The impact of traffic disturbance on the personality traits of the tungara frog (*Engystomops pustulosus*) in Trinidad

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Introduction

From June – August 2018 I spent 10 weeks conducting fieldwork in Trinidad as part of my PhD. The purpose of this investigation was to determine whether the levels of road traffic disturbance have an effect on the behavioral traits found in tadpoles inhabiting water bodies next to these roads. My research aimed to examine both how the behavioural traits of individuals within a species can determine the environment they inhabit and the contribution traffic disturbance can have on the distribution of individuals within the environment.

Background

Over the past decade, research within behavioral ecology has revealed that individuals within a species differ in their expression of behavioural traits, with some being consistently more aggressive, bold or active than others (Bell 2007; Carere & Maestripieri 2013; Pruitt 2014; Beekman & Jordan 2017). This has been termed "animal personality" and the field is primarily concerned with the causes of individual variation in behaviour and the consequences this variation may have on both individuals and populations (Stamps & Groothuis 2010; Réale et al. 2010).

A potential consequence of animal personality is that the behavioural traits that an organism displays may limit the types of environments that an individual can inhabit (Cote et al. 2010; Sih et al. 2012). As a result, the mean expression of a behavioural trait may restrict an individual's ability to cope with certain environmental stressors (Réale et al. 2007). Consequently a non-random distribution of behavioural traits may be found across a species' environment where individuals settle within habitats that most suit their suite of behavoural traits (Jacob et al. 2015; Edelaar et al. 2008). An example of this is that bolder individuals can frequently be found in more risky environments than shyer individuals (Holtmann et al. 2017; Bonnot et al. 2018).

Human disturbed environments can inflict a high degree of stress on organisms (Zhang 2016; Hamer & McDonnell 2008; Kark et al. 2007; Lowry et al. 2013) . Extended periods of light and noise pollution (McMahon et al. 2017; Kempenaers et al. 2010; Naguib 2013) in urban areas may disrupt foraging behaviour and increased pollution of air and water bodies by vehicle exhausts can lead to a decrease in fitness within these environments (Lough et al. 2005). Amphibians in particular are known to be highly susceptible to the negative effects of roads and motor vehicle traffic (Rytwinski & Fahrig 2015; Spellerberg 1998), being both sensitive to chemical contaminants in the water and soil (Adlassnig et al. 2013) as well as being at increased risk of mortality when crossing roads (Hamer et al. 2015).

Owning to the high level of disturbance caused by roads, heavy levels of vehicle traffic may act as an environmental filter on populations (Rytwinski & Fahrig 2015). This may lead to high disturbance

areas only being inhabited by individuals which have the behavioural traits necessary to cope with high levels of disturbance. This may affect the distribution of behavioural triats in two ways. Firstly, there may be an overall difference in the expression of a behaviour between disturbed and non-disturbed areas. For example, individuals inhabiting disturbed environments may be expected to be more active and bold compared to individuals within less disturbed locations. Such an effect has been found when urban and rural individuals have been compared across numerous species, with urban environments selecting for more bold, aggressive and active individuals (Hardman & Dalesman 2018; Kralj-Fišer et al. 2017; Sepp et al. 2018; Chejanovski et al. 2017; Miranda et al. 2013). Secondly, the extent that a behaviour may vary among and within individuals may differ between populations. Within a disturbed environment, animals can be expected to respond in a more consistent manner when repeatedly confronted with the stressor (Holtmann et al. 2017). As a result individuals in disturbed located can expected to show less within within-individual variance in their behaviour.

In this study we examined whether the vehicle traffic levels on Trinidad roads impacted the behavioural traits of tadpoles inhabiting nearby temporary water bodies. We used the tungara frog, *Engystomops pustulosus*, because it is a common species of frog found throughout Trinidad and can be found in both high and low disturbed environments. Adults lay their eggs in foam nests constructed within predator free, temporary water bodies such as potholes and road side ditches. Consequently, larvae of the species can be found in high numbers within human disturbed environments close to the road edge where they are more prone to disturbance by vehicle traffic. Figure 1 shows an example of a tungara foam nest, tadpole and adult of the species.

Figure 1. A tungara frog foam nest (top left), tungara frog tadpoles (top right) and an adult male tungara frog calling to attract a female (bottom left).

Experiment aims and hypotheses

Our aim was to collect a minimum of 120 *E.pustulosus* foam nests from at least 30 different sites identified as receiving high or low volumes of traffic. Upon hatching, one tadpole from each nest would be reared under common laboratory conditions and their activity, exploratory and neophobia behaviour recorded at six time points over the course of their larval development.

