed. To predict the association between FID_category and Perch Height a different GZLM was created. In this model only Perch Height was a covariate. For the association between Perch Height and Flight Attempt, a logistic binary regression was used.

For the association between Perch Height and PC1, the variable Perch Height has been transformed. A square root transformation was used to make this variable normally distributed. This transformed variable was used only when Perch Height was the dependent variable, in order to be able to conduct parametric tests. Next, a linear regression was used to predict the association between Perch Height and PC1.

3. Results

A total of 125 *O. pumilio* have been successfully caught and processed in this study, 12 of which were recaptures. These recaptures have been excluded from the analysis unless mentioned otherwise.

3.1. Colour intensity and escape behaviour

The significantly justified PCA ($p=0.000$) on residual RGB scores resulted in the extraction of one principal component, PC1 (Figure 3). This component explains 65.3% of the variance in corrected RGB colour measurements (Attachment 2: table 1). Only components with an Eigenvalue greater than 1 were extracted. The PC1 shows that when green values are high, also red and blue values will be high (Attachment 2: table 2).

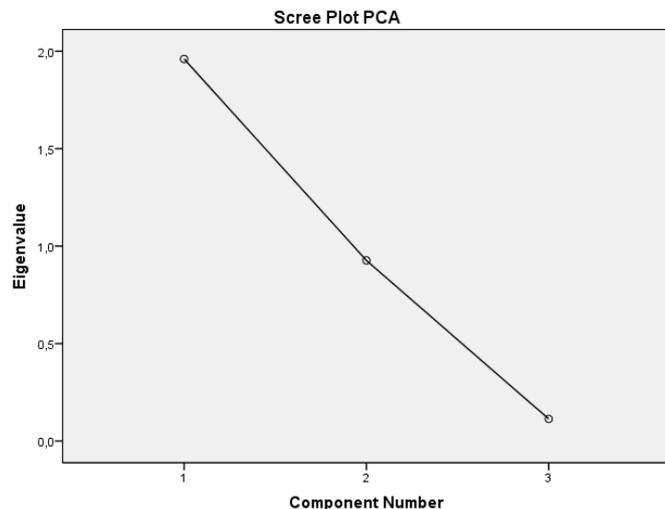


Figure 3. Scree plot of the PCA on residual RGB scores. Only components with a Eigenvalue greater than 1 have been extracted. This graph has been created in SPSS v.20

The association between PC1 and FID was found to be positive, but non-significant ($B=0.020$, $p=0.840$) (Attachment 2: table 3). The GZLM that predicted this association was a significantly reliable model ($p=0.026$). The association between PC1 and FID was found non-significant in every model created in the analysis. Out of the 113 frogs included in the analysis, 75 attempted escape and 38 did not ($FID = 0$). The output of the binary logistic regression shows with a 66.4% accuracy that PC1 is not significantly associated with flight attempt ($p=0.333$).

3.2. Perch height and escape behaviour

A square root transformation was performed, to turn the variable Perch Height into a normally distributed variable (Figure 4). Perch height was found significantly and negatively associated with FID ($p=0.007$, $B= -0.010$, Attachment 3: table 1). This negative association is shown in the linear regression line fitted in a graph that shows the association between FID and Perch Height (Figure 5). Also, a binary logistic regression shows with a 69.9% accuracy that Flight Attempt is significantly and negatively associated with Perch Height ($B= -0.022$, $p=0.002$, Attachment 3: table 2&3). Thus, frogs on a higher perch were less likely to attempt escape than frogs on a lower perch.

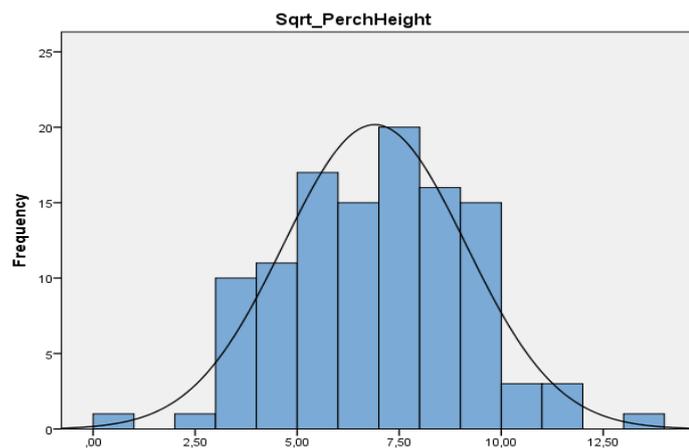


Figure 4. Histogram of the transformed variable Perch Height with the normal curve included. A square root transformation was used on Perch Height. The graph has been created in SPSS v.20

