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# How the guppy got his spots.

Practical Report

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## Introduction

Male guppies (*Poecilia reticulata*) have brightly coloured spots, whereas females are cryptically coloured. This sexual dimorphism is likely a result of bright spots being used by males to attract a mate. However, observations of natural guppy populations in a system of river beds show differences in distributions of male spot brightness (figure 1). In this system the Lower East was fed by the Upper East, as well as by Meir Creek (where no guppies were recorded) and the Lower West was fed by the Upper West. Both lower river sections were separated from the upper sections by large waterfalls; lower populations were affected by upper populations but there was no migration of individuals upstream.

In these preliminary observations location explained substantially more variability than what we would expect to see by chance (ANOVA,  $Df = 3$ ,  $F = 350.63$ ,  $P < 0.001$ ). Differences in community composition and environment were also observed (table 1). The population where only *P. reticulata* were present (Upper East), had the highest spot brightness

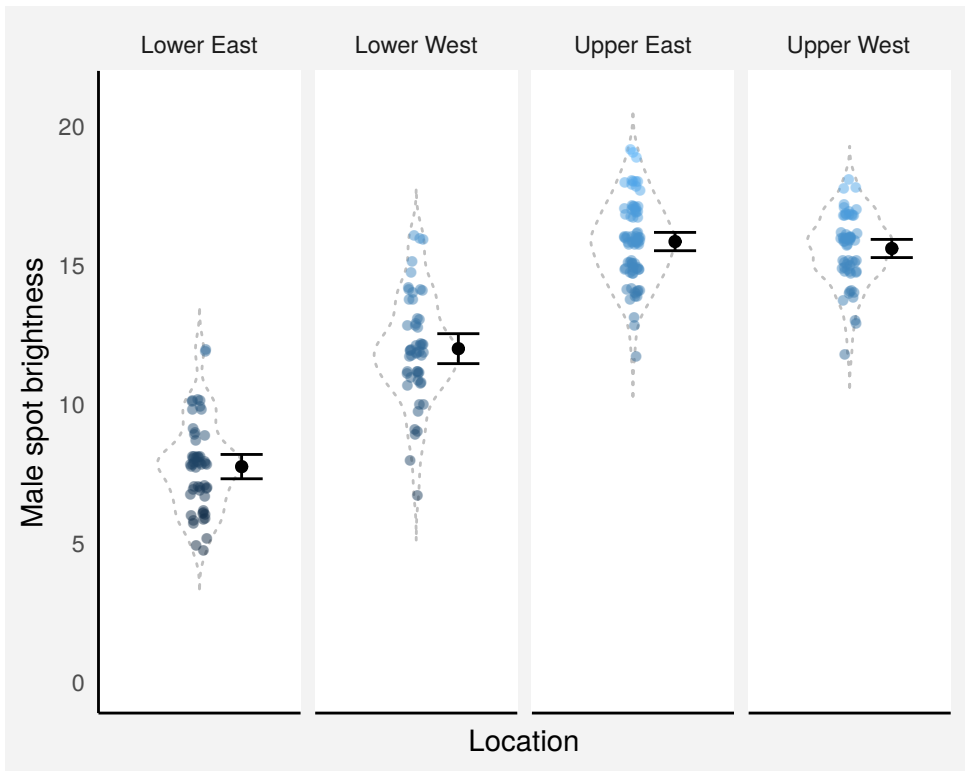
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which would be expected if selection favours males with brighter spots due to female mate choice. Where *Rivulus hartii* were also found (Upper West) spot brightness was similarly high. If this species did not predate upon adult guppies it's presence may have had no selective effect. In other populations that included *Crenicichla punctata* (Lower East) or *Aequidens pulcher* (Lower West) spot brightness was significantly reduced. These species could be effective predators of adult guppies as this would impose negative selection pressures on spot brightness due to the brightest guppies becoming easier prey targets. However, substrate of these lower river sections also differed from the upper sections where spot brightness was higher; spot brightness in the section with vegetation (Lower West) was higher than that with mud (Lower East). It's not clear from these observations whether vegetation may be buffering the negative pressure of predation by offering refugia, or whether mud does the opposite by making their bright spots more obvious, or whether the predator species simply exert differing selective pressure on the guppies due to physiological or behavioural differences. Indeed, a combination of these effects could be true. Total numbers of other species varied in each river section between a range of two and ten. Predator density was also likely to amplify any selective pressure of predation.

This study aims to discern the contributions of predator species, predator density and substrate to differences in male spot brightness, via controlled tank experiments which vary these factors.