We had two hypotheses we wanted to investigate as part of this project:
1) The mean level of activity, exploratory and neophobia behaviours would be higher in tadpoles originating from high compared to low disturbance sites.
2) The among-individual and within individual variance in activity, exploratory and neophobic behaviours would be smaller in tadpoles originating from high compared to low disturbance sites.

Methods

Collection sites

We collected *E. pustulosus* foam nests between June and August 2018 from road side locations across Trinidad. We assigned sites as high or low disturbance based on the volume of traffic passing the collecting site (see Traffic disturbance). Between one and eight foam nests were collected from each site and collection locations were considered separate sites if they were located 400 meters apart (2x the average dispersal distance of *E. pustulosus* adults) (Marsh et al. 2000; Marsh et al. 1999). Figure 2 shows an example of the types of temporary water bodies *E. pustulosus* can frequently be found in and Figure 3 shows the location of high and low disturbance collection sites. Following collection, all nests were brought back to our field research station (William Beebee Tropical Research Station; Arima, Trinidad) where all individuals were raised under similar conditions. One tadpole from each nest was transferred into its own container where it was assigned its own number for identification.

Figure 2. An example of road side ditch and potholes where *E.pustulosus* foam nests could commonly be found in Trinidad.

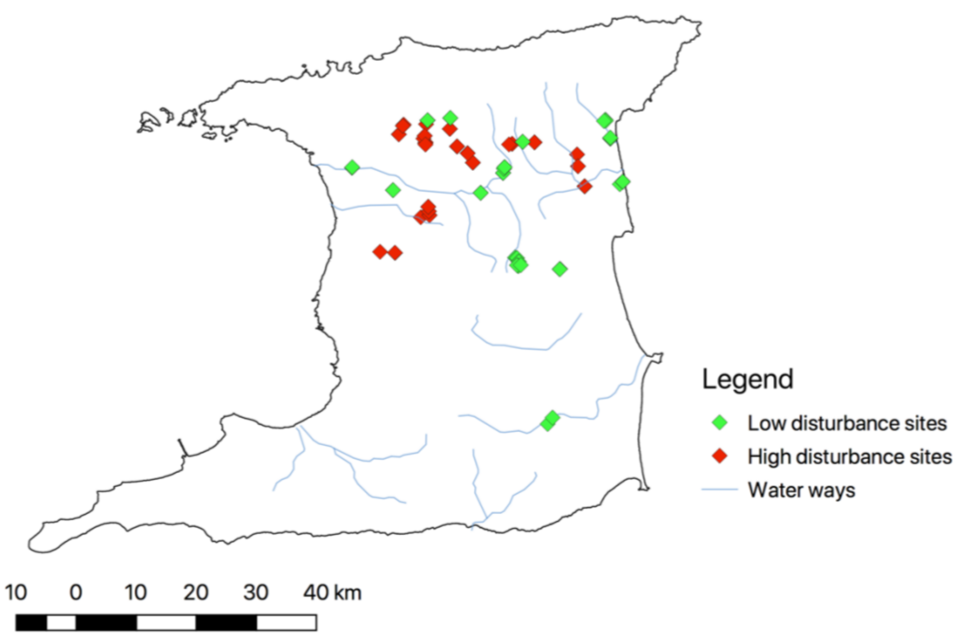

Figure 3. Map of Trinidad showing the location of high and low disturbance collection sites of *Engystomops pustulosus* nests.

Behavioural assays

We recorded tadpole behaviour in activity, neophobia and exploration assays six times from each tadpole. All assays were conducted in the same order with activity conducted first and exploration last to avoid any carry over effects from more potentially disruptive assays. Activity and neophobia assays were filmed on the same day and exploration assays were filmed either directly after the neophobia assay or the following day. Assay replicates were separated by 24-48 hours. Tadpoles were filmed in pairs to increase the number of tadpoles which could be filmed at once. Examples of the assays conducted can be found in Figure 4a and 4b.

Activity assay

We measured activity levels of tadpoles by recording their movements within their home tanks for a duration of 10 minutes. A 10 minute acclimation period was included to account for disturbance effects of moving tadpole's containers to an adjacent recording room.

Neophobia assay

Neophobia assays were conducted in a tadpole's home tank. We isolated tadpoles at one end of their tank with an opaque covering and allowed tadpoles to acclimate for 10 minutes. During the acclimation period, an object, unfamiliar to the tadpole, was placed at the opposite end of the tank. After acclimation, the cover was lifted and the tadpole's behaviour was filmed over a 20 minute period.