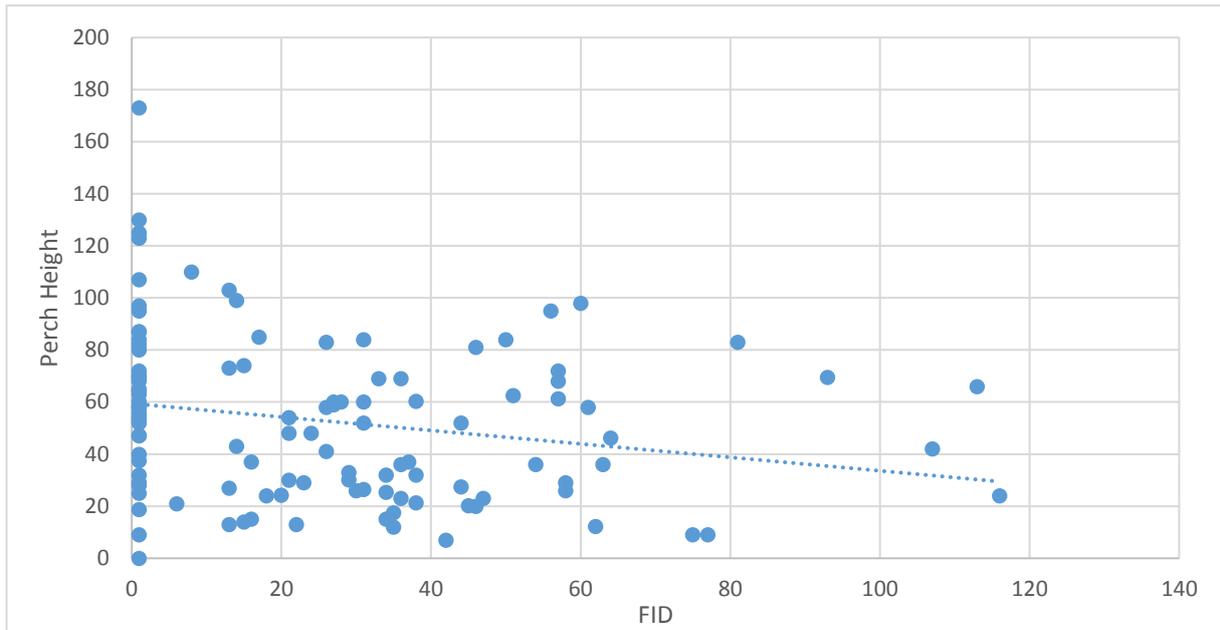


Figure 5. FID and Perch Height of all the frogs combined. The data of in total 113 frogs are presented in this graph. A linear regression line is fitted in the graph.

The overall average perch height was 52.6 cm (Std. dev.= 31.2). Males had a lower average perch height (48.6 cm, Std. Dev.=33.0, n=61) than females (57.3 cm, Std. Dev.=28.5, n=52), however this difference was found to be non-significant ($p=0.071$). Also the correlation between sex and FID was not significant ($p=0.934$), however the model that predicted this correlation was also non-significant ($p=0.062$).

The linear regression shows a low positive correlation between Perch Height and PC1 of 0.158 (Figure 6) (Attachment 3: table 4). However, the scatterplot shows that there is no clear correlation between PC1 and Perch Height. PC1 accounts for 1.6% of the variance in Perch Height (Attachment 3: table 5). Therefore, the correlation between PC1 and Perch Height was non-significant ($p=0.095$).

