Behaviour and morphology in wild guppies from populations with high and low predation pressure in Trinidad
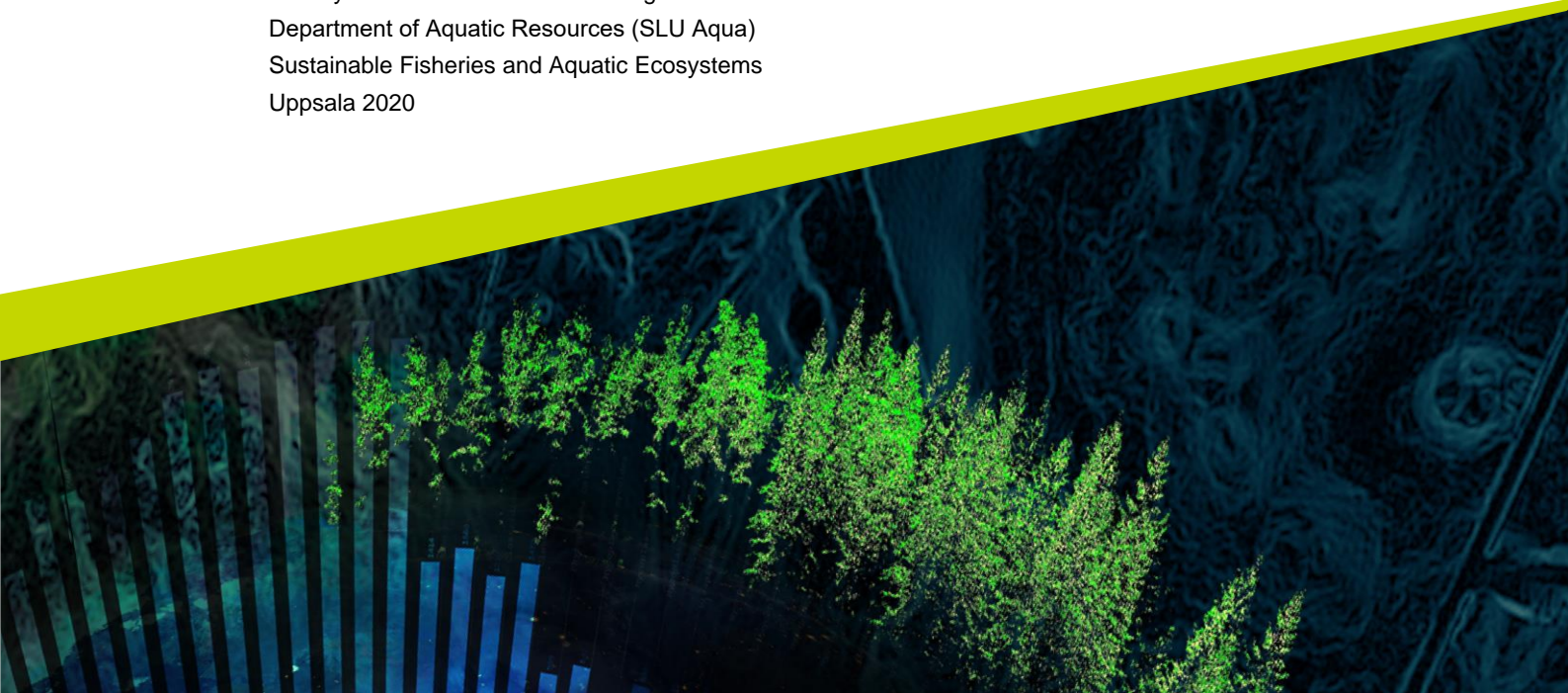
William Ashworth

Behaviour and morphology in wild guppies from populations with high and low predation pressure in Trinidad

Beteende och morfologi hos vilda guppies från populationer med högt och lågt predationstryck i Trinidad

William Ashworth

Supervisor:	**Josefin Sundin, SLU, SLU Aqua**
Assistant supervisor:	Mirjam Amcoff, Stockholm University, Department of Zoology
Examiner:	Andrea Belgrano, SLU, SLU Aqua

Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

Department of Aquatic Resources (SLU Aqua)

Archiving and publishing

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. When you have approved, metadata and full text of your thesis will be visible and searchable online. When the document is uploaded it is archived as a digital file.

☒ YES, I hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work. https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/

☐ NO, I do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract:

Behaviour and morphology can vary significantly within a species as a result of the predation pressure individuals are exposed to. Wild populations of guppies (*Poecilia reticulata)* exposed to high and low predation can be found in the streams of northern Trinidad, providing a useful study system for investigating how behaviour and morphology are affected by predation. In the present study, I investigated how behaviour, colouration and size in male guppies from 3 Trinidadian streams (Aripo, Tunapuna/Tacarigua, Turure) was affected by predation pressure. This was achieved by quantifying the behaviour of males from high and low predation regimes in the presence of a female conspecific from the same population. I also measured the amount of orange and black colouration on each side of the males. I found that behaviour and morphology were only affected by predation regime in guppies from one of the streams, namely Turure. Males from the high predation environment of Turure spent more time in proximity of the female conspecific, had less total black colouration, and were smaller than males from the low predation environment of the same stream. Males from Turure also had less total orange colouration, and less orange colouration symmetry than males from the other two streams (Aripo and Tunapuna/Tacarigua), regardless of predation regime. These results are interesting as the stream Turure belongs to a different drainage (Oropuche) than the streams Aripo and Tunapuna/Tacarigua (Caroni). This investigation highlights the possibility that predation regime may affect behaviour and morphology differently depending on the populations which are being studied, potentially due to significant ecological variation between drainages.

Popular scientific summary:

It is in the best interest of all prey animals to avoid being eaten by predators, therefore many prey animals have developed anti-predatory strategies. Such strategies and adaptations can be changes to their physical appearance (morphology), for example camouflage to avoid detection by predators, or changes in their behaviour, for example being less active when a predator is nearby. While these strategies and adaptations benefit the survival of the individual, they often come at a cost. When an organism is less active to avoid predators, it is sacrificing time it could have used to forage or find a mate. Similarly, if an organism has evolved inconspicuous colouration to avoid detection by predators, there might be a trade-off between such colours and those favoured by a mate, potentially making a dull individual less likely to reproduce. Because of this, anti-predatory adaptations are more frequent amongst animals living in environments with a high threat of predation. Furthermore, members of the same species can often appear notably different if they live in an environment with high or low levels of predation. Guppies (*Poecilia reticulata*) provide an excellent opportunity to study such differences. Guppies is a small freshwater fish, common in the aquarium trade, that occur naturally in streams in Trinidad. The predators of guppies (mostly larger species of fish) only occur in the downstream environments, as they are unable to pass the waterfalls that divide upstream and downstream habitats. I can therefore compare guppies from upstream and downstream environments to see if and how the varying predation pressure has led to differences in their morphology and behaviour.

To do this males and females were collected from the upstream and downstream environments of 3 streams in northern Trinidad (Aripo, Tunapuna/Tacarigua and Turure). The behaviour of males was observed in the presence of a female. I primarily recorded the total amount of time the pair spent close to each other, and the total amount of time the male in an observation would spend showing either one of his sides to the female. Photos were then taken of each side of the male, so that the total amount of black and orange colouration on both sides of the male, and their length could later be measured. By measuring the amount of time a male spent showing both of his sides to a female and measuring the total colouration on each of his sides, I could see if males prefer to show their more colourful side to the female.

Using these measurements, I could test if the behaviour and/or morphology of guppies was affected by predation. The results showed that only males from one stream, Turure, were different in their morphology and behaviour between high and low predation environments. Specifically, males from the high predation environment of Turure spent more time close to the female, had less total black colouration, and were smaller than males from the low predation environment of Turure. Additionally, regardless of the level of predation, males from Turure had less total orange colouration, and less orange colouration symmetry than males from the other two streams (Aripo and Tunapuna/Tacarigua). No males showed any preference to show their more colourful side to the female, regardless of their native stream, or level of predation.

The fact that fish from stream Turure were different compared to the other two streams is interesting when one considers the geography of the streams. Aripo and Tunapuna/Tacarigua are tributaries of the Caroni drainage, while Turure is a tributary of the Oropuche drainage. The fact that these streams are geographically isolated from one another means that environmental differences could exists between Turure and the other two streams that could explain the results found in this study.

Table of contents (should start on an odd page number)

List of tables

List of figures

Introduction

Predation pressure can induce morphological (Endler, 1978) and or behavioural changes (Komers, 1997) in almost all prey species. Morphological changes would usually come in the form of changes in size, shape or colouration which would make the organism harder to either locate, attack or consume (Ruxton *et al*., 2004). Anti-predatory behaviours can often occur in the form of reduced activity, aggressive displays, increased grouping or increased vigilance (Ruxton *et al*., 2004). These behaviours aim to decrease the likelihood of any attack succeeding or even occurring in the first place, ultimately leading to an increased chance of survival (Ruxton *et al*., 2004). These morphological and behavioural changes however are costly, and so only occur in environments with high enough predation pressure to justify the trade-off (Endler, 1978; Komers, 1997). Trinidadian streams provide a unique opportunity for studying how behaviour may be affected by predation, as conspecifics from naturally high and low predation environments can be easily obtained. This is because the majority of aquatic predators on the higher trophic levels are unable to migrate upstream due to natural physical barriers (Magurran, 2005), resulting in low predation in upstream environments, and high predation in downstream environments. The Guppy, *Poecilia reticulata*, is a freshwater fish species commonly found throughout these Trinidadian streams. Their wide distribution, hardiness and comparative boldness combined with an abundance of existing literature make guppies and ideal model organism when studying behavioural ecology (Houde, 1997; Magurran, 2005). The primary predator of guppies in Trinidad, *Crenicichla frenata*, is confined to the lower areas of these streams (Magurran, 2005). Consequently, guppy populations in these upstream areas are subject to far less predation pressure than their counterparts downstream. Under high predation, courtship behaviour decreases (Farr, 1975; Magurran & Seghers, 1994a), and anti-predatory behaviour increases (Magurran & Seghers, 1991, 1994b). Guppies exposed to a high predation pressure also have a less colourful appearance and are generally smaller (Endler, 1978).

Male guppies have sexual ornaments in the form of eye-catching colour (Houde, 1997). This colouration is an essential part of a male's courtship display (Houde, 1997). Sigmoid displays are performed by males with the intention of emphasising their colour to impress females (Houde 1997, Table 1).

Table 1. Reproductive behaviour observable in P. reticulata. (Houde, 1997)

Behaviour	Description
Side showing	Male specifically shows a side of its body to a female, displaying his colours, with the intention of impressing the female.
Following	Male will commonly follow the female as a sign of interest. The female may also follow the male if she is impressed.
Gonopodal thrusts	Male will thrust his gonopodium towards a female, in an attempt to inseminate the target.
Sigmoid	Male will bend his body into a sigmoid shape, with the intention of making his colours more eye catching to a female. Sigmoids may also be followed by a dash away. If interested, the female will follow the male after he dashes.
Stiff quivering	The male's whole body will quiver stiffly. This indicates a successful copulation.

Numerous studies have observed that colourful males are significantly more desirable as mates to female guppies (Houde, 1997 and references therein; Endler 1983). Symmetry has also identified to be a preferable trait in males to female conspecifics (Møller & Pomiankowski, 1993; Watson & Thornhill, 1994).There is evidence that symmetry is also favoured in male guppies by sexual selection (Sheridan

& Pomiankowski, 1997). It should also be noted that it is theoretically possible for a significantly asymmetrical male to still appear as desirable to females, by performing sigmoids showing its most colourful side, while concealing a less impressive and notably different other side (Gross, *et al.*, 2007). This would produce a deceptive signal which would mis-inform potential female recipients of the males quality, to the benefit of the male (Gross, *et al.*, 2007).