*The predictions tested were as follows:*

- Predation will select against spot brightness.
- Predation at higher densities will select more strongly against spot brightness.
- Different predator species will apply differing amounts of selective pressure.



**Figure 1.** Location as a function of guppy spot brightness: Data points represent individual male guppies surveyed at each location. Points are jittered around both X and Y values and are coloured according to spot brightness. Adjacent error bars show the mean with confidence intervals at 95%. Dotted grey violin plot outlines have widths relative to frequency counts of the data points.

- Substrate will interact with predation, either positively or negatively.

## Methods

Sequential experiments in tanks containing each substrate type (sand, vegetation and mud) were run where predator species and number were varied. Each tank began with 44 random guppies. Sequential waves of experiments had random predators of one species (*R. hartii*, *A. pulcher*, or *C. punctata*) added (first 0, 2, 5, then 10). Each tank observed was

**Table 1.** Observations regarding wild environments. N column is the count of male guppies present in the community. N for other species is the total count for that species.

| Location   | Mean male spot brightness | N  | Other species   | Substrate  |
|------------|---------------------------|----|---|------------|
| Lower East | 7.8                       | 54 | <i>Crenicichla punctata</i> (N = 6).                                  | Mud        |
| Lower West | 12.1                      | 52 | <i>Rivulus hartii</i> (N = 5)<br><i>Aequidens pulcher</i> (N = 5).    | Vegetation |
| Upper East | 15.9                      | 78 | None present.   | Sand       |
| Upper West | 15.7                      | 59 | <i>Rivulus hartii</i> (N = 5).  | Sand       |
| Meir Creek | 0.0                       | 0  | <i>Rivulus hartii</i> (N = 5)<br><i>Crenicichla punctata</i> (N = 2). | Sand       |

completely independent of the others (new random individuals were added each time). All experimental conditions were replicated twice so that spot brightness of eight separate guppy populations were recorded for each predator treatment and six populations were recorded for each density.

Spot brightness for the entire guppy population was recorded at the beginning, then at roughly 250 day intervals until 1500 days had passed. This time span covered 12 *P. reticulata* generations (lifespan approximately 120 days) so that selection on spot brightness could be properly observed. Measurements at time intervals were taken so that in addition to examining the overall differences in selective pressures, the differences in rate of change over time could also be examined.

## Results

### *Predator species*

As expected, predation imposed negative selection on the spot brightness of male guppies. Figure 2 shows the results from all populations at the end of each experiment (Day > 1400). Predator species explained substantially more variability than what we would expect to see by chance (ANOVA, Df = 3, F = 3425, P < 0.001). Presence of *R. hartii* did not significantly impact spot brightness whereas presence of *A. pulcher*

**Table 2.** linear model coefficients from single factor classification of predator species as a function of spot brightness ( $R^2 = 0.730$ ).

| Predator                    | B      | 95% Confidence |        | t       | P       |
|-----------------------------|--------|----------------|--------|---------|---------|
|                             |        | Lower          | Upper  |         |         |
| None                        | 19.038 | 18.900         | 19.177 | 268.902 | < 0.001 |
| <i>Rivulus hartii</i>       | -0.170 | -0.370         | 0.030  | -1.669  | > 0.05  |
| <i>Aequidens pulcher</i>    | -4.820 | -5.026         | -4.614 | -45.873 | < 0.001 |
| <i>Crenicichla punctata</i> | -9.386 | -9.594         | -9.178 | -88.293 | < 0.001 |

significantly reduced mean spot brightness by 4.82. Presence of *C. punctata* had the largest negative effect on spot brightness, reducing the mean by 9.39 (almost half). See table 2 for test statistics and coefficients from the model which explains 73% of the variation in spot brightness ( $R^2 = 0.730$ ).