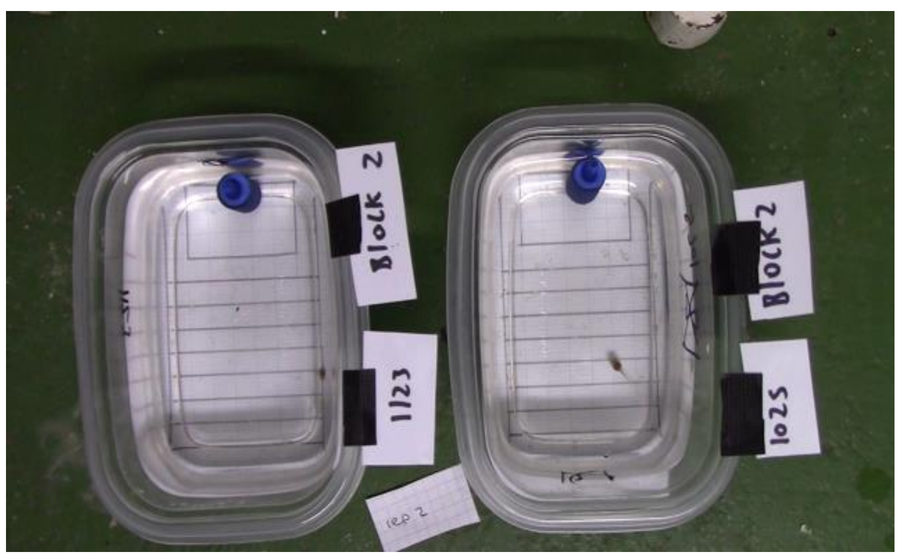

Figure 4a. Picture stills of Activity assay (top) and neophobia assay (bottom). Description of assay provided to left of images.

Exploration assay

To assess a tadpole's willingness to investigate a new environment, tadpole movements were recorded in a novel, semi-closed tank. The figure opposite shows an image of the tank which consisted of an acclimation chamber and 12 zones which included a central corridor and 8 rooms. To start a trial, we transferred tadpoles into the acclimation zone and after 10 minutes lifted an opaque barrier and filmed the tadpole's behaviour over 30 minutes.

Figure 4b. Picture still of the exploration assay. Description of assay provided to left of image.

Traffic Disturbance

We used traffic surveys to assess the levels of vehicle disturbance at foam nest collection sites. We conducted traffic surveys at each collection sites on two separate dates during a period from June to August 2018. Where two sites were close to one another along the same section of road, traffic surveys were conducted between the two sites. On each survey day, we counted the number of cars, mini-buses and lorries which passed the collection site for a period of one hour at 12pm and 5pm. An afternoon and evening survey were used to account for differences in traffic volume at different times of the day. Surveys were only conducted from Monday – Friday to avoid sampling some sites on weekends when the traffic volume may be altered. Figure 5 provides some examples of high and low traffic disturbance sites.

Figure 5. Example of a low disturbance site (left) and a high disturbance site (right). Arrows indicate where the foam nests were collected from.

Data Analysis

Behavioual assay footage

Footage from the behavioural assays will be reduced to 1 frame per second and converted into image stacks for analysis by the tracking software plugin, AnimalTracker (Gulyás et al. 2016) in FIJI (Schindelin et al. 2012). Figure 6 shows an example of a tracking line generated for a tadpole within the activity assay. The results from the tracking data will be used to calculate the total distance a tadpole moved within the activity assay and the total time a tadpole spent within a 1.8cm radius (3x times the average snout to vent length of tadpoles) of the object in the neophobia assay. The total number of new zones visited within the exploration arena will be used as a measure of exploratory behaviour.

