

1 Researchers as predators: are field biologists altering traits of the populations that they
2 study?

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Abstract

Field biologists rarely directly measure the long-term impact of their work on the populations they study. However, heavy or repeated sampling (with or without release) could potentially alter population traits and even bias study conclusions. Trinidadian guppies (*Poecilia reticulata*) are a frequently-studied species in evolutionary biology, particularly in the context of variation in predation. Here, we tested how research sampling intensity might be associated with behavioral traits (boldness, exploration, and shoaling), coloration (relative black and orange area), or size (body mass) in two low predation guppy populations. We carried out a literature review and identified paired sites in the Aripo and Paria rivers that have been either weakly-sampled or heavily-sampled for research, collected guppies from these sites, and measured traits. None of the behavioral traits, orange colouration, or mass showed any detectable impact of sampling intensity, which is reassuring for long-term guppy research. However, guppies from heavily-sampled sites had greater relative black area than did those from weakly-sampled sites. This last result suggests that guppy researchers could have unintended impacts on some guppy traits, and highlights the importance of considering long-term researcher-induced effects in study design.

Keywords: Researcher effects, sampling effects, natural selection, biological fieldwork, experimental design

Introduction

Scientific field studies are rarely, if ever, truly neutral for the study populations; researchers' presence and methods can influence the very systems that they study (e.g., Crofoot et al. 2010; Hoy & Brereton, 2022). Although fieldwork, research and ethical guidelines already acknowledge and attempt to minimize the impact of researchers (e.g. "Guidelines for the Ethical Treatment of Nonhuman Animals in Behavioural Research and Teaching," 2026), specific long-term consequences of animal fieldwork remain largely unexplored and poorly accounted for. Existing studies on these so-called 'researcher effects' have primarily focused on short-term ecological, environmental, or behavioral impacts, and tests to quantify researcher effects beyond the temporal scope of a given study are rare. Yet such effects can persist even after a study ends. For example, Rinehart et al. (2023) found that researcher-trampled plots in tide marshes differed 10 months later in plant cover, composition, and plant traits, compared to untrampled controls. On even longer timescales, from altering the environment to repeated sampling and even sometimes experimental translocations, researchers can introduce unintentional selective pressures that could lead to evolved changes in behavioral, morphological, or demographic characteristics of the populations that they study (Espeland et al., 2017; Gordon et al., 2009; Hutchings, 2014). Researcher-induced pressures could therefore alter the predictions that one would make regarding the evolutionary trajectories of various populations and lead to unexpected results. This possibility makes it important to ask: to what extent are we influencing the patterns we aim to explain?

Trinidadian guppies (*Poecilia reticulata*) are an important model organism in evolutionary biology, with guppy research providing substantial insights into basic ecological and evolutionary principles for over half a century (Haskins et al., 1961; review: Magurran,

2005). Much of this pioneering research has focused on the selective role of predation, enabled by the environments that guppies inhabit in Trinidad, where physical barriers have generated guppy populations that differ in predation intensity (Reznick et al., 1996). Studies leveraging these high versus low predation environments have generated strong evidence demonstrating that predation selects for variation in a number of guppy traits, including color (e.g., Endler, 1980), behavior (e.g., Magurran & Seghers, 1990, 1991), and life history traits (e.g., Reznick et al., 1996). Recently, however, some studies using this high predation versus low predation dichotomy have noted that the extent of phenotypic parallelism is highly variable even in these “textbook examples” of phenotypic parallelism (e.g., guppy color; Heckley et al., 2022; Weese et al., 2010; Yong et al., 2021).