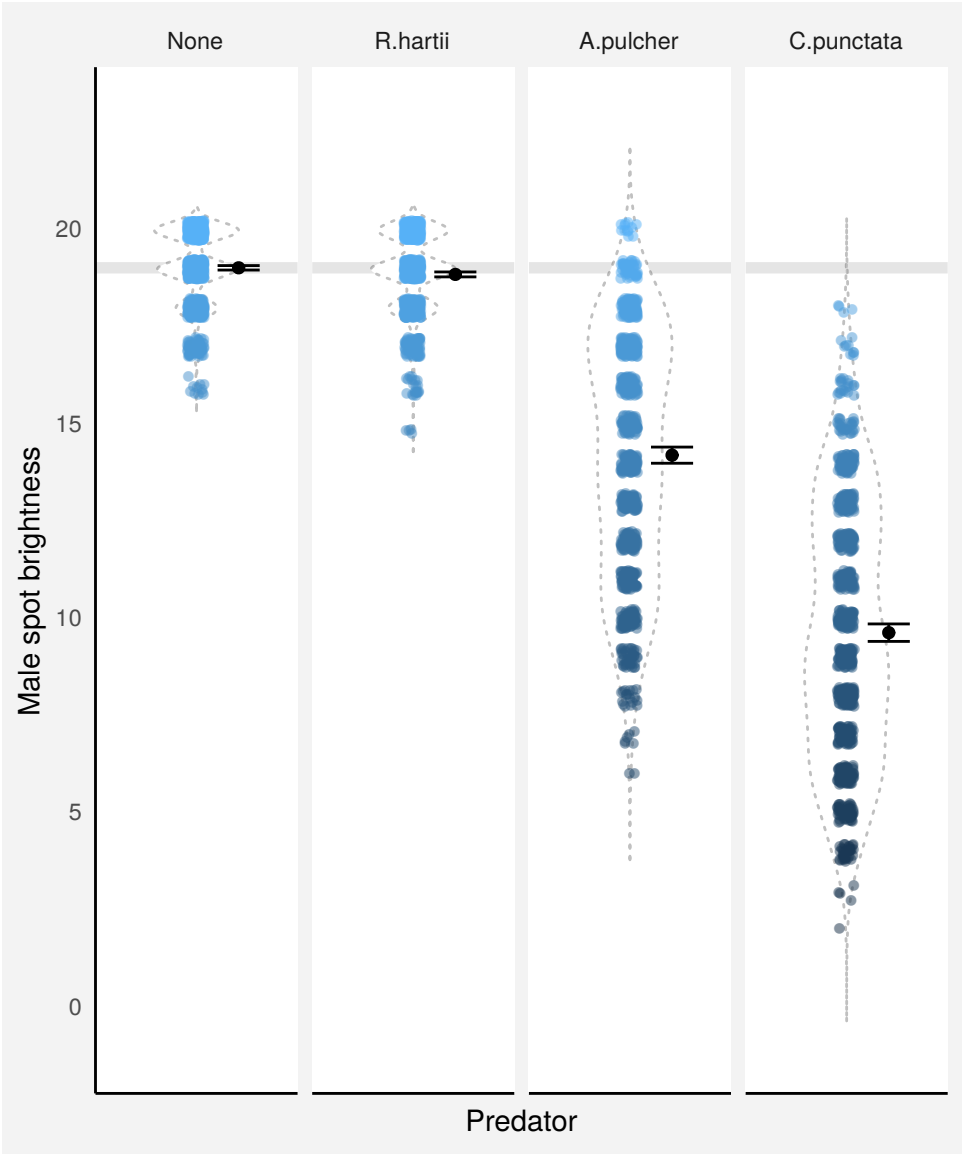
*Predator density*

The effect of predator species on spot brightness was amplified as numbers of the predator increased. Figure 3 shows the negative relationship of predator number as a function of spot brightness for each species. This amplification is steeper relative to the size of the predator species effect (seen in figure 2). A quadratic term was added to the model to fit curvilinear predictions and the final model explains 91% of the variation in spot brightness ( $R^2 = 0.907$ ). Coefficients from the model can be found in table 3.