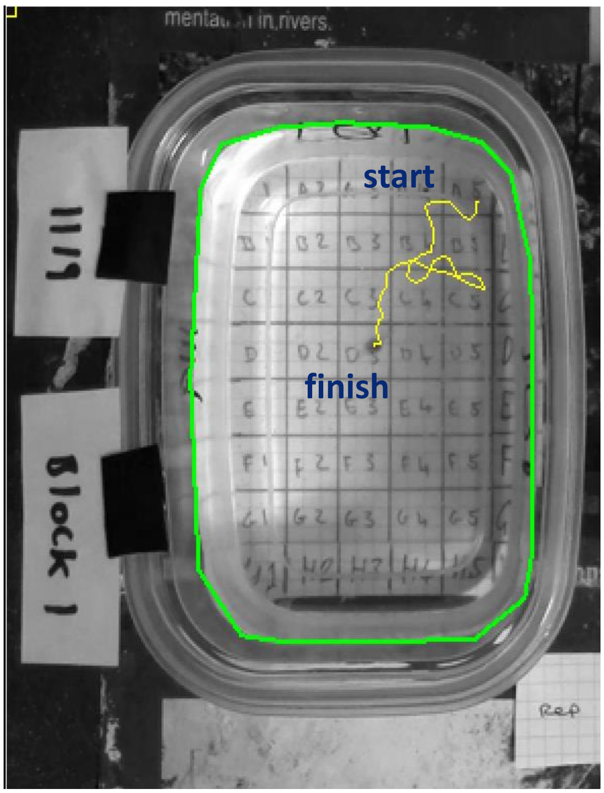

Figure 6. Example of the use of tracking software to track the movement and position of a tadpole over the course of an activity recording (yellow line). The green circle identifies the trackable area.

Statistical analysis

To investigate the effect of traffic disturbance on an individual's mean level of behaviour, three mixed models will be constructed with either measures of activity, neophobia or exploration as the response variable. Traffic disturbance will be included as a fixed factor and tadpole identity and its

site of origin will be included as random factors. Body size will be included as additional explanatory fixed effect as the size of an individual is expected to contribute towards a tadpole's behaviour.

To investigate the effect of traffic disturbance on the individual variance in behavior, mixed effect models will be constructed with either measures of activity, neophobia or exploration as the response variable. Traffic disturbance will be included as a fixed factor and tadpole identity will be included as a random factor. This "null" model where the variance components are not allowed to vary between site type, will be compared to an alternative model where the variance is allowed to vary with site type. Comparing these two models statistically will allow us to determine whether there is an effect of increased or decreased variance in tadpole behaviour in one treatment. Furthermore by partitioning the variance components due to the effect of tadpole identity will allow us to determine whether this variance is predominantly the result of an increase in the among or within individual differences.

Preliminary Results

A full data set of behavioural assays was collected from 129 tadpoles, each collected from a separate foam nest. 65 tadpoles were collected from low disturbance sites and 64 tadpoles were collected from high disturbance sites. Across the study, foam nests were collected from a total of 18 low disturbance and 21 high disturbance sites. Table 1 lists the total number of tadpoles which were collected from each unique site.

From each of the 129 tadpoles assayed, six replicates of three behavioural recordings were taken. In total this amounts to 2,160 separate videos which were recorded over the course of the study period and the number of videos which will be anaylsed so that the behavioral variables of interest can be extracted.

Table 1. The number of tungara foam nests collected per site in Trinidad.

Site name	Disturbance level	No. foam nests collected per site
ArenaForest	Low	2
AripoSavanna	Low	5
ChicheRoad	High	1
CouraRoad	High	3
CouraRoad2	High	4
CumberbatchRoad	High	1
DemeraraRoad1	High	3
DemeraraRoad2	High	3
EcoResort	Low	4
EdinburghRoad	High	2
Fishingpond1	High	5
FishingpondForestWalk	Low	5
LasLomasRoad	High	2
LasLomasRoad2	High	3
LasLomasRoad3	High	3
Lopinot1	High	2
Lopinot2	High	2
Lopinot3	High	3
Lopinot4	High	6
LopinotRural	Low	8
MeturaForest1	Low	1
MeturaTurtleRoad	Low	2
MeturaTurtleSideRoad	Low	3
RioClaroFarRuralRoad	Low	1
RioClaroFarRuralRoad2	Low	1
RioClaroForestPathRoad1	Low	6
SanJuanAgriField	Low	2
Simla	Low	5
SimlaRoad	High	4
SimlaRoad2	High	3
Tamana Cave	Low	4
TamanaSideTrack1	Low	8
TamanaSideTrack2	Low	4
TamanaSideTrack4	Low	1
TocoRoad1	High	2
TocoRoad2	High	3
ValenciaRoad1	High	5
ValenciaRoad2	High	4
WarrenField	Low	3
Total		129

Traffic surveys were used to determine the level of vehicle disturbance where tungara foam nests were collected from. Table 2 reports on the median number of vehicles which were recorded from high and low disturbance sites, demonstrating that there was a clear difference in the level of traffic between the two sites types.

Table 2. median number of vehicles recorded per hour from low and high traffic disturbed sites

Site type	Number of sites	Median number of vehicles per hour
Low disturbance	18	0
High disturbance	21	168

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Top image: Toby Dighero, Greig Muir and myself (Cammy Beyts). Bottom image: Outside grounds of the William Beebe Tropical Research Station.