Inconsistencies in study outcomes could be – at least in principle – partly due to one overlooked source of selection: the researchers themselves via their sampling. Several studies have shown that the likelihood of an organism being captured during sampling can be associated with particular traits (Biro, 2013; Carter et al., 2012; Stuber et al., 2013). For example, even non-size selective nets are two times more likely to capture fast-growing fish than slow-growing fish (Biro, 2023), and some capture methods are more likely to catch individuals with certain behavioral traits (e.g., bolder individuals; Álvarez-Quintero et al., 2021; Wilson et al., 1993). Many guppy researchers use hand nets (especially butterfly nets) for sampling, such that behavioral (e.g., boldness, activity levels, habitat preferences) and morphological traits (e.g., size or conspicuous coloration) could impact the likelihood of capture. Due to the popularity of guppies as a model study system some sites have been especially heavily-sampled throughout the years, with long-standing concerns raised about possible impacts (Magurran, 2005). Researcher

sampling could exert selection that mimics predation in two ways. First, repeated removal of fish could increase overall mortality, which, like predation, can favor traits such as earlier maturation and greater reproductive investment. Second, if the fish most likely to be caught by researchers have the same traits that increase predation risk, such as boldness, activity or conspicuous coloration, then long-term intensive sampling could select against these traits similar to as predators do. These effects would likely be especially pronounced in sites that only have low natural levels of predation.

The possibility that researcher sampling influences selection in these low predation sites has implications for how guppy studies are interpreted, and might lead to deviations from the assumptions underlying some studies. For instance, these selective effects could mean that low predation sites might now be closer in their selection environment to high predation sites, compared to before researchers started sampling them. Sites with higher sampling intensity likely experience higher mortality which is a major driving force behind trait differences in low and high predation guppy populations (Reznick et al., 1996; Weese et al., 2010). Different predator species can have similar effects on guppy trait evolution suggesting that if researcher sampling also leads to increased mortality, it could result in shifts in traits similar to predation (Reznick et al. 1996). Additionally, the impact of individual traits on sampling likelihood (i.e., if certain traits make individuals more trappable or more conspicuous to researchers) could lead to researcher induced directional selection. This systematic sampling bias could also impact the conclusions made from a study, for example, by influencing population size estimates, trait means and distributions, and the size of correlations between traits of interest. Much of the work in guppies has been foundational to our understanding of evolutionary biology (Endler, 1995;

Houde, 1987, 1997; Magurran, 2005; Reznick et al., 1996); so it is important to understand the extent to which any systematic bias in sampling or selection induced by researcher sampling might be impacting the results of guppy research conducted in Trinidad.

In the present study, we investigate how researcher sampling might have impacted the traits of low predation populations by comparing behavioral and morphological traits of guppies from heavily-sampled sites to guppies from weakly-sampled sites. We tested the prediction that individuals from heavily-sampled low predation sites will exhibit phenotypes more similar to those previously reported for high predation localities than fish from weakly-sampled sites. Therefore, we focused on traits that have been repeatedly found to vary between high and low predation sites such as: size (low predation guppies are larger: Reznick et al., 1996; Reznick et al., 2001), male coloration (low predation male guppies are more colorful: Haskins et al., 1961; Endler, 1980), bold behavioral traits (low predation guppies are less bold: Harris et al., 2010), and shoaling (high predation guppies shoal more: Jacquin et al., 2016; Magurran & Seghers, 1991, 2005). In short, phenotypes in heavily-sampled low predation sites might move to resemble high predation phenotypes owing to high rates of removal, selective sampling or other impacts of guppy research.

Materials and methods

Sample site selection and guppy sampling

To identify low predation sites where researcher sampling could be reflected in the population's current phenotype, we conducted a literature review to estimate which sites in Trinidad have been most heavily-sampled over the last 20 years. We focused on this timeframe because we assumed that these sampling events were recent enough for historical sampling to have lasting effects on phenotypes at the time of our sampling. On October 10th, 2023, we searched Web of Science, SCOPUS, and CAB Direct using the following terms and Boolean operators: “(guppy OR guppies OR "poecilia reticulata") AND (trinidad OR trinidadian)”. For SCOPUS, we searched title, abstract, and keyword fields, whereas for Web of Science and CAB Direct we searched all fields (i.e., the entire searchable record). This search generated 844 unique studies (1383 total studies including duplicates).