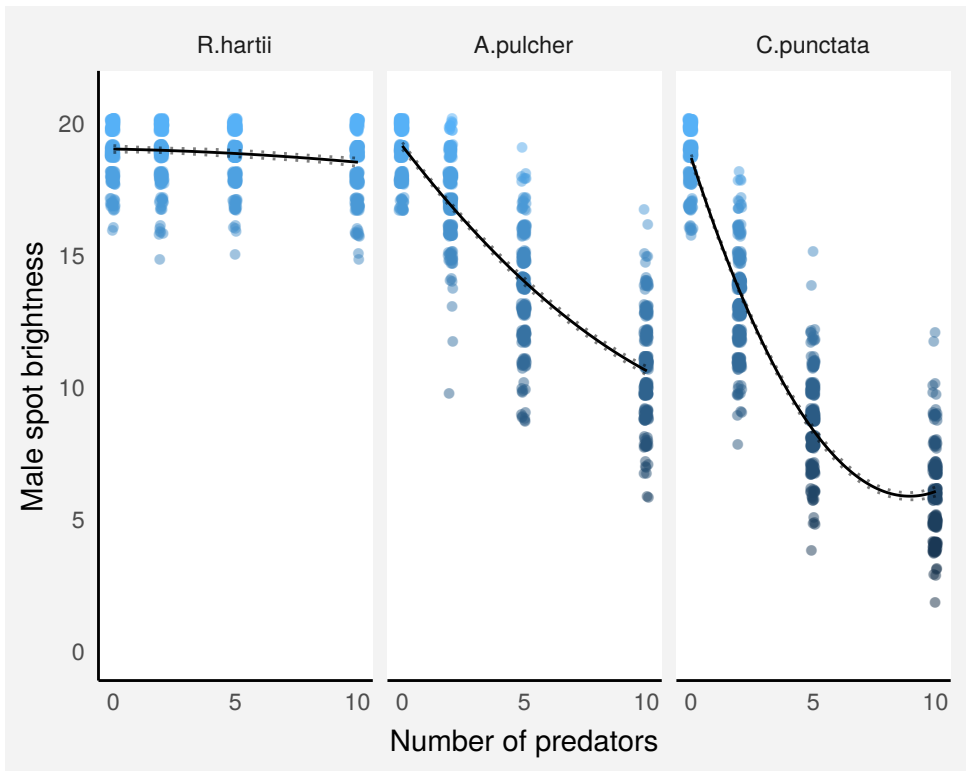
*Substrate*

*Time*

Model simplification steps for all models along with the data analysed can be seen in supporting digital information (?).



**Figure 2.** Predator species as a function of spot brightness: Data points represent individual male guppies from the end point of populations exposed to a minimum of 2 predators. Points are jittered around both X and Y values and are coloured according to spot brightness. Adjacent error bars show the mean with confidence intervals at 95%. Dotted grey violin plot outlines have widths relative to frequency counts of the data points. The grey horizontal line behind each plot shows the mean spot brightness of control populations where no predators were added. *R. hartii* N = 997, *A. pulcher* N = 886, *C. punctata* N = 849,  $R^2 = 0.730$ .



**Figure 3.** Predator number as a function of spot brightness: negative for all species. Data points represent individual male guppies from the end point of all populations. Points are jittered around both X and Y values and are coloured according to spot brightness. Black regression lines show the predictions from the model with confidence envelope at 95% marked in dotted lines around the fit. *R. hartii* N = 1350, *A. pulcher* N = 1241, *C. punctata* N = 1206,  $R^2 = 0.907$

## Discussion

## References

Cite references like this: (Chaib 2017) to put them inside round brackets, or do Author says (2017) to just put the year. The references will appear at the end of the document automatically containing only those from the bib file that are actually cited. For more advanced citation techniques, and how to add **nocite** references for a bibliography see the [RMarkdown citation guidelines](#).

**Table 3.** Linear model coefficients including quadratic effect of predator number (model simplification via sequential f-tests can be seen in supporting digital information,  $R^2 = 0.907$ ).

| Term  | B      | 95% Confidence |        | t       | P       |
|---|--------|----------------|--------|---------|---------|
|   |        | Lower          | Upper  |         |         |
| <i>Rivulus hartii</i>   | 19.089 | 18.958         | 19.220 | 285.252 | < 0.001 |
| <i>Aequidens pulcher</i>  | 0.119  | -0.067         | 0.305  | 1.259   | > 0.05  |
| <i>Crenicichla punctata</i>   | -0.340 | -0.526         | -0.154 | -3.582  | < 0.001 |
| Predator number <sup>2</sup>  | -0.003 | -0.010         | 0.004  | -0.939  | > 0.05  |
| Interaction between <i>R. hartii</i> and predator number                | -0.018 | -0.089         | 0.053  | -0.492  | > 0.05  |
| Interaction between <i>A. pulcher</i> and predator number               | -1.205 | -1.279         | -1.130 | -31.722 | < 0.001 |
| Interaction between <i>C. punctata</i> and predator number              | -2.868 | -2.942         | -2.794 | -75.636 | < 0.001 |
| Interaction between <i>A. pulchens</i> and predator number <sup>2</sup> | 0.038  | 0.029          | 0.048  | 7.685   | < 0.001 |
| Interaction between <i>C. punctata</i> and predator number <sup>2</sup> | 0.164  | 0.154          | 0.174  | 32.597  | < 0.001 |



## **References**

Chaib F. (2017) *Uganda ends Marburg virus disease outbreak*. World Health Organization. Available: <https://www.who.int/en/news-room/detail/08-12-2017-uganda-ends-marburg-virus-disease-outbreak> [Accessed: 19 January 2019].