As previously mentioned, guppies are an excellent model organism when it comes to the study of behaviour. As a result, there is an abundance of literature in the field of behavioural ecology that uses *P. reticulata* as a model organism (Reviewed in: Endler, 1995; Houde, 1997; Magurran, 2005). Furthermore, variation in behaviour (De Santi *et al.,* 2000; Farr, 1975; Magurran & Seghers, 1991, 1994a, 1994b) and colour (Endler, 1978, 1983, 1991; Millar, Reznick, Kinnison, Hendry, & Persson, 2006) under differing predation regimes has also been extensively studied. There is however limited research which has combined these two related fields: Gross *et al.,* (2007) conducted an investigation on *P. reticulata* with a genetic context, concluding that males show their best (most attractive to potential mates) side in courtship trials. Similarly, Amcoff *et al.,* (2009) used *Corynopoma riisei* to investigate the preference of a male to show its best side to a female, determining the best side of specimens based on specialised paddles used in courtship, yielding matching results. Furthermore, Rezucha and Reichard (2015) investigated how asymmetry in Endler's guppies, *Poecilia wingei*, influenced courtship behaviour, finding that males preferentially show their more attractive side, mainly when competition is strong. Hence, the few studies that exists to date suggest that freshwater teleost fish which must show one of their sides during courtship, are aware of their most attractive side and preferentially display it. However, more research is needed before general conclusions can be made. In addition, none of the aforementioned studies, which investigated the relationship between physical asymmetry and courtship behaviour, analysed how this relationship may change under different predation regimes.

This investigation focuses on the reproductive behaviour exhibited by guppies from high and low predation environments. Guppies readily exhibit reproductive behaviour (Houde, 1997), therefore differences between individuals exposed to high and low predation pressures should be observable in such behaviours if present. The main observable behaviours associated with courtship in guppies are displayed by males and are listed in Table 1. It should be noted that some behaviours can occur without the underlying courtship intentions, such as side showing and following. Such behaviours may occur when individuals remain close to each other for schooling/anti-predatory purposes, rather than for potential mating. This thesis aims to investigate how the relationship between behaviour and morphology changes in high and low predation regimes. This was achieved with the study of behaviour and colouration in Trinidadian guppies.

Courtship behaviour and side showing preference of male guppies was analysed in the presence of a female conspecific (video analysis), using guppy pairs collected from high and low predation environments to allow predation regime to be tested. The area of colour of each male was subsequently quantified. Orange and black colouration was measured as studies have identified these two colours to be the most attractive to females (Houde, 1997 and references therein). These colours are an effective form of communication due to the fact that they are costly to produce, and therefore represent the quality of the individual displaying them (Doorn & Weissing, 2015).

I predict that males from low predation environments will be more colourful, as without significant predatory threat, colourful ornamentation is favoured by sexual selection (Endler, 1978). I also predict males under low predation will be more symmetrical, as a lack of symmetry may be more detrimental to a male's courtship success in low predation more competitive environments, than it would be in high predation less competitive environments (Endler, 1995). If asymmetry in colour is present, I would expect the male to show it's more colourful, and therefore best side to the female, for a greater period

of time than it shows its less colourful side (Amcoff *et al.*, 2009; Gross *et al.,* 2007; Rezucha & Reichard, 2015), regardless of predation regime.

Hypotheses:

H1: There is a correlation between the more colourful side of a male guppy and the side the same guppy choses to display more frequently a female conspecific.

> H_0: There is no correlation between the more colourful side of a male guppy and the side the same guppy choses to display more frequently a female conspecific.

H2: There is an effect of predation regime on the total colouration of a male guppy.

> H_0: There no effect of predation regime on the total colouration of a male guppy.

H3: There is an effect of predation regime on the colouration symmetry of a male guppy.

> H_0: There no effect of predation regime on the colouration symmetry of a male guppy.

Materials and Methods

Data collection background:

Wild adult male and female guppies were collected in 2015 from 3 different Trinidadian streams; Aripo, Turure, and Tunapuna/Tacarigua (two tributaries of the same stream). Specimens were collected from 6 sites in total, one high predation site and one low predation site from each stream (Table 2). High predation sites were located downstream, and low predation sites were located upstream. Natural predators of the guppy are only abundant in downstream environments, due to their inability to pass natural barriers, preventing any potential upstream migration (Magurran, 2005). Once collected, specimens were transported back to the University of West Indies and put into glass tanks at 24°C and fed flake food ad libitum at the start and middle of each day to maintain uniform levels of satiation.

Table 2. Number of guppy pairs collected and studied from each stream and predation regime.

Stream	Low predation	High predation	Total
Aripo	22	20	42
Tunapuna/Tacarigua	28	26	54
Turure	11	11	22
Total	61	57	118

Behavioural trials were conducted in glass tanks (46×23×23 cm), divided in two arenas, each arena 23×23×23 cm, water level 4 cm, temperature 24°C. The sides and bottom of the tank were covered in white opaque material to prevent external stimuli from influencing the behaviour of the fish. Females were introduced to the arena (one female per arena) and given 10 min to acclimatize. A male conspecific was added to each arena and given approximately 3 minutes to acclimatize. The trials were video recorded (using a GoPro Hero 4) and lasted 9 minutes (Videos were 12 minutes long, 3 minutes acclimatization + 9 minute trials). Four replicates were filmed simultaneously (i.e., 4 arenas, 2 tanks, were used at a time). Each arena was labelled with a unique male ID number to allow blind analysis of the videos. After trials, males were netted from the arenas and sedated in an ice slurry. Thereafter, the males were placed on measuring paper together with the ID note and a photo was taken of both the left and right sides of each fish (using a Canon EOS 7D with a Canon EF-S lens) (Figure 1). The females were weighed after trials to obtain a measurement of size, excess water was gently wiped off and the fish was weighed to the nearest 0.01 g.
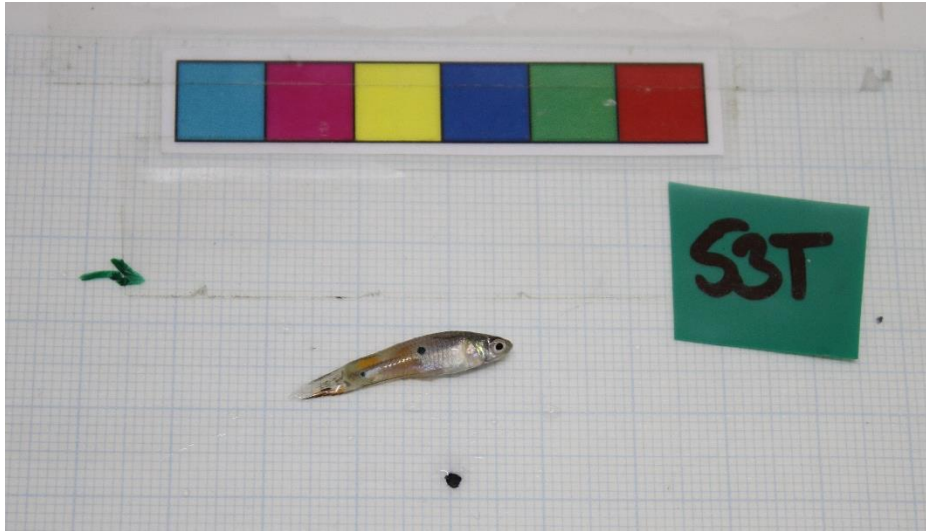
Figure 1. Photograph of a sedated male guppy from the analysis of colour ornaments (black and orange colouration on left and right side) and length. Photo: M Amcoff.

Video analysis

In order to quantify whether males preferentially displayed their left or right side towards the female, videos were analysed using BORIS software (Friard & Gamba, 2016). Several additional behavioural parameters were also quantified (Table 3). An appropriate protocol was designed (detailed below) using information from existing literature and from observing a sample of the videos (Table 3). Since each arena was labelled with the male ID, this allowed videos to be analysed blind regarding predation and stream origin by an individual (myself) with no involvement in this or any previous elements of the experiment.

Behaviour protocol

Behaviours were Identified and defined by observing a sample of videos, and with reference to previous literature (Houde, 1997. Table 1). Behaviours were recorded as either states or events. States were continuously recorded behaviours which were measured as the number of seconds an individual was displaying the state behaviour in a trial (Table 3). Events were behaviours which can be identified as distinct occurrences and were measured in frequency per trial (Table 3).

Individuals were defined to be within proximity when they were within 3 (male) body lengths of each other. When the individuals were within proximity of each other, the side of the male which was facing the female was recorded as left, right, or no clear side. The side the male was showing was determined using two 20° cones originating from the centre of the male (Figure 2A). If the female was within the range of either cone, no clear side was recorded. If the female was outside the range of both cones, the side of the male facing the female was recorded. This defines 3 behavioural states which were recorded during the video analysis: Proximity L (Individuals in proximity, male showing left side), Proximity R (Individuals in proximity, male showing right side) and Proximity N (individuals in proximity, male showing no clear side) (Table 3).

When individuals were within proximity of each other, and the male was following the female, this was recorded as a separate state. The male was defined as following when the female moved away from the male, and the male responded with a movement which reduced the distance between the 2 individuals. The head of the male also had to be behind the middle point of the female (Figure 2B) for the male to be defined as following, otherwise proximity was recorded as above. The side the male was showing to

the female was also recorded when the male was following, using the same system as previously described. This defines 3 more behavioural states: Following L (male following, showing left side) Following R (Male following showing right side) and Following N (Male following, showing no clear side i.e. directly behind) (Table 3). In some rare cases, the female followed the male. As it was rare, and the side of the female which faces the male is not the focus of this study, female following was recorded as a single state (Female following) (Table 3). This state was recorded simultaneously alongside Proximity N, Proximity L or Proximity R, so that the side the male was presenting to the female continued to be recorded. The definition of following remained the same for this behaviour. If a leading individual left the proximity of its follower, the following individual was allowed 2 seconds to re-enter proximity before they were considered to be no longer following, to avoid the recording of excessive stops and starts.

If the heads of both individuals in a pair were simultaneously touching the same wall of the experimental arena and both individuals are active, proximity and following states were recorded as non-directional (N), as the side of the male facing the female would change frequently, and seemingly randomly, making this behaviour challenging to record accurately.

Aggressive behaviour exhibited by the female was also recorded. This was either in the form of a female bite or female chasing. A female bite was recorded as a single point (Table 3) and defined as a rapid movement of the female's head towards and making contact with the male. The male would then typically react with its own short rapid movement away, or by swimming away entirely. Female chasing was recorded as a state (Table 3) and defined as continuous rapid movement of the female towards the male, where the male responds by rapidly moving away from the female. Chases in bouts occurring within 2 seconds of each other were recorded as a single period of chasing. Female chasing was recorded simultaneously to proximity and following behaviours. Chasers were also allowed 2 seconds to re-enter proximity if the fleeing individual left proximity, again, to avoid excessive stops and starts.