Abstracts of all 844 studies were screened by two authors (JAF and AMH) to ensure they were published after 2002 and guppies were sampled in the wild in Trinidad. If the sampling country was unclear, we retained those studies to read in full. One hundred and eighty-four studies passed this initial abstract screening. Studies conducted without removing fish from the stream (*e.g.*, *in situ* behavioral assays) were excluded. Each of these 184 studies was then read in full including supplemental materials when necessary. To include these studies in our final dataset, we required GPS coordinates or grid references for the sampling locations, as well as the sampling year to ensure that guppies were sampled during or after 2003. For papers that gave a range of sample sizes for the number fish collected from the stream, we used the highest number in the range to calculate mean sample size. For papers that aggregated sample size across sites or years, we divided the given sample size by the number of sites or years to provide an estimate for each site. As the majority of papers did not report whether sampled fish were returned to the

original site, we could not distinguish between sampling with return and sampling without return. Therefore, both sampling types are included in our analysis. We used these results, which aligned with our personal knowledge of heavily-sampled sites in Trinidad, to select one candidate heavily-sampled, low predation site in four rivers across both the Northern and Southern slopes: Yarra, Paria, Aripo, and Quare.

For each river we also required at least one weakly-sampled site. Whereas the heavily-sampled sites were selected using the results of the literature review, the weakly-sampled sites were largely selected based on what was logistically feasible in the field. For instance, we were unable to include weakly-sampled sites in the Yarra or the Quare because small pools did not exist upstream or downstream of the heavily-sampled sites, the rivers were unsafe to navigate, or the tributaries off the main river we searched were dry. We therefore only sampled the Paria and the Aripo, where we sampled two weakly-sampled sites in each river, one upstream and one downstream from the heavily-sampled site (Table 1). To ensure that sites were accurately labeled as weakly versus heavily sampled, we cross-referenced each site against the sampling locations in our literature review by counting sampling instances where reported coordinates placed them within 250 m (straight-distance) of a site, given the precision of the coordinates as reported (e.g., excluding coordinates reported only to the nearest arcminute or kilometre). We found no precisely located records within 250 m (straight-line distance) of one of the weakly-sampled sites from each river (AWS2 and PWS1). The other two weakly-sampled sites each had three sampling instances with 73 fish sampled from AWS1 and 57 from PWS2. Likely due to ease of sampling (Magurran 2005), all heavily-sampled sites were close to a road and had open canopy,

190 whereas weakly-sampled sites were further from a road with much thicker vegetation and a
191 closed canopy.

192 In the Paria the weakly sampled sites were 393 m and 418 m from the heavily sampled
193 site, while in the Aripo they were 718 m, and 1173 m (straight line distances). Some gene flow
194 likely occurs within these stretches of the rivers (Blondel et al. 2018; Crispo et al. 2006; Barson
195 et al. 2009). However, most guppies are philopatric, showing minimal movement within rivers,
196 and our between-site distances exceed maximum dispersal distances reported for both low-
197 predation male and female guppies over relatively long time frames (i.e., >1 year; Borges et al.
198 2022). Further, strong selection can lead to rapid local adaptation or strong spatial signatures of
199 locally adapted guppy traits (e.g., Fitzpatrick et al. 2015; Blondel et al. 2018). Therefore, if
200 researchers are affecting the evolution of guppy phenotypes, then, even with some gene flow,
201 these distances should be sufficient to detect signatures of adaptation.

202 Between February 14 and 25, 2024, we used butterfly nets to collect between 30 and 60
203 fish within a 30 m stretch of each site (Table 1) with approximately half males and half females.
204 We typically fished for guppies from the side of the stream to minimize sediment disturbance,
205 but in deeper or wider sections we waded in the water to catch fish. At each site, we collected
206 several additional females to use for the shoaling test (see below). Guppies were transported to
207 the William Beebe Tropical Research Station (Simla) in individual whirl-pak bags with Stress
208 Coat (API) to prevent parasite transfer. At Simla, guppies were housed, still in their individual
209 whirl-pak bags, in larger tanks filled with rainwater and outfitted with bubblers. Twenty holes
210 were pricked in each whirl-pak bag using a small lancet needle to allow for the movement of
211 oxygenated water and for the exchange of chemical cues among guppies held in the same tank to

reduce any isolation stress and its potential behavioral impacts. We allowed guppies at least two days to acclimate to captivity in these conditions before they were used in the behavioral assays described below. In captivity, guppies were fed flake food (TetraMin, Tetra) once daily but were not fed on the day of the behavioral assays until after testing was completed. Guppies were returned to their site of origin after behavioral assays and processing were completed.