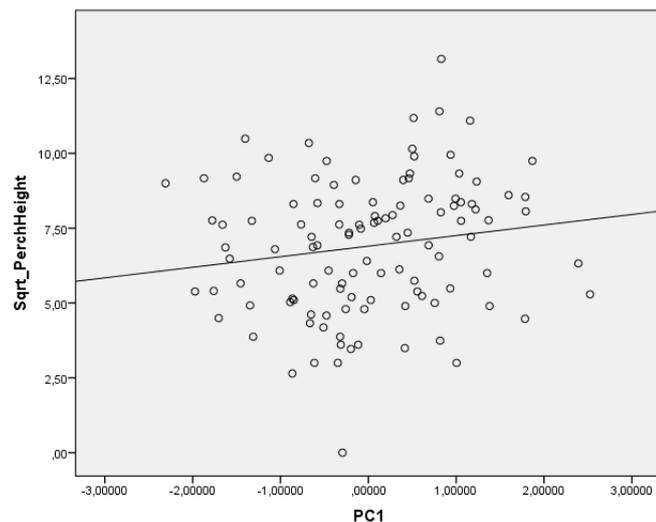


Figure 6. Scatterplot of PC1 and Sqrt_Perch Height fitted with a regression line. This Graph has been created in SPSS v.20

In the transect there is a wooden boardwalk of about 500 m in length that is elevated from the ground varying between 50-85 cm. A total of 29 frogs were found on this boardwalk, 27 of which were female and only two of which were male.

3.3. Recaptures

A total of eight frogs were caught more than once. The observations in Perch Height and FID were not the same with each capture for individuals (Attachment 4: table 1). In particular, frogs that were only caught two times varied a lot in their results. The individual with the maximum number of recaptures, five, showed a minimum FID of 0 cm, and a maximum FID of 98 cm, and perch height varied

between 69 and 75 cm. One individual with three recaptures showed more variation in FID: 30, 47 and 69 cm, correlating to Perch Heights of 62, 86 and 84 cm, respectively. In four cases the FID decreased with subsequent recapture, while only one instance of FID increasing with subsequent recaptures were recorded. Only one individual showed FID of 0 in all instances of recapture.

4. Discussion

The results show a negative and significant association between Perch Height and FID ($p=0.007$). Thus, frogs that occupied a higher perch allowed a closer simulated predator approach than frogs that occupied a lower perch. It also indicates that frogs on higher perches were less likely to attempt an escape. Similar results have been found for the males of an *O. pumilio* population on the island of Bastimentos, Panama (Dugas et al., 2015). Previous studies only focused on males, but our results indicate that this is true for both sexes. This suggests that frogs of both sexes that occupy higher elevated perches place more trust in their defence mechanism, based on their toxicity, which is linked to their colour intensity (Maan & Cummings, 2012; Summers & Clough, 2001). Therefore, it was expected that frogs with higher colour intensity would allow a closer predator approach than frogs with lower colour intensity. While our results show a slightly positive association between PC1 and FID, this correlation was found to be non-significant ($p=0.840$), and thus not sufficient to prove association. Additionally, PC1 was not significantly associated with flight attempt ($p=0.333$).

One possible explanation for the non-significant association between PC1 and Perch Height is that colour intensity is simply not a reliable indicator of escape behaviour of *O. pumilio*. As mentioned before previous studies found mixing results supporting and not supporting this explanation (Dugas, et al., 2015; Breed, 2008). Therefore, there have to be different and additional explanations for the differences in escape behaviour.

Variation in dorsal brightness across *O. pumilio* populations are detectable and informative (in terms of toxicity levels) to their main predators, birds (Maan & Cummings, 2012). This suggests that these predators influence the colour variation between populations by conscious prey selection based on colouration. However, it is likely that these birds do not detect the variation in colour intensity within populations (Crothers & Cummings, 2013). This has been observed in one of the brightest *O. pumilio* populations there is, located in Isla Solarte, Panama. Thus, it is likely that in places where there is only one distinct population of *O. pumilio* birds do not necessarily predate based on colour intensity. Therefore, individuals with higher colour intensity might not have a better defense and thus might not be bolder. This suggests that selection on colour intensity by predators might not be the only explanation of the diversity and intensity in colours in *O. pumilio*.