Reproductive behaviour was recorded in the form of "sigmoids". Sigmoids are a courtship display performed by the male (Houde, 1997), which were present in some of the analysed videos. Sigmoids were recorded as a single point, however this behaviour was split into strong sigmoids and weak sigmoids (Table 3), due to observed variation in the behaviour. In some instances, the male was observed to perform a sigmoid motion, and then dash away from the female conspecific. This dash away following a sigmoid is an additional behaviour of significance which was also recorded (Post sigmoid dash).

Lastly, a few individuals were observed to jump out of the water, so this was also recorded as a single event, using a separate code for each sex (male jump and female jump) (Table 3).
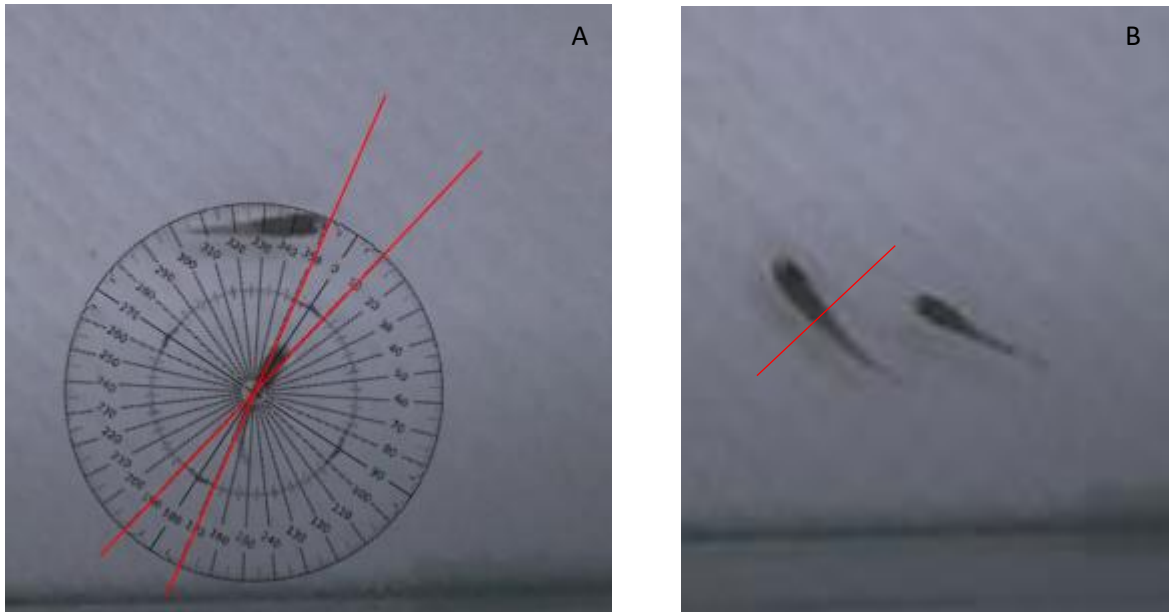
Figure 2. Method used to determine side showing and the following behaviour in the video analysis. A) a male guppy with two 20° cones originating from the centre of the male to determine whether left or right side was shown to the female. B) a female guppy (left), with a mark across its middle point determining male (left) following behaviour.

Table 3. List of all recorded behaviours, and whether they were recorded as states or events.

Behaviour	State or Point event
Proximity L	State
Proximity N	State
Proximity R	State
Following L	State
Following N	State
Following R	State
Female following	State
Female chasing	State
Female bite	Point
Weak sigmoid	Point
Strong sigmoid	Point
Male jump	Point
Female jump	Point

Photo analysis

The photographs of male specimens were analysed to investigate potential differences in colour/symmetry between populations of guppies exposed to varying levels of predation. Corresponding to the video analysis, as the photos included the male ID numbers, they could be analysed blind with regard to predation and stream origin of the male. In order to quantify whether the male sexual colour ornaments differed between males, and between the left and right side of individual males, ImageJ software (Schindelin *et al*., 2012) was used to analyse the photos. The standard length of each individual was also measured. I quantified the frequency and total area of black/orange ornaments present on each male (yellow outlines seen in Figures 3 - 7). This process was carried out with photos of both sides of every specimen. Black and orange patches were identified by eye. At 100% magnification, specimens took up between 14% and 22% of the width of a 5184x3456 pixel image.

Additionally, black was left unmeasured if it was identified as the general colouration of the specimen (Figure 3), rather than a specific patch of concentrated melanic pigment. Dark horizontal strips of colour, located along the centre of the rear half of some specimens, were also left unmeasured (Figure 4, 5, 6). These strips varied markedly in their colouration and were often difficult to define as black or dark purple/blue/green, therefore they were left unmeasured in all occurrences. Orange and black patches on the tail have been left unmeasured since the tail was not fully spread in all photos (Figures 3-6). If a colouration patch was unidentifiable by eye from 100% magnification, it was left unmeasured (Figure 7).
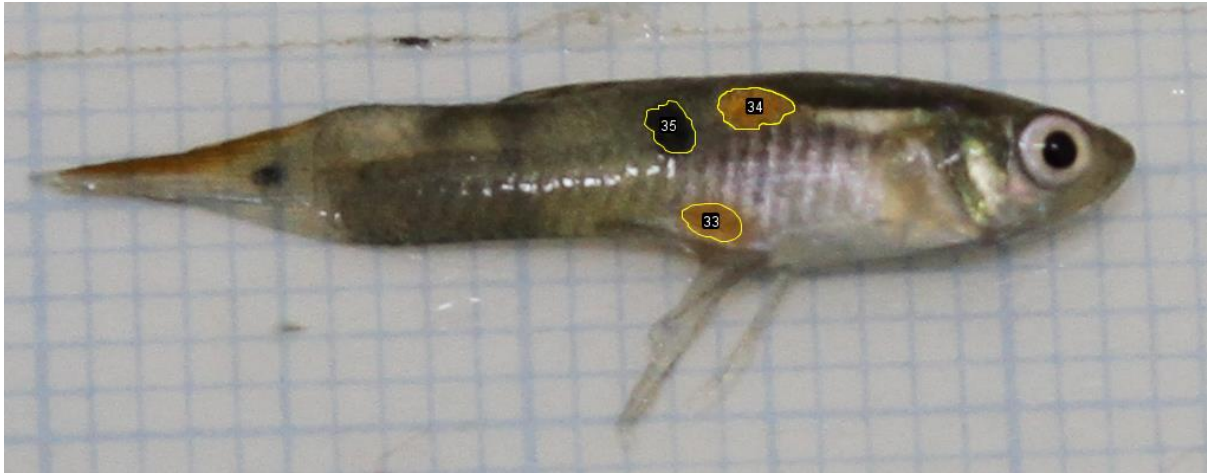
Figure 3. Photograph of a male guppy from the colour analysis (100% magnification) with distinct and indistinct patches of black colouration. The distinct patch has been measured (shape 33-35), and the indistinct have been left unmeasured.
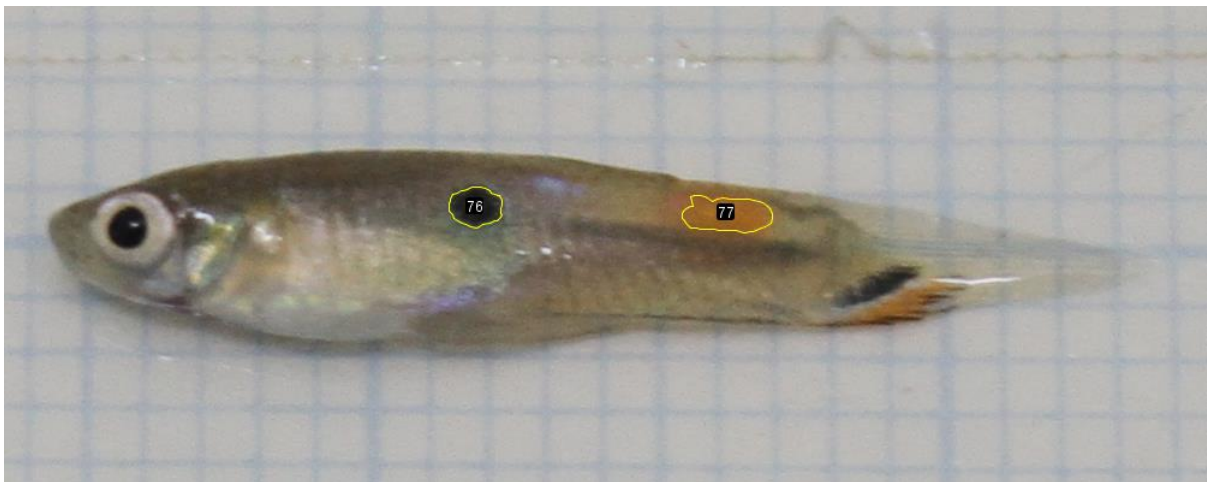
Figure 4. Photograph of a male guppy from the colour analysis (100% magnification), showing one clear patch of black (shape 76) and one clear patch of orange (shape 77) that have been measured. The faint grey patch running through the centre of the rear end of the specimen is difficult to identify as black or not. It was therefore left unmeasured.

Figure 5. Photograph of a male guppy from the colour analysis (100% magnification) with a dark strip running along the centre of the rear end of the specimen. The dark strip fades into purple colouration and is difficult to identify as black or not. It was therefore left unmeasured.
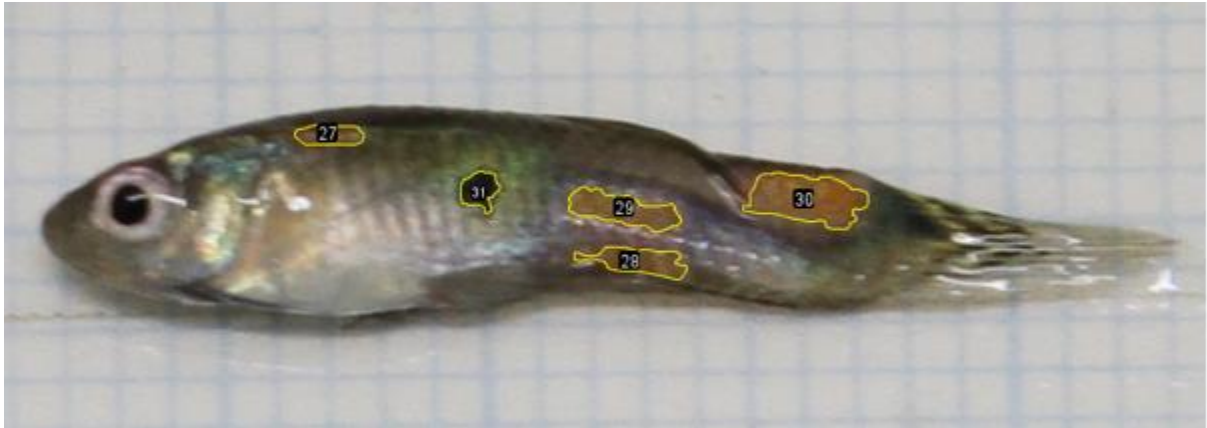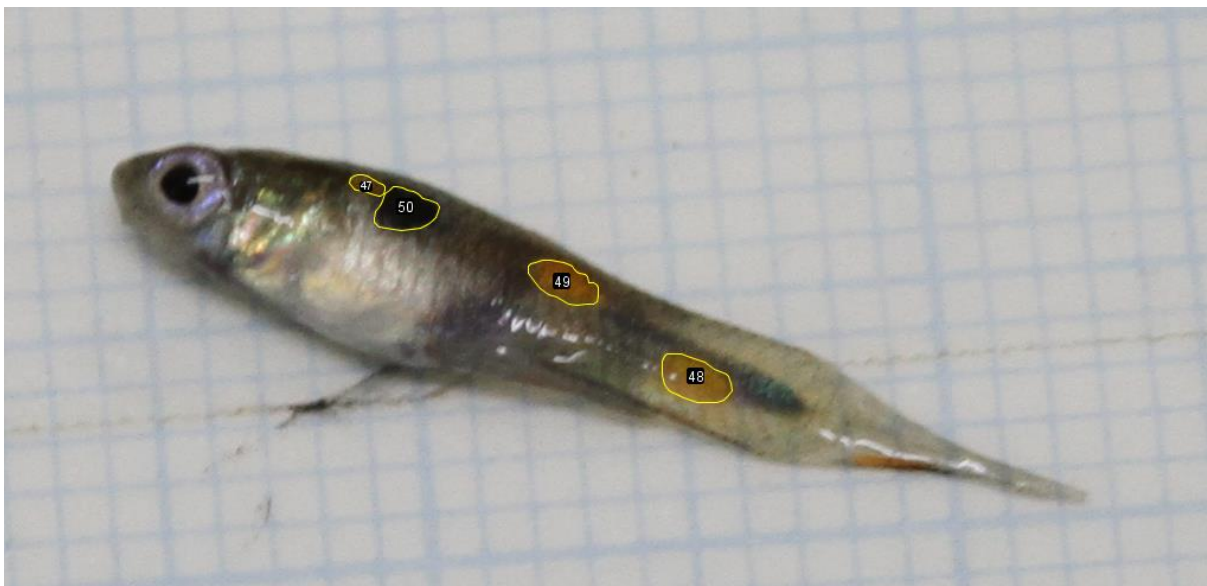