Behavioral assays and processing

Behavioral assays were conducted in 20 L rectangular glass tanks with the internal sides covered in white contact paper to prevent reflections. Tanks were filled with 7 cm of fresh rainwater and light-colored gravel was loosely scattered along the bottom. Water was fully changed at the start of each day and partially changed after each individual. Assays were conducted from 7 am to 6 pm, to ensure that they aligned with the period at which guppies are most active, which is consistent with other behavioral work in guppies (e.g., Endler et al., 2022). Each assay lasted for 5 minutes and was recorded using a 1080P HD Model N5 webcam (HDZIYU, Shenzhen, China) positioned 60 cm above the tank. Individuals from heavily-sampled and weakly-sampled sites were alternated (one heavily-sampled then one weakly-sampled, repeating) throughout the day.

Behavioral assays were conducted in a fixed order to standardize possible carryover effects between assays (Bell, 2013). The first behavioral assay was latency to emerge from a shelter. Fish were acclimated for three minutes in two 20 oz (~591 mL) white stacked Styrofoam cups placed in the center of the tank and weighed down by gravel. After the three-minute

acclimation period, the outer cup was lifted by hand to reveal the second cup that had a square hole in it (approximately 3 cm x 3 cm), allowing the fish to emerge into the open field (these methods are similar to other emergence tests conducted with fish; Wilson & Godin, 2009; Harris et al., 2010; Bensky & Bell, 2018). We recorded the total amount of time (up to five minutes) for the whole body of the fish (excluding caudal fin) to emerge from the holding container. If a fish did not emerge, the cup was removed and the subject was recorded as taking at least 300 seconds. Upon entering the arena, the next assay immediately began. The second behavioral assay was an open field test, where the fish was allowed to explore the tank for 5 minutes (Burns, 2008). Following this assay, fish were immediately transferred to a new tank for a shoaling assay (Chapman et al., 2008; Laskowski & Bell, 2014; Fox et. al., 2025). This new tank contained two clear solid plastic containers (14.3 cm L x 9.6 cm W x 22.1 cm H) placed inside the tank, with one container at each end: one container just contained water and the other also contained a shoal of four female fish collected from the same site as the ‘focal’ individual. The side of the tank where each container was in was randomized for the first trial on each day and switched between assays. Fish were again given a three-minute acclimation period but this time in a small transparent container which was then raised, again by hand, allowing the individual to swim around the tank for five minutes.

After the behavioral assays, fish were anaesthetized using clove oil. Anaesthetized fish were then visually scanned under a VanGuard dissecting microscope at 2-4.5x magnification for *Gyrodactylus* spp. ectoparasites for a separate study and weighed to 0.01 g using an Ascher scale. *Gyrodactylus* prevalence did not differ between the rivers or sampling regimes (unpublished data). In the Aripo only, we noticed that several guppies also had nematode

parasites, and so the presence or absence of nematodes was recorded. Nematode prevalence did not vary between sampling regimes (unpublished data). We assessed the behavior of as many fish as possible in behavioral assays within the time available for each site (one to two days); fish that could not be assayed were weighed, and males were additionally photographed for color analysis.

Photography and color data extraction

After the behavioral trials and *Gyrodactylus* scans, photos of all anaesthetized males were taken using a Nikon D850 digital SLR camera equipped with a 60-mm macro lens. The photos were taken in a white photo light tent, and the fish were illuminated with two fluorescent lamps outside of the tent and a Nikon Speedlight Commander Kit R1C1 flash. Fish of both sexes were placed in a small well filled with water and clove oil on a white background, and the right side of the fish was photographed (Gotanda et. al., 2018). A ruler and non-commercial color standard were included in the photos for subsequent color analyses.