Sexual dimorphism in brightness within a population has been observed (Maan & Cummings 2009). In the previously mentioned *O. pumilio* populations in Isla Solarte and Isla Bastimentos females preferred brighter males during mate selection. This suggests that aposematic colouration is not just the product of natural selection, but is also influenced by directional intersexual selection (Maan & Cummings, 2008, 2009; Summers et al., 1999). Further, colour intensity influences male on male interactions within *O. pumilio* (Crothers et al., 2010). This may lead to intrasexual selection based on colour intensity as well.

Another possible explanation is that the RGB measurements in this study were not accurate. This can have a number of reasons, such as picture quality, lighting and white balance. Because of the changing and challenging circumstances for photography in the jungle, not all the pictures were of the same desired quality. A selection has been made to exclude pictures that were not up to standard. Also, the RGB measurements have been corrected with the help of a 18% grey scale card. However, it is possible that small defects or errors in the measurements in the pictures have been missed.

Furthermore, it is possible that the correlation between colour intensity and escape behaviour differs between populations. For example, a population of small and less conspicuous *O. pumilio* on Isla Popa, Panama, showed the same amount of aggressive exploration behaviour as frogs from different populations with higher colour intensity (Breed, 2008). It is possible that conspicuous frogs below a certain body size are perceived by predators as not worth eating. Thus, a population of small individuals could experience less predatory pressure, in turn making them bolder in their escape behaviour. Additionally, every area features varying abundance and diversity of predators, and higher predation pressure can result in a longer FID within the same species (Diego-Rasilla, 2002). However, more research is needed to support the theory that there are measurable differences in escape behaviour between different populations.

Special consideration should be paid to that fact that every frog used in this study was perching. Perches are valuable for both male as female *O. pumilio* (Meuche et al., 2011). A male's mating success is strongly associated with perch height (Pröhl & Hödl, 1999), while females mainly use territorial behavior to defend their feeding grounds (Meuche et al., 2011). However, manning these perches comes at a price: *O. pumilio* are slow and they escape their predators by jumping down in the leaf litter (Dugas et al., 2015). Returning to a higher perch costs more time and energy, which may be why the individuals that occupy higher perches allow a predator to come closer before they attempt escape.

Furthermore, the results of the recaptures show that FID and Perch Height alters between different captures. One possible explanation is that the frog may have acquired a better or worse territory in the time between captures. Male *O. pumilio* will ferociously defend a secured territory and fight other males for a better one (Baugh & Forester, 1994; Gardener & Graves, 2005). More aggressive males win more battles and thereby have a greater chance of securing higher quality perches (Breed, 2008). Also females are aggressive towards other females to defend their territory (Meuche et al., 2011). Secondly, the frog may still have been climbing up a perch when it was found. All individuals sampled were perching and not actively moving, but not all of them were calling. It is possible that they stopped moving once they spotted the observer. This may have been mistaken with actual perching behaviour.

It should also be mentioned that of all the frogs found on the boardwalk, only 7% were male. The boardwalk height is between 50 and 85cm, higher than the average Perch Height of males within this study (48.6 cm). Considering that perch height is an important factor in perch quality (Pröhl & Hödl, 1999; Graves et al., 2005), it was expected that males might also use this boardwalk to perch on. One possible explanation for the males not using the boardwalk as a perch is that it is too exposed. When males are perching they are usually calling to defend their territory or to attract females (Crothers et al., 2013; Pröhl, 2003). However, calling is risky behaviour as it is easier for acoustic predators to spot them (Ryan et al., 1981; Jaeger, 1976; Tuttle & Ryan, 1981; Tuttle et al., 1980). Therefore, open habitat, such as the boardwalk, may be too risky to use as a calling perch. However, further study is needed to fully understand the relationship between perch cover and perch quality.

5. Conclusion

This study shows that there is a significant association between perch height and escape behaviour in *O. pumilio*, for both sexes, whereas before this was only investigated for males. Frogs that occupy higher perches allow a closer simulated predator approach and are less likely to flee at all. We predicted that this could be explained by colour intensity, because of a previously demonstrated link to toxicity (Maan & Cummings, 2012; Summers & Clough, 2001). However, the results of this study do not support that explanation. In previous studies this association has had mixed support as well (Dugas et al., 2015).