Figure 6. Photograph of a male guppy from the colour analysis (100% magnification) with a dark strip running along the centre of the rear end of the specimen. The dark strip fades into blue colouration and is difficult to identify as black or not. It was therefore left unmeasured.
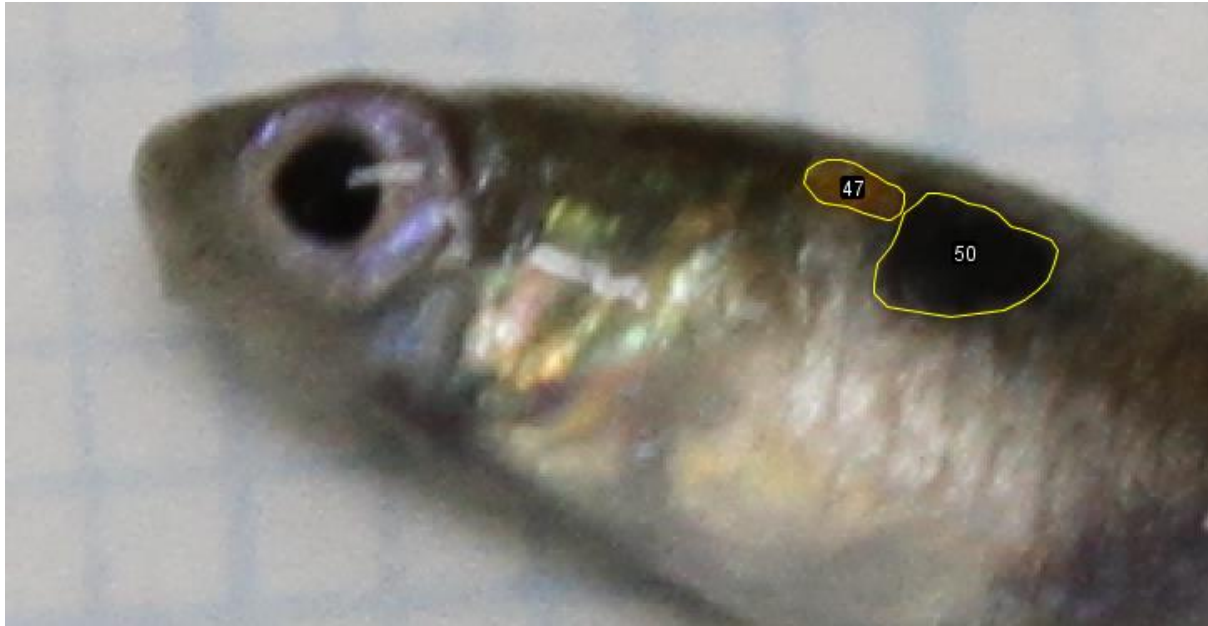
Figure 7. Photograph of the same individual male guppy as in Figure 6 (200% magnification). This photograph shows small patches of orange, which were left unmeasured as they were not easily identifiable at 100% magnification.

Statistical analysis

To calculate a males preference to show one side over the other, the total amount of time a male spent showing its left side to the female (Proximity right + Following right) was subtracted from the total amount of time a male spent showing its right side to the female (Proximity right + Following right). This created a variable where negative values represent an individual's preference to show its left side to a female, and positive values represent an individual's preference to show its right side to a female. This variable was called Side showing preference. All recorded behaviours where the male was in proximity of the female were added together (Proximity left + Proximity neutral + proximity right + following left + following behind + following right) to create a variable called all proximity behaviour, representing the total amount of time individuals spent close to each other in every observation of the behavioural experiment.

In order to test whether male colouration was affected by predation regime or stream, several variables were constructed from the raw data where appropriate to simplify subsequent models. Firstly, the total amount of black and orange on each side of every male was added together to construct a variable for total black colouration and total orange colouration on each specimen. To investigate symmetry in colouration between the left and right side of each male, the difference between black/orange on each side was calculated in two different ways. The total amount of a colour on the left side of every specimen was subtracted from the total amount of the same colour on the right side. This created a variable where negative values indicate that an individual had more of that colour on its left side, and positive value indicate that the individual had more of that colour on its right side. These variables were called black colouration difference and orange colouration difference. The second way in which the difference in colour was calculated was comparable, with the difference being that the absolute number was used for all values (i.e., all negative values were made positive), creating a variable that represents the absolute difference in colour between either side of an individual. These variables were named black colouration symmetry, and orange colouration symmetry. This allowed symmetry to be investigated, regardless of which side of a male had more colour.

To investigate the effect of predation regime and stream on behaviour and colouration, linear models (LMs) were used. Within these models the interaction between predation regime and stream was also

included. If the interaction was non-significant, it was then removed using stepwise backward elimination. Where appropriate, data was square root transformed so that the distribution of residuals of every model remained normal. The normality of residuals was assessed visually using histograms and gg-plots, and statistically with the use of Shapiro tests. LMs were used to investigate the effects of the factors predation regime (high and low) and stream (Turure, Tunapuna/Tacarigua, Aripo) on the constructed variables for colour (total black colouration, total orange colouration, black colouration symmetry and orange colouration symmetry). Male standard length was included as a covariate in all of these models. For side showing preference, an LM was ran with predation regime and stream as fixed factors. Here all proximity behaviour was included as a covariate to control for differences in the total amount of time males spent showing either side. Then, two LMs were used to investigate the effect of predation regime and colouration difference on side showing preference, one with black colouration difference as the response variable, and one with orange colouration difference as the response variable. All proximity behaviour was again included as a covariate here. Three more LMs were similarly used to look for the effect of predation regime, total black colouration and total orange colouration on all proximity behaviour (response). Stream also remained a fixed factor here. Finally, LMs were used to establish whether predation regime and stream had an effect on male standard length and female weight. Tukey's honest significant difference post hoc tests were used to further investigate significant results where appropriate.

Results

Colouration

The total amount of black colouration on male guppies was significantly affected by the interaction between predation regime and stream (P=0.047, Figure 8, Table 4). Post-hoc tests revealed that individuals living in the higher predation environment of the stream Turure (i.e., from the downstream population) had less black colouration than their conspecifics belonging to the low predation environment (the upstream population) in the same stream, and compared to the high and low predation populations in the other streams (Figure 8, Table 5). Black colouration symmetry was also significantly affected by the interaction between predation regime and stream (P=0.019, Figure 9, Table 4). Similarly to total black colouration, a post-hoc test revealed that individuals from the high predation (downstream) environment in the stream Turure were significantly more symmetrical in their black colouration than individuals from low predation (upstream) environments in Turure and in the other streams (Figure 9, Table 6). There was, however, no significant difference in black colouration symmetry between the high predation population from Turure and any of the other high predation populations (Figure 9, Table 6).

Figure 8 The effect of predation regime and stream on total black colouration.
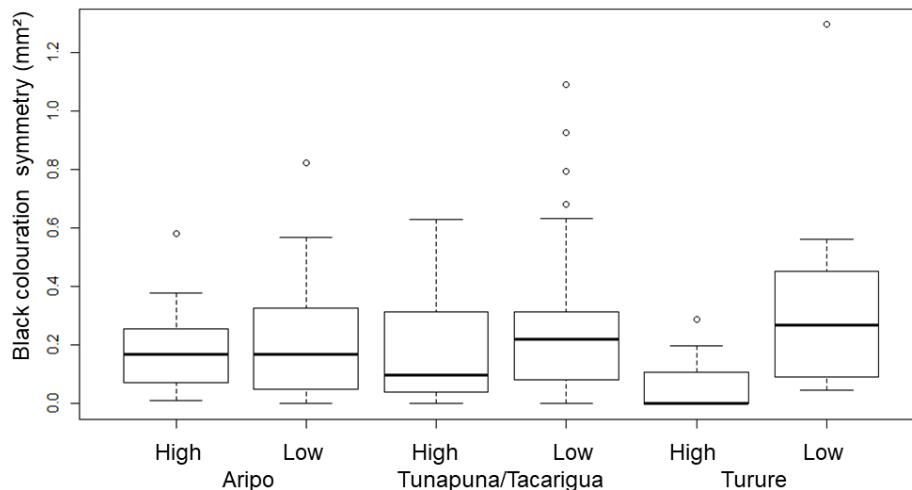
Figure 9. The effect of predation regime and stream on black colouration symmetry.