Images of male fish only were processed in ImageJ (Schneider et al., 2012). We used the freehand selector tool to outline the fish's body, excluding fins, as well as orange, red, yellow, black, fuzzy black, violet, green, and blue color spots for every photograph (n = 93). While this assessment may not be reflective of guppy eyesight (White et al. 2003), these color categorizations and methods are consistent with past guppy work (e.g., Gotanda et al., 2013; Weese et al., 2010) and therefore are most useful for understanding if past assessments have

been influenced by researcher sampling. We extracted body area, the total number of spots of each color, the area of *each* color, and the area for *all* colors for each individual. For each individual, we then divided the area of each color by body size to obtain relative area values. Assigning color categories by eye and measuring relative area of each color is a straightforward and common approach for assessing guppy color that has been established through decades of research (Houde, 1997). Due to a lens malfunction in the field, brightness was not consistent across all images; therefore, although we are able to accurately extract spot size and numbers, we do not consider brightness or hue in the present study.

Color data were extracted by two authors (JAF and AMH). To ensure consistency between extractors, 29% (28/93) of the images were double scored. We calculated inter-rater reliability (IRR) from these double-scored images using the *irr* package in R (Gamer et al., 2019; R Core Team, 2022). Black (including black and fuzzy black) and orange had high IRR (IRR = 0.84, and IRR = 0.86, respectively). Other colors had weaker IRR, including when collapsed into functional color groupings (e.g., structural – blue, green, and violet: $R = 0.53$). We therefore focussed subsequent analyses on black and orange. These two colors are recognized as important colors for guppy fitness, affecting both mate choice preference and predation risk (Godin & McDonough, 2003; Houde, 1987; Houde & Endler, 1990; Millar et al., 2006; Weese et al., 2010).

Behavioral video analysis

We used EthoVision XT v11.567 to quantify distance travelled (cm) and time spent frozen (s) in the open field test. Time spent frozen was classified from velocity using EthoVision's movement detection which applies thresholds to reduce tracking noise from rapidly switching between moving and not moving states. A fish is considered frozen when its velocity falls below 1.75 cm/s and remains classified as frozen until its velocity increases above 2 cm/s. Using EthoVision, we also overlaid a 5 x 8 grid of even-sized squares and counted the number of squares entered and time spent (s) in the squares along the outer edge of the tank. This software has previously been validated for automated tracking in the setup used, and all tracks were manually checked to ensure the automated tracking was accurate. For the shoaling test, a single observer (MDC) used BORIS v7.12.2 (Friard & Gamba, 2016) to record time spent within 5 cm, or roughly two to three body lengths, of each container; this is a commonly used shoaling metric (e. g., Chapman et al., 2008; Fox et al., 2025). Due to some fish being removed due to issues with the behavioral assay or video analysis, the final sample sizes varied for each test (Table 2).

Statistical analyses

Analyses were conducted in the R environment v 4.4.2 (R Core Team, 2022). Prior to modelling, we calculated Spearman correlations between behavioral variables as several were measured in the same behavioral assay and therefore could be highly correlated. Distance travelled, time frozen, and number of squares explored were highly correlated ($r > 0.7$); so we retained only distance travelled in further analyses. The rest of the behavioral variables were not highly

318 correlated ($r < 0.2$). Therefore, for behavioral variables, we analyzed latency to emerge from
319 shelter, distance travelled and time spent on the outer edge in the open field test, and preference
320 for shoal. Preference for shoal was calculated by subtracting time spent near the empty container
321 from the time spent near the container containing the shoal (Fox et al., 2025). Within each river,
322 we used a t-test to check if fish preferred the shoal over the empty container, and one-sample
323 Wilcoxon signed-rank tests to check if fish preferred the edge of the arena over the 55% of time
324 expected by chance (the outer 22 of 40 squares, 55% of the arena area).