One likely explanation is that frogs that occupy higher perches have invested more time and energy in getting up the perch, and may be less likely to waste that investment by jumping down to escape from a predator. Another important possible explanation for this is that the differences in colour intensity in *O. pumilio* may also be a product of sexual selection instead of only natural selection. It is strongly advised that more research will be done in the amount of colour variety accounted for by sexual selection in *O. pumilio*.

Given the high variety in colouration between populations, escape behaviour will likely differ between populations as well. However, it is also possible that the accuracy of the RGB measurements have played a role in this study. Therefore, we suggest that in future studies pictures should be taken under standard conditions, as has been previous done by Loiseau et al. (2008). This ensures a uniform illumination on the entire frog and it minimizes glare. Finally, no conclusive reason has been found for the large amount of females and the small amount of males on the boardwalk. As such, more research is needed on the preferences and deterrents of a perch for both male and female *O. pumilio*.

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Attachment 2: SPSS output colour intensity and escape behaviour.

In this attachment output of the different statistical tests used to analyse the associations between colour intensity and escape behaviour is shown.

First, the output of the Principal Component Analysis (PCA) on corrected residual RGB measurements is displayed. The total variances explained by the different components is shown (*Table 1*). The first column shows the Eigenvalues. The one component that was extracted, PC1, explains 65.3% of the variance in the used variables.

Table 1. Total variance explained table. Output created by a PCA in SPSS v.20. Only component 1 was extracted.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1,960	65,334	65,334	1,960	65,334	65,334
2	,927	30,884	96,218			
3	,113	3,782	100,000			

Second, the correlation between the three different variables in the PCA within the extracted component is shown (*Table 2*).

*Table 2. Component matrix table.
Output created by a PCA in SPSS v.20.
The correlation between the three variables used for the PCA within the extracted component is shown.*

	Component
	1
RES REd	,694
RES Green	,970
Res Blue	,733

Next the output of the Generalized Linear Model that was used to explain the association between FID and PC1 is shown (Table 3). In the second column, B, a positive association of 0.020 between PC1 and FID_category can be seen. However, this association is not significant as can be seen in the last column ($p=0.840$).

Table 3. Parameter estimates table. Output created by a Generalized Linear model with a negative binomial distribution and Log link function created in SPSS v.20. The dependent variable was FID_category, the two covariates used in this model are PC1 and Perch_Heightcm.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3,758	,2104	3,346	4,170	319,125	1	,000
PC1	,020	,0966	-,170	,209	,041	1	,840
Perch_heightcm	-,010	,0036	-,017	-,003	7,403	1	,007
(Scale)	1 ^a						
(Negative binomial)	1 ^a						

Next, the output of a Logistic Binary Regression that explains the association between PC1 and Flight_attempt is shown (Table 4). The classification table shows that the regression's output is for 66,4% accurate.

Table 4. Classification table. Output of logistic binary regression with Flight_attempt as dependent variable and PC1 as the independent variable. Created by SPSS v.20.

Observed	Predicted		
	Flight_attempt?1=n2=y		Percentage Correct
	no	yes	
Step 1 Flight_attempt?1=n2=y no	0	38	,0
yes	0	75	100,0
Overall Percentage			66,4

The Logistic Binary Regression shows that there is no significant association between PC1 and Flight_attempt ($p=0.333$) (Table 5).

Table 5. Significance of the variables in the equation. Created by a Logistic Binary Regression in SPSS v.20.

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a PC1	-,196	,202	,938	1	,333	,822
Constant	,686	,200	11,725	1	,001	1,986

Attachment 3: SPSS output perch height and escape behaviour.

In this attachment output of the different statistical tests used to analyse the associations between perch height and escape behaviour is shown.

The output of the Generalized Linear Model that was used to explain the association between FID and Perch Height is shown (Table 1). In the second column, B, a negative association of -0.010 between Perch Height and FID_category can be seen. The association was found significant ($p=0.007$).

Table 1. Parameter estimates table. Output created by a Generalized Linear model with a negative binomial distribution and Log link function created in SPSS v.20. The dependent variable was FID_category and the covariate used in this model is Perch_Heightcm.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3,753	,2088	3,344	4,162	323,007	1	,000
Perch_heightcm (Scale) (Negative binomial)	-,010 1 ^a 1 ^a	,0035	-,017	-,003	7,397	1	,007

Next, the output of a Logistic Binary Regression that explains the association between Perch_Height and Flight_attempt is shown (Table 2). The classification table shows that the regression's output is for 69.9% accurate.