For the analysis of total amount of orange colouration, the interaction between predation regime and stream was non-significant and therefore removed from the model (P=0.284, Figure 10, Table 4). There was also no significant effect of predation regime alone on total orange colouration (P=0.153, Figure 10, Table 4). Conversely, there was a significant effect of stream (P<0.001, Figure 10, Table 4). A post hoc test revealed that guppies from Turure had significantly less orange than guppies from Aripo (P=0.028, Figure 10, Table 7) and Tunapuna/Tacarigua (P<0.001, Figure 10, Table 7) regardless of predation regime. For the analysis of orange colour symmetry, the interaction between predation regime and stream was again non-significant and removed from the model (P=0.792, Figure 11, Table 4). There was no significant effect of predation regime on orange colouration symmetry (P=0.617, Figure 11, Table 4). Following the same pattern, there was a significant difference in orange colouration symmetry between streams (P=0.001, Figure 11, Table 4). Another post hoc test showed that guppies from Turure had significantly less orange symmetry than guppies from Aripo (P=0.001, Figure 11, Table 8) and Tunapuna/Tacarigua (P=0.031, Figure 11, Table 8) regardless of predation regime.
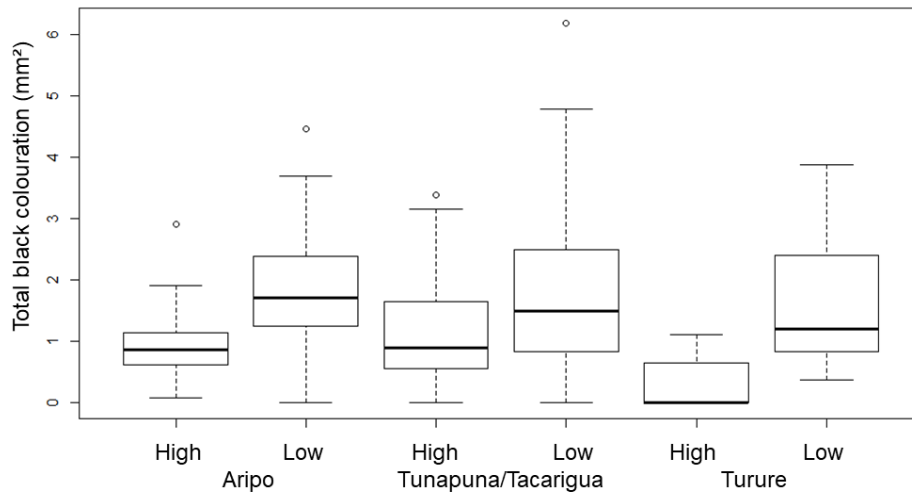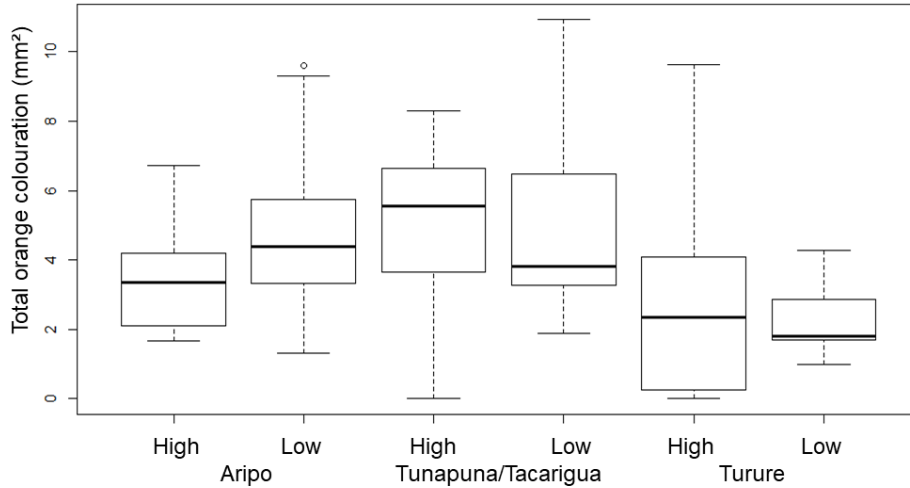
Figure 10. The effect of predation regime and stream on total orange colouration.
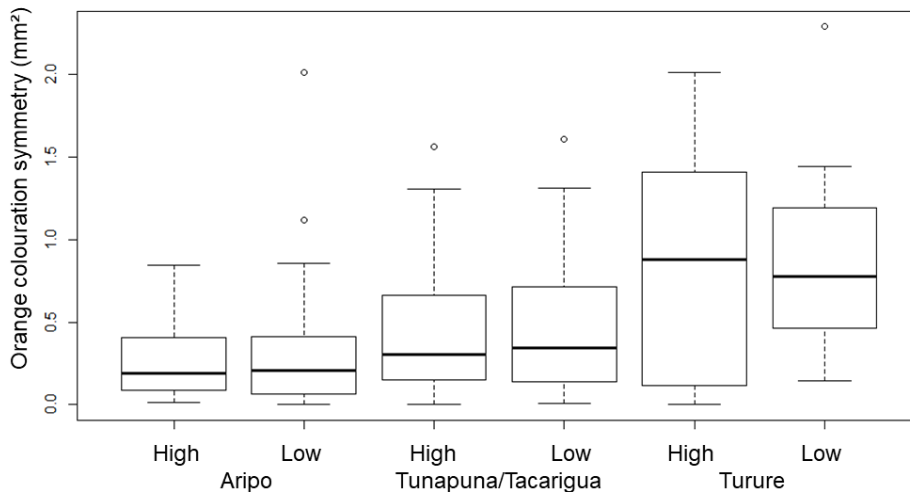
Figure 11. The effect of predation regime and stream on orange colouration symmetry.

Behaviour

The interaction effect of predation regime and stream on the preference of a male to show one side over its other was non-significant and subsequently removed from the model (P=0.231, Figure 12, Table 4). There was also no significant effect of predation regime (P=0.498, Figure 12, Table 4) or stream alone (P=0.805, Figure 12, Table 4) on the side showing preference of males. Likewise, there was no significant interaction effect of predation regime and black (P=0.937, Figure 13A, B, Table 4) or orange (P=0.662, Figure 13C, D, Table 4) colouration difference on the side showing preference of an individual, and so the interaction was removed. There was also no significant effect of black (P=0.443, Table 4) or orange (P=0.230, Table 4) colouration difference alone on the side showing preference of an individual. There was however a significant interaction effect of predation regime and stream on the total amount of time a male spent within 3 body lengths of the female (all proximity behaviour) (P<0.001 Figure 14, Table 4). Specifically, individuals from the low predation (upstream) environment of Turure had significantly lower all proximity behaviour values, than individuals from the high

16

predation (downstream) environment of Turure (P=0.001, Figure 14, Table 9), and the low predation environment of Aripo (P=0.001, Figure 14, Table 9).
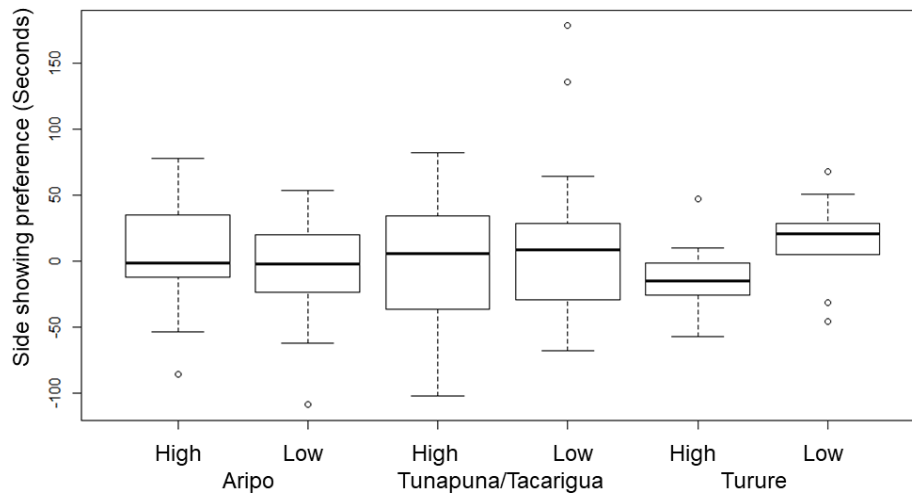
Figure 12. The effect of predation regime and stream on side showing preference.
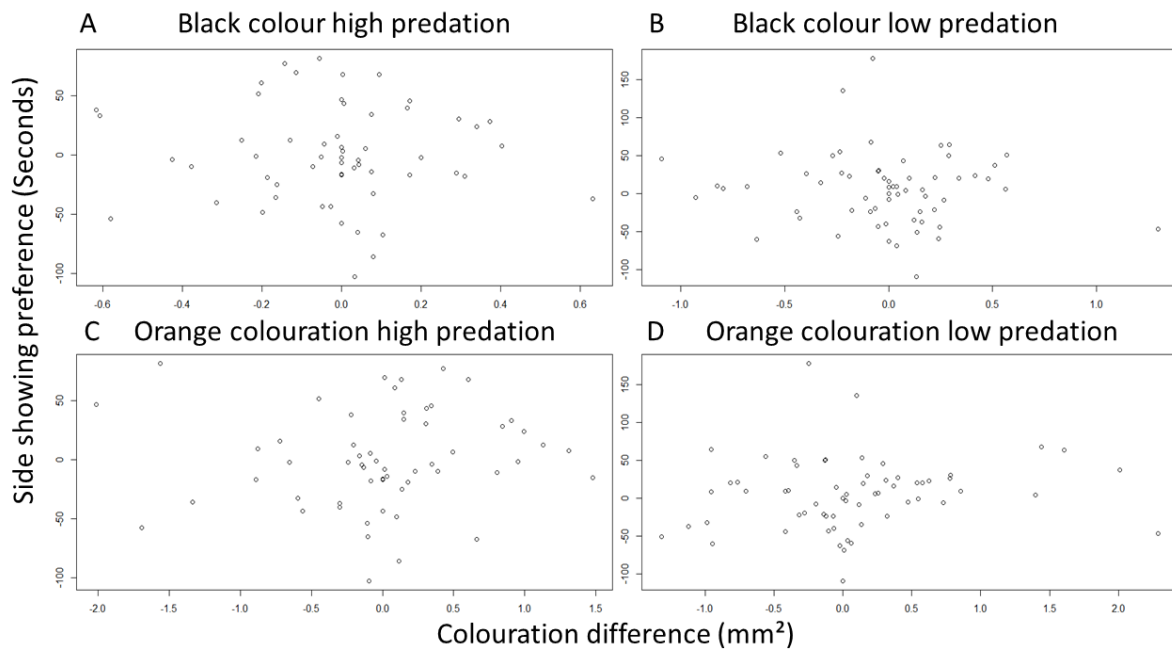
Figure 13. Correlation between black (A, B) and orange (C, D) colouration difference and side showing preference in male guppies from high (A, C) and low (B, D) predation environments. If left or right side showing preference was correlated to the left or right side of the fish with more black or orange coloration, a positive relationship would be present.
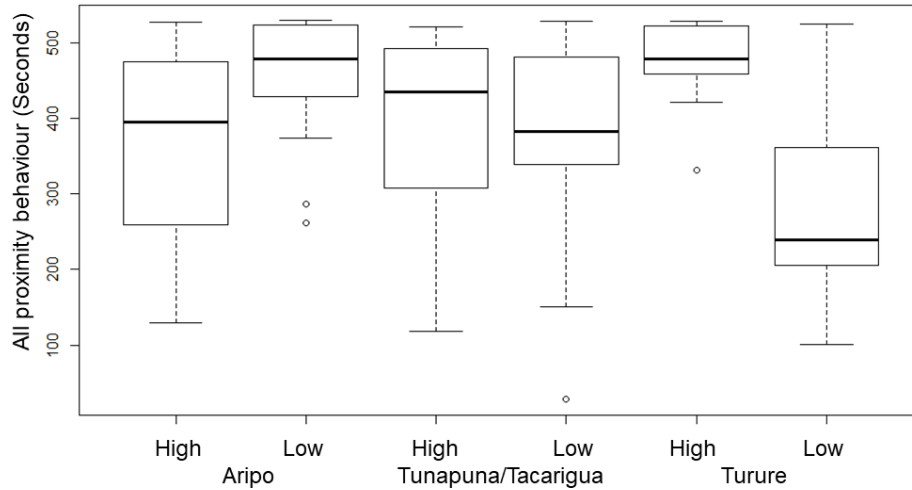
Figure 14. The effect of predation regime and stream on all proximity behaviour.

Size

There was a significant interaction effect of predation regime and stream on both male standard length (P<0.001, Figure 15, Table 4) and female weight (P=0.004, Figure 16, Table 4) Post-hoc tests showed that males from the low predation (upstream) environments of Aripo and Turure were significantly larger than males from any other population (Figure 15, Table 10). There was no significant difference in male standard length between the low predation populations of Aripo and Turure (Figure 15, Table 10). Males from high predation (downstream) environments in Aripo were significantly smaller than males from any other population (Figure 15, Table 9). There was no significant difference in size between males from Tunapuna/Tacarigua (regardless of predation regime) and males from the high predation (downstream) environment of Turure (Figure 15, Table 10). Furthermore, females from the lower predation (upstream) environments of Aripo were significantly larger than females from any other population (Figure 16, Table 11). Conversely, females from the high predation (downstream) environments of Tunapuna/Tacarigua were smaller than females from almost every other population, with the exception of the high predation (downstream) Aripo population (P=0.050, Figure 16, Table 11).
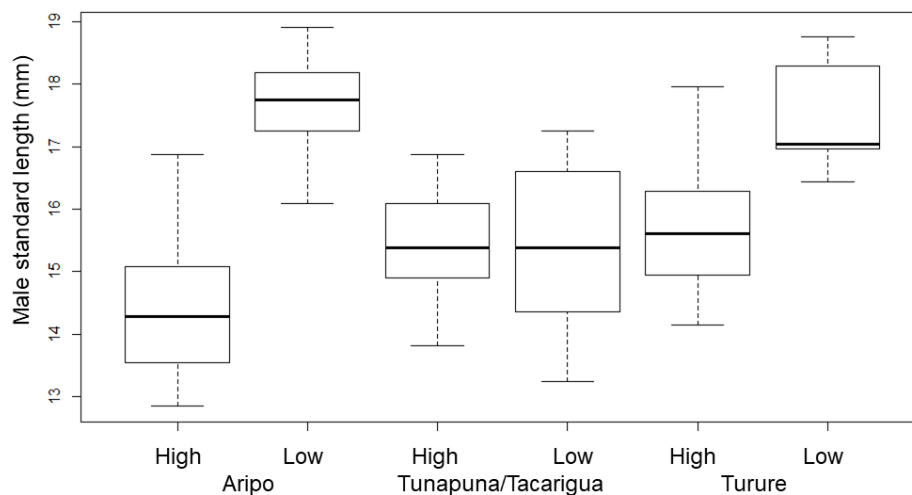
Figure 15. The effect of predation regime and stream on male standard length.
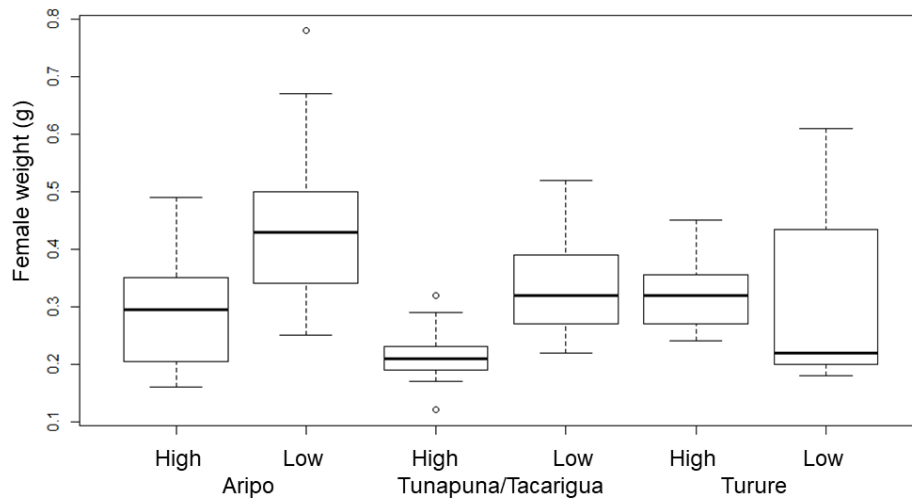
Figure 16. The effect of predation regime and stream on female weight.

Female aggression

It should be noted that while female aggression occurred too infrequently to analyse, it was mostly recorded in individuals from Tunapuna/Tacarigua. 73% (11/15) of replicates where at least one female bite was observed, and 100% (4/4) of replicates where female chasing was observed were conducted using individuals from the Tunapuna/Tacarigua population.

Table 4. Statistical model outputs. See Table 2 for sample sizes. Significant results highlighted in bold.

Response : predictor/covariate	F	Df	P
Total black colouration			
-Predation regime	6.862	1	**0.010**
-Stream	6.328	2	**0.003**
-Male standard length	5.190	1	**0.025**
-Predation regime x stream	3.151	2	**0.047**
Black colouration symmetry			
-Predation regime	7.362	1	**0.008**
-Stream	0.511	2	0.602
-Male standard length	0.389	1	0.534
-Predation regime x stream	4.139	2	**0.019**
Total orange colouration			
-Predation regime	2.069	1	0.153
-Stream	14.323	2	**<0.001**
-Male standard length	14.939	1	**<0.001**
-Predation regime x stream	1.272	2	0.284
Orange colouration symmetry			
-Predation regime	0.252	1	0.617
-Stream	7.283	2	**0.001**

-Male standard length	0.071	1	0.421
-Predation regime x stream	0.234	2	0.792
Side showing preference			
-Predation Regime	0.462	1	0.498
-Stream	0.217	2	0.805
-Black colouration difference	0.592	1	0.443
-Orange colouration difference	1.456	1	0.230
-Predation regime x stream	1.487	2	0.231
-Predation regime x black colouration difference	0.006	1	0.937
-Predation regime x orange colouration difference	0.192	1	0.662
All Proximity Behaviour			
-Predation regime	0.126	1	0.724
-Stream	0.866	2	0.424
-Predation x stream	12.860	2	**<0.001**
-Total black colouration	0.001	1	0.974
-Total orange colouration	0.974	1	0.326
Male Standard length			
-Predation regime	72.077	1	**<0.001**
-Stream	13.373	2	**<0.001**
-Predation x stream	34.359	2	**<0.001**
Female Weight			
-Predation regime	43.048	1	**<0.001**
-Stream	9.563	2	**<0.001**
-Predation x stream	5.833	2	**0.004**

Table 5. Post-hoc comparison of total black colouration between predation regimes and streams. Significant results highlighted in bold.

Total black colouration post-hoc comparison	Diff	P value
High predation Turure - Low predation Turure	0.191	**0.002**
High predation Turure - Low predation Aripo	-0.211	**<0.001**
High predation Turure - High predation Aripo	-0.148	**0.009**
High predation Turure - Low predation Tunapuna/Tacarigua	-0.217	**<0.001**
High predation Turure - High predation Tunapuna/Tacarigua	-0.161	**0.002**
Low predation Turure - Low predation Aripo	-0.020	0.997
Low predation Turure - High predation Aripo	0.043	0.912
Low predation Turure - Low predation Tunapuna/Tacarigua	-0.026	0.986
Low predation Turure - High predation Tunapuna/Tacarigua	0.030	0.976
High predation Aripo - Low predation Aripo	0.063	0.467
High predation Aripo - Low predation Tunapuna/Tacarigua	0.069	0.294
High predation Aripo - High predation Tunapuna/Tacarigua	0.013	0.999
Low predation Aripo - Low predation Tunapuna/Tacarigua	0.007	0.998
Low predation Aripo - High predation Tunapuna/Tacarigua	-0.050	0.645
High predation Tunapuna/Tacarigua - Low predation Tunapuna/Tacarigua	0.057	0.444

Table 6. Post-hoc comparison of black colouration symmetry between predation regimes and streams.
Significant results highlighted in bold.

Black colouration symmetry post-hoc comparison	Diff	P value
High predation Turure - Low predation Turure	0.373	**0.005**
High predation Turure - Low predation Aripo	-0.257	**0.046**
High predation Turure - High predation Aripo	-0.237	0.092
High predation Turure - Low predation Tunapuna/Tacarigua	-0.288	**0.012**
High predation Turure - High predation Tunapuna/Tacarigua	-0.196	0.206
Low predation Turure - Low predation Aripo	0.115	0.777
Low predation Turure - High predation Aripo	0.135	0.653
Low predation Turure - Low predation Tunapuna/Tacarigua	0.085	0.916
Low predation Turure - High predation Tunapuna/Tacarigua	0.177	0.312
High predation Aripo - Low predation Aripo	0.020	0.999
High predation Aripo - Low predation Tunapuna/Tacarigua	0.050	0.978
High predation Aripo - High predation Tunapuna/Tacarigua	-0.041	0.992
Low predation Aripo - Low predation Tunapuna/Tacarigua	0.030	0.998
Low predation Aripo - High predation Tunapuna/Tacarigua	-0.062	0.947
High predation Tunapuna/Tacarigua - Low predation Tunapuna/Tacarigua	0.092	0.716

Table 7. Post-hoc comparison of total orange colouration between streams. Significant results
highlighted in bold.

Total orange colouration post-hoc comparison	Diff	P value
Turure - Aripo	-1.494	**0.028**
Turure - Tunapuna/Tacarigua	-2.228	**<0.001**
Aripo - Tunapuna/Tacarigua	0.735	0.234

Table 8. Post-hoc comparison of orange colouration symmetry between streams. Significant results
highlighted in bold.

Orange colouration symmetry post-hoc comparison	Diff	P value
Turure - Aripo	-0.138	**0.001**
Turure - Tunapuna/Tacarigua	-0.190	**<0.001**
Aripo - Tunapuna/Tacarigua	0.052	0.182

Table 9. Post-hoc comparison of all proximity behaviour between predation regimes and streams.
Significant results highlighted in bold.

All proximity behaviour post-hoc comparison	Diff	P value
High predation Turure - Low predation Turure	8.858	**0.001**
High predation Turure - Low predation Aripo	-1.005	0.994
High predation Turure - High predation Aripo	-5.373	0.052
High predation Turure - Low predation Tunapuna/Tacarigua	-4.525	0.114
High predation Turure - High predation Tunapuna/Tacarigua	-4.038	0.217
Low predation Turure - Low predation Aripo	7.852	**0.001**
Low predation Turure - High predation Aripo	3.485	0.424
Low predation Turure - Low predation Tunapuna/Tacarigua	4.332	0.146
Low predation Turure - High predation Tunapuna/Tacarigua	4.820	0.082
High predation Aripo - Low predation Aripo	-4.368	0.057
High predation Aripo - Low predation Tunapuna/Tacarigua	-0.848	0.992
High predation Aripo - High predation Tunapuna/Tacarigua	-1.335	0.944
Low predation Aripo - Low predation Tunapuna/Tacarigua	3.520	0.135
Low predation Aripo - High predation Tunapuna/Tacarigua	3.033	0.288
High predation Tunapuna/Tacarigua - Low predation Tunapuna/Tacarigua	0.488	0.999

Table 10. Post-hoc comparison of male standard length between predation regimes and streams. Significant results highlighted in bold.

Male standard length post-hoc comparison	Diff	P value
High predation Turure - Low predation Turure	1.782	**0.001**
High predation Turure - Low predation Aripo	-1.997	**<0.001**
High predation Turure - High predation Aripo	1.371	**0.004**
High predation Turure - Low predation Tunapuna/Tacarigua	0.284	0.965
High predation Turure - High predation Tunapuna/Tacarigua	0.303	0.956
Low predation Turure - Low predation Aripo	-0.216	0.991
Low predation Turure - High predation Aripo	3.154	**<0.001**
Low predation Turure - Low predation Tunapuna/Tacarigua	2.066	**<0.001**
Low predation Turure - High predation Tunapuna/Tacarigua	2.085	**<0.001**
High predation Aripo - Low predation Aripo	3.368	**<0.001**
High predation Aripo - Low predation Tunapuna/Tacarigua	1.086	**0.004**
High predation Aripo - High predation Tunapuna/Tacarigua	1.068	**0.005**
Low predation Aripo - Low predation Tunapuna/Tacarigua	-2.282	**<0.001**
Low predation Aripo - High predation Tunapuna/Tacarigua	-2.300	**<0.001**
High predation Tunapuna/Tacarigua - Low predation Tunapuna/Tacarigua	0.019	0.999

Table 11. Post-hoc comparison of female weight between predation regimes and streams. Significant results highlighted in bold.