Table 2. Classification table. Output of logistic binary regression with Flight_attempt as dependent variable and PC1 as the independent variable. Created by SPSS v.20

Observed		Predicted		
		Flight_attempt?1=n2=y		Percentage Correct
		no	yes	
Step 1	no	9	29	23,7
	yes	5	70	93,3
Overall Percentage				69,9

The Logistic Binary Regression shows that there is negative significant association between Perch_Height and Flight_attempt ($B=-0.022$; $p=0.002$) (Table 3).

Table 3. Significance of the variables in the equation. Created by a Logistic Binary Regression in SPSS v.20.

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	Perch_heightcm	-,022	,007	9,750	1	,002	,978
	Constant	1,920	,460	17,391	1	,000	6,822

Next, the output of the linear regression shows the association between Sqrt_PerchHeight and PC1 (Table 4). There is a positive but non-significant association ($B=0.158$; $p=0.095$).

Table 4. Coefficients table linear regression. Dependent variable is sqrt_PerchHeight and the independent variable is PC1. Created by SPSS v.20.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	6,901	,209		33,094	,000
	PC1	,353	,209	,158	1,685	,095

The Adjusted R square shows that PC1 accounts for 1.6% of the variance in the variable sqrt_PerchHeight (Table 5).

Table 5. Model summary linear regression. Dependent variable is sqrt_PerchHeight and the independent variable is PC1. Created by SPSS v.20.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,158 ^a	,025	,016	2,21652

Attachment 4: Recaptures.

In this attachment all the collected data of the recaptures is shown (*Table 1*). All the frogs with the same colours are the same individuals. The last six columns show the colour intensity measurements obtained by ImageJ.

Table 1. Data recaptures. All the frogs with the same colours are the same individuals.

FROG#	Date	Calling?	Seks	FID(cm)	Perch_height(cm)	Flight_attempt?1=n2=y	Red	Green	Blue	Grey_R	Grey_G	Grey_B
18	26-jan	n	m	0	29,2	1	122.842	10.730	3.843	148.735	151.148	149.502
57	2-feb	n	m	0	29	1	131.647	16.437	9.308	128.025	128.907	128.893
113	1-mrt	n	m	0	10	1	125.626	18.971	9.485	125.358	125.713	126.043
20	26-jan	n	m	0	47,2	1	113.238	24.740	5.756	134.240	138.570	137.525
62	2-feb	y	m	47	50	2	103.514	22.012	8.526	136.545	135.675	135.593
24	26-jan	n	m	15	58,3	2	183.540	25.640	3.867	138.683	140.560	143.695
66	2-feb	y	m	0	173	1	184.100	38.316	8.875	152.430	156.685	152.970
34	1-feb	n	f	0	68,1	1	143.636	30.436	12.491	140.333	144.035	144.928
84	15-feb	n	f	0	110	1	131.323	22.506	11.437	124.403	123.385	123.375
33	1-feb	n	f	98,3	76,4	2	171.587	31.108	6.467	127.055	131.553	133.548
95	18-feb	n	f	0	69	1	155.800	36.721	18.550	142.567	148.553	148.400
118	1-mrt	n	f	0	66	1	145.484	33.295	8.547	154.525	158.685	157.155
127	3-mrt	n	f	0	68	1	164.210	32.331	8.503	138.947	140.673	142.820
142	19-mrt	n	f	0	75	1	139.854	28.610	11.547	134.330	135.495	131.705
21	26-jan	n	m	0	60,2	1	101.546	17.028	3.367	151.208	155.222	158.238
116	1-mrt	n	m	0	75	1	143.142	33.883	7.294	165.857	166.803	167.130
86	15-feb	n	f	69	84	2	153.421	30.506	14.003	147.280	148.590	146.312
129	3-mrt	n	f	47	86	2	139.671	28.637	8.151	141.917	147.385	150.715
161	7-apr	n	f	30	62	2	176.020	32.486	8.745	138.425	140.900	140.032