Female weight post-hoc comparison	Diff	P value
High predation Turure - Low predation Turure	-0.009	0.999
High predation Turure - Low predation Aripo	-0.096	**0.025**
High predation Turure - High predation Aripo	0.032	0.908
High predation Turure - Low predation Tunapuna/Tacarigua	-0.019	0.986
High predation Turure - High predation Tunapuna/Tacarigua	0.103	**0.009**
Low predation Turure - Low predation Aripo	-0.105	**0.011**
Low predation Turure - High predation Aripo	0.023	0.975
Low predation Turure - Low predation Tunapuna/Tacarigua	-0.028	0.934
Low predation Turure - High predation Tunapuna/Tacarigua	0.095	**0.023**
High predation Aripo - Low predation Aripo	0.128	**<0.001**
High predation Aripo - Low predation Tunapuna/Tacarigua	0.051	0.287
High predation Aripo - High predation Tunapuna/Tacarigua	-0.071	0.050
Low predation Aripo - Low predation Tunapuna/Tacarigua	-0.077	**0.018**
Low predation Aripo - High predation Tunapuna/Tacarigua	-0.120	**<0.001**
High predation Tunapuna/Tacarigua - Low predation Tunapuna/Tacarigua	0.123	**<0.001**

Discussion

The presence of predators is known to affect the morphology and behaviour of prey (Endler, 1978; Komers, 1997). I hypothesized that differences in morphology and behaviour would be noticeable between individuals taken from low and high predation environments. The majority of results however showed no significant effects of predation regime on morphology and behaviour, and for the significant effects that were detected there was great variation between streams.

Morphology

There was an interaction effect of predation regime and stream on both total black colouration and black colouration symmetry. Further investigation revealed that individuals from the higher predation environment of Turure had significantly less total black colouration than all other populations. Individuals from this same environment also had significantly more black colouration symmetry than their conspecifics from low predation environments. This pattern might be caused by this population having many individuals with no black colouration (High predation Aripo: 0/20, Low predation Aripo: 1/22, High predation Tunapuna/Tacarigua: 1/26, Low predation Tunapuna/Tacarigua: 2/28, High predation Turure: 6/11, Low predation Turure: 0/11), heavily influencing the mean total colouration, and representing perfect symmetry due to the methods used to calculate colouration symmetry. There were no other significant differences in total black colouration or black colouration symmetry. Furthermore, there was a significant effect of stream on total orange colouration and orange symmetry, while predation regime had no effect. Males from the stream Turure had significantly less total orange colouration, as well as significantly less orange colouration symmetry than males from the other streams. This was the only significant difference in total orange colouration and orange colouration symmetry revealed by the models.

There was also an interaction between predation regime and stream on both male standard length and female weight. Males from the lower predation environments of Aripo and Turure were significantly larger than males from all other environments, while males from the higher predation environment of Aripo were significantly smaller than males from the other environments. Female weight followed a different pattern. Females from the lower predation environment of Aripo were significantly heavier

than females from all other environments, while females from the higher predation environment of Tunapuna/Tacarigua were significantly smaller than females from all other environments with the exception of the higher predation environment of Aripo. Potentially, these results could also suggest that there are significant ecological differences between streams.

Any amount of colouration that makes an individual stand out in its environment will also make the individual easier for visually oriented predators to detect, and attack (Endler, 1978). Colour also plays a vital role in social interactions between guppies and is especially important in males (Houde, 1997 and references therein). Guppies in high predation environments are therefore faced with a trade-off between having inconspicuous colouration to avoid predation and using eye-catching sexual ornamentation in social interactions (Endler, 1991). I would therefore predict that males from high predation environments would have significantly less total colouration, as the value of inconspicuous colouration is theoretically greater than that of sexual ornamentation in such environments (Endler, 1978, 1983). Males from low predation environments would not face the same trade-off, as elaborate colouration would theoretically be less detrimental to their survival (Endler, 1978, 1983). I would expect individuals from these environments to be more colourful (Endler, 1978, 1983). However, since males from only the Turure population showed significantly less total black colouration under higher levels of predation, while this could not be seen in the total orange colouration of any population, those theoretical predictions based on previous literature were not supported overall by the data.

The significantly lower quantity of total black colouration observed in individuals from the higher predation environment of Turure could indicate that males from this environment have reduced total black colouration to minimize the risk of detection by an increased predation threat. For example, a lower turbidity would make it easier for visually oriented predators to detect guppies (Endler, 1987).Furthermore, males from this population were significantly smaller than males from the low predation environment of the same stream. Some prey species increase in size in response to predation pressure (Richards & Bull, 1990) thereby preventing consumption by gape limited predators. Previous literature however suggests that guppies are generally smaller in the presence of predators, as larger individuals are preferentially consumed (Endler, 1978). The latter is supported by the data, suggesting that the differences in colour and size in males from the stream Turure most likely is a response to predatory threat. I had anticipated a similar pattern for female size (female weight), however this was not the case. This contradictory result could be explained by the difference in predation pressure experience by male and female guppies. Females generally lead predation inspection and could therefore be considered to be more aware of predatory threats than male conspecifics (Magurran & Nowak, 1991). Conversely, males may experience greater predation pressure due to their colourful sexual ornamentation (Endler, 1983). This could explain why male standard length was affected by predation pressure in Turure, while female weight was not. The lower total orange colouration of males from Turure regardless of predation regime may suggest that orange colouration is either more detrimental to survival in Turure than in Aripo or Tunapuna/Tacarigua, regardless of predation regime, or that the dietary components needed to express orange colouration (Grether *et al*., 1999) are scarce throughout this stream. Either way this could suggest that there are ecological differences between streams.

Symmetry can be a desirable trait in a mate and is likely preferred by female guppies (Sheridan and Pomiankowski, 1997). When predation is low and competition between males for mates is high, I would expect symmetry to favoured by sexual selection (Endler, 1995). Symmetry in black and orange colouration varied between stream and predation regime in a similar manner to total colouration. Contrary to my predictions and previous literature, black colouration symmetry was significantly greater in the high predation environment of Turure than in any other low predation environment. As previously discussed, a greater proportion of males from high predation Turure expressed no black colouration compared to other populations. While this was not statistically tested, the perfect symmetry value produced by individuals with no black colouration could potentially mis-represent differences in

black colouration symmetry between predation regimes and streams. Conversely, males from Turure, regardless of predation regime, were significantly less symmetrical in their orange colouration than males from Aripo and Tunapuna/Tacarigua. This could well compliment the aforementioned theory, that total orange colouration is reduced in Turure. Regardless of why total orange colouration may be reduced in Turure, I would expect a population with less orange colouration to have a reduced orange colouration symmetry. By concentrating colouration onto one side, and preferentially showing this side, a male guppy could create the illusion to females that he has large quantities of orange colouration, and is therefore an optimal choice as a mate (Gross *et al*., 2007). If this was the case however, I would also expect males to consistently show their most colourful side, which was not the case.

Behaviour

There was no significant effect of predation regime, black colouration difference or orange colouration difference on a male's preference to show one side more than the other. The Interaction effects between predation regime and black/orange colouration difference were also non-significant. There was however a significant interaction effect between predation regime and stream on the total amount of time a male spent within proximity of a female. Pairs from the low predation environment of Turure spent significantly less time in proximity of one another than pairs from the high predation environment of Turure.

Contrary to my hypotheses and past literature (Gross *et al.*, 2007. Amcoff *et al.,* 2009. Rezucha and Reichard, 2015), I found no relationship between a males morphological "best side" and the side a male chooses to display most frequently to a female conspecific, regardless of if best side was defined by black or orange colouration difference, and regardless of predation regime. This would suggest that the colouration difference between each side of a male guppy does not affect its preference to show its best side, regardless of predation regime. While this does largely contradict past literature, Rezucha and Reichard (2015) specifically Identified that *Poecilia wingei* males only consistently showed their best side when other males were present. As the methodology of this investigation used only a single male and female guppy per behavioural trial, it is possible that the lack of competition is the reason why males were not consistently observed showing their best side. When reviewing previous papers there are some experimental differences which could also explain these inconsistent results. The specimens used in previous investigations were lab-reared (Gross *et al*., 2007. Amcoff *et al*., 2009. Rezucha and Reichard, 2015), which may have had an effect on the results. Several important factors can be controlled in lab-reared stocks, such as virginity, prior exposure to predation and to an extent the health of individuals, yet studies using wild caught specimens are still useful as such results can be expected to be more representative of the natural environment. It is possible that one or more of the controlled factors in a laboratory could explain why the results of this study differ to previous papers (Gross *et al*., 2007. Amcoff *et al*., 2009. Rezucha and Reichard, 2015). Ultimately, using lab-reared and wild-caught specimens is necessary to obtain a more comprehensive understanding of the natural world, as this study indicates when put in the context of previous literature. Further research is necessary to advance this field of study, evident due to the aforementioned contrast in results, and lack of research in this area.

Models investigating factors affecting all proximity behaviour did produce significant results. Pairs from the higher predation environment of Turure spent significantly more time in proximity of each other compared to pairs from the lower predation environment of Turure. All proximity behaviour in Aripo and Tunapuna/Tacarigua was not affected by predation regime. Furthermore, there were no significant differences between streams. By measuring the time the pair spent in proximity of each other (all proximity behaviour) I obtained data representative of schooling behaviour, an anti-predatory adaptation. The trend observed in Turure is consistent with past literature(Magurran & Seghers, 1991; Seghers, 1974), that individuals from high predation environments spend more time schooling than conspecifics from low predation environments. It should be noted that all proximity behaviour could also represent a male's intention to court the female, rather than the pairs intention to school. However

the amount of time spent in proximity should theoretically still represent the tendency of the individuals to school through the behaviour of the female, as the female is more likely to stay in proximity when schooling, than when being courted (Magurran & Seghers, 1994b). While this study may be limited in its contributions to existing knowledge on the topic of behaviour, this is yet another result potentially highlighting ecological differences between streams.

Aggressive behaviour has been identified to vary between streams. Magurran and Seghers (1991) identified males from the stream Tunapuna to be significantly more aggressive than males from the streams Turure and Aripo. While I did not observe aggression in males during this study, aggression in females was observed, predominantly in females from Tunapuna/Tacarigua. In the same study, Magurran and Seghers (1991) also investigated whether female aggression varied between streams. However this was tested on a smaller subset of streams, of which Tunapuna and Turure were not included. Their results indicated that female aggression also varies between streams. It may be that both males and females from Tunapuna/Tacarigua exhibit greater aggression than in most other streams. One potential explanation for this could be the water quality, which was identified by Pettersson et al., (2004) to be poor in Tacarigua, potentially increasing competition and the need for aggressive interactions. While I am only able to speculate here due to the lack of sufficient female aggression observations, the data suggests that further investigation into the factors influencing female aggression in guppies could yield useful results.

By contributing to the knowledge of behaviour in guppies, this investigation further develops a useful study system, with great value to future research. The contrasting results of this study compared to previous literature suggest that morphology, behaviour, predation pressure and their complex interactions should be carefully considered in future ecological studies, especially those using model organisms with highly variable colouration and symmetry, such as *P. reticulata*. This is particularly important due to the effect guppy phenotype can have on an ecosystem. Bassar et al., (2010) showed that a range of variables such as algal and invertebrate standing stocks, gross primary productivity and nutrient cycling, can be significantly affected by the phenotype of guppies present (I.e. low predation or high predation phenotype guppies). This further emphasises the significance of understanding this study system, and its value to ecological studies.

Differences between streams

For most of the results in this study, morphology and behaviour were stream dependent. The variation between streams becomes more interesting when geography is taken into consideration. Significant differences were seen mainly within Turure only (total black colouration, black colouration symmetry, all proximity behaviour), or between Turure and the other streams (total orange colouration, orange colouration symmetry). A noteworthy difference between Turure and the other streams is that they are geographically isolated from one another. Aripo and Tunapuna/Tacarigua are tributaries of the Caroni drainage, while Turure is a tributary of the Oropuche drainage. There is no connection between the Caroni drainage and the Oropuche drainage. Differences between tributaries within the same drainages (Bassar et al., 2010; Pettersson, Ramnarine, Becher, Mahabir, & Magurran, 2004) and between the Caroni and Oropuche drainages (Neff *et al.*, 2008; El-sabaawi *et al.*, 2012) have been identified, prompting the need for further study. These environmental differences may mask the predicted observable trends in behaviour and morphology variation between predation regimes.

A handful of transplants of guppies between populations have occurred in these north Trinidadian streams. In 1976, an introduction of 200 guppies took place (Endler, 1980). The fish were taken from downstream (high predation) Aripo and moved to an upstream (low predation) area of Aripo, where guppies had previously been absent. As this may have ultimately resulted in the mixing of high and low predation populations in Aripo, it could potentially explain the lack of significant differences in

behaviour and colouration between upstream and downstream (low predation and high predation) Aripo. Earlier, in 1957, 200 guppies were taken from a downstream (high predation) location of the stream Arima, located in the Caroni drainage, and relocated to an upstream (low predation) site of Turure, also previously uninhabited by guppies (Carvalho, Shaw, Hauser, Seghers, & Magurran, 1996). The effects of the 1957 transplant are observable in more recent genetic analyses (Shaw, Carvalho, Magurran, & Seghers, 1991; Suk & Neff, 2009).

Studies into the genetics of the guppy populations present in the Caroni and Oropuche drainages have interestingly revealed that individuals from the stream Turure are actually more closely related to populations from the Caroni drainage than they are to other populations from the Oropuche drainage (Shaw *et al*., 1991; Suk & Neff, 2009). It could be assumed that this apparent anomaly is the result of the aforementioned introduction in 1957 (Carvalho *et al.,* 1996). As the guppies were introduced upstream (low predation), it could also be argued that the significant differences recorded between high and low predation environments are actually a result of a comparison between a Caroni-Oropuche mixed population, and a pure Oropuche drainage. Arguing against this however, Suk and Neff (2009) also showed that both upstream and downstream (low predation and high predation) Turure populations are more closely related to each other than any other population. While these artificial introductions and the genetic evidence of similarity may suggest that the populations of Turure, Aripo and Tunapuna/Tacarigua are not significantly different, the environments the different stream populations are exposed to could certainly vary enough to induce significant differences in behaviour and morphology (Pettersson *et al*., 2004; Neff *et al*., 2008; Bassar *et al*., 2010; El-sabaawi *et al*., 2012). Furthermore, the level of anthropogenic disturbance could vary between these drainages, thus it is worth noting that the Caroni drainage is closer to the more heavily developed areas of Trinidad.

Conclusion

While the high and low predation regimes of Trinidadian streams is a popular and useful study system, the present study emphasises a potentially overlooked detail, that significant variation exists between streams and drainages, which could potentially lead to inaccurate interpretations if not accounted for. The results from this investigation matched the predictions for one of the three studied streams, Turure, the only stream from the Oropuche drainage studied. This could suggest that the Caroni drainage populations are subject to significantly different environmental conditions which mask expected trends, perhaps due to anthropogenic disturbance. Studies which yield consistent results using guppies from the Caroni drainage, the Oropuche drainage, and ideally other Trinidadian populations can be confident in saying that their results are due to high and low predation pressure, and not an artefact of some stream or drainage variable that was not accounted for. Future studies may wish to investigate the observable differences in the results of experiments using lab reared and wild caught guppy populations. This could be achieved either in the form of a literature summary, or by conducting experiments, e.g. replicating the methodology of this study, using lab reared and wild caught guppies for a direct comparison. Furthermore, multiple investigations quantifying the environmental variation between streams and thereby quantifying the environmental variation between the Caroni and Oropuche drainages would be beneficial to development of this valuable study system.

References

Amcoff, M., Arnqvist, G., & Kolm, N. (2009). Courtship signalling with a labile bilateral signal : males show their best side. *Behavioral Ecology and Sociobiology*, *63*, 1717–1725.

Bassar, R. D., Marshall, M. C., López-sepulcre, A., Zandonà, E., Auer, S. K., Travis, J., … Reznick, D. N. (2010). Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences*, *107*, 3616–3621.

Carvalho, G. R., Shaw, P. W., Hauser, L., Seghers, B. H., & Magurran, A. E. (1996). Artificial introductions , evolutionary change and population differentiation in Trinidadian guppies (Poecilia reticulata : Poeciliidae). *Biological Journal of the Linnean Society*, *57*, 219–234.

De Santi, A., Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2000). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Italian Journal of Zoology*, *67*, 175–178.

Doorn, G. S. Van, & Weissing, F. J. (2015). Sexual Conflict and the Evolution of Female Preferences for Indicators of Male Quality. *The American Naturalist*, *168*, 742–757.

El-sabaawi, R. W., Travis, J., Marshall, M. C., Kohler, T. J., Zandona, E., Thomas, S. A., … Flecker, A. S. (2012). Environmental and Organismal Predictors of Intraspecific Variation in the Stoichiometry of a Neotropical Freshwater Fish. *PLoS ONE*, *7*, e32713.

Endler, J. A. (1978). A Predator ' s View of Animal Color Patterns. *Evolutionary Biology*, *11*, 319–364.

Endler, J. A. (1980). Natural selection on colour patterns in Poecilia reticulata. *Evolution*, *34*, 76–91.

Endler, J. A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes*, *9*, 173–190.

Endler, J. A. (1987). Predation , light intensity and courtship behaviour in Poecilia reticulata (Pisces : PoecUiidae). *Animal Behaviour*, *35*, 1376–1385.

Endler, J. A. (1991). Variation in the appearance of Guppy color patterns to Guppies and their predators under different visual conditions. *Vision Research*, *31*, 587–608.

Endler, J. A. (1995). Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution*, *10*, 22–29.

Farr, J. A. (1975). The role of predation in the evolution of social behaviour of natural populations of the guppy Poecilia reticulata (pisces: poeciliidae). *Evolution*, *29*, 151–158.

Friard, O., & Gamba, M. (2016). BORIS : a free , versatile open-source event-logging software for video / audio coding and live observations. *Methods in Ecology and Evolution*, *7*, 1325–1330.

Grether, G. F., Hudon, J., & Millie, D. F. (1999). Carotenoid Limitation fo Sexual Coloration along an Environmental Gradient in Guppies. *Proceedings of the Royal Society B*, *266*, 1317–1322.

Gross, M. R., Suk, H. Y., & Robertson, C. T. (2007). Courtship and genetic quality: asymmetric males show their best side. *Proceedings of the National Academy of Sciences*, *274*, 2115–2122.

Houde, Anne, E. (1997). *Sex, Color, and Mate Choice in Guppies*.

Komers, P. E. (1997). Behavioural plasticity in variable environments. *Canadian Journal of Zoology*, *75*, 161–169.

Magurran, A. E. (2005). *Evolutionary Ecology : The Trinidadian Guppy*.

Magurran, A. E., & Nowak, M. A. (1991). Another Battle of the Sexes : The Consequences of Sexual Asymmetry in Mating Costs and Predation Risk in the Guppy , Poecilia reticulata. *Proceedings*

of the Royal Society B, *246*, 31–38.

Magurran, A. E., & Seghers, B. H. (1991). Variation in Schooling and Aggression Amongst Guppy (Poecilia reticulata) Populations in Trinidad Author(s): Anne E. Magurran and Benoni H. Seghers Source: *Behaviour*, *118*, 214–234.

Magurran, A. E., & Seghers, B. H. (1994a). Predator Inspection Behaviour Covaries with Schooling Tendency Amongst Wild Guppy , Poecilia reticulata , Populations in Trinidad. *Behaviour*, *128*, 121–134.

Magurran, A. E., & Seghers, B. H. (1994b). Sexual Conflict as a Consequence of Ecology : Evidence from Guppy , Poecilia reticulata , Populations in Trinidad. *Proceedings of the Royal Society B*, *255*, 31–36.

Millar, N. P., Reznick, D. N., Kinnison, M. T., Hendry, A. P., & Persson, L. (2006). Disentangling the Selective Factors That Act on Male Colour in Wild Guppies. *Oikos*, *113*(1), 1–12.

Møller, A. E., & Pomiankowski, A. (1993). Fluctuating asymmetry and sexual selection. *Genetica*, *89*, 267–279.

Neff, B. D., Pitcher, T. E., & Ramnarine, I. W. (2008). Inter-population variation in multiple paternity and reproductive skew in the guppy. *Molecular Ecology*, *17*, 2975–2984.

Pettersson, L. B., Ramnarine, I. W., Becher, S. A., Mahabir, R., & Magurran, A. E. (2004). Sex Ratio Dynamics and Fluctuating Selection Pressures in Natural Populations of the Sex ratio dynamics and fluctuating selection pressures in natural populations of the Trinidadian guppy , Poecilia reticulata. *Behavioral Ecology and Sociobiology*, *55*, 461–468.

Rezucha, R., & Reichard, M. (2015). Strategic exploitation of fluctuating asymmetry in male Endler ' s guppy courtship displays is modulated by social environment. *Journal of Evolutionary Biology*, *28*(2), 356–367.

Richards, S. J., & Bull, C. M. (1990). Size-Limited Predation on Tadpoles of Three Australian Frogs. *Copeia*, *1990*, 1041–1046.

Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2004). Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning signals and Mimicry.

Schindelin, J., Arganda-carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji : an open-source platform for biological-image analysis. *Nature Methods*, *9*, 676–682.

Seghers, B. H. (1974). schooling behavior in the guppy (poecilia reticulata): an evolutionary response to predation. *Evolution*, *28*, 486–489.

Shaw, P. W., Carvalho, G. R., Magurran, A. E., & Seghers, B. H. (1991). Population differentiation in Trinidadian guppies (Poecilia reticulata): patterns and problems. *Journal of Fish Biology*, *39*, 203–209.

Sheridan, L., & Pomiankowski, A. (1997). Female choice for spot asymmetry in the Trinidadian guppy. *Animal Behaviour*, 1523–1529.

Suk, H. Y., & Neff, B. D. (2009). Microsatellite genetic differentiation among populations of the Trinidadian guppy. *Heredity*, *102*, 425–434.

Watson, P. J., & Thornhill, R. (1994). selection. *Trends in Ecology & Evolution*, *9*, 21–25.