325 For distance travelled, time spent on the outer edge, and preference for shoaling, we used
326 the *lme4* package (Bates et al., 2015) to fit linear mixed models with sampling (heavily/weakly),
327 river (Paria/Aripo), mass, and sex as fixed effects and site as a random effect. The shoaling
328 model additionally included which side of the tank the shoal was on (left or right) as a fixed
329 effect. For latency to emerge, we used a mixed-effect Cox proportional hazards model as the data
330 were right-censored (i.e., fish that had not emerged within the five-minute trial). Using the
331 *survival* package (Therneau & Grambsch, 2000), we created a survival dataset with time to
332 emergence and an indicator variable that indicates whether the fish had emerged. The *coxme*
333 package (Therneau et al., 2003) was used to fit the model. The proportional hazards assumption
334 was assessed using scaled Schoenfeld residuals. Proportional hazard assumptions were not
335 violated for sex, mass, and globally, but were violated for river; therefore, we stratified the
336 model by river. Final model estimates were interpreted as hazard ratios (HR).

337 For the color and body mass analyses, we first calculated the relative area of black
338 (including black and fuzzy black) and orange on each male by dividing the total area of each
339 color by the body area. For pictures that were double scored, we used the average values. We

built separate linear models for each color, with relative color area as the response variable, sampling, river, and extractor (AMH/JAF/both) as fixed effects, and site as a random effect. Relative orange area was log transformed to meet homogeneity of variance assumptions, and no transformations were required for black to meet linear model assumptions. Lastly, we log-transformed mass to correct for strong left-skew and then ran a model predicting mass with sampling, river, and sex as fixed effects, and site as a random effect.

Model assumptions were verified using the *DHARMA* package (Hartig, 2022). We used the *car* package (Fox & Weisberg, 2019) to calculate Chi-square/F and *p* statistics for each model using Type 2 sum of squares. Effect sizes are reported as 95% confidence intervals (CI) of estimated differences between levels of each factor, or, for mass, the change per 0.1 g using Kenward-Roger degrees of freedom. To determine the variance explained, we calculated marginal and conditional R^2 (Nakagawa & Schielzeth, 2013; Nakagawa et al., 2017) with the *performance* package (Lüdtke et al., 2021), and semi-partial R^2 with 95% CIs for each fixed effect using *r2glmm* (Jaeger et al., 2017) with the Nakagawa–Schielzeth method. Site variance in the model of black coloration was estimated as zero, so we report only marginal R^2 for that model. We did not correct for multiple testing because each response tests its own *a priori* prediction about sampling effects (Moran, 2003).

Ethical considerations

We received permits for field sampling from the Water and Sewage Authority of Trinidad and the work was approved by institutional protocols at McGill University (protocol MCGL-7708), and followed applicable laws and ASAB/ABS guidelines. To minimize the risk of cross-contamination of *Gyrodactylus spp.* between rivers, nets were fully air-dried in direct sunlight between sampling each river (Scottish Executive & DEFRA, 2005).

Results

Literature review

We required site-specific sampling data, but many studies did not include GPS coordinates or grid references for any sampling site (99/184), and a further 29 studies did not report the year of sampling, leaving a total of 482 sampling incidents from 56 papers. Further, many papers did not report a sample size for their sampling (25 studies covering 94 sampling instances), or did not report sample size consistently (e.g., incomplete details or aggregating numbers across rivers or sites). There are also likely many other sampling instances that are not recorded in the literature (such as collections for captive stocks or unpublished studies). Therefore our site-specific estimates of sampling intensity are likely below the true sampling intensity but we expected them to correlate with true sampling intensity. We retained all 482 sampling instances for our estimation of sampling intensity near our sites, which was based on reported coordinates alone. Instances without a reported sample size contributed to counts of sampling instances but not to totals of fish sampled.

Across all sampling instances in our dataset, 22031 fish were sampled in total (covering captures with and without return). The range of reported sample sizes of fish collected per sampling instance across all rivers was 1 - 757 with a mean of 56.80 (SD = 81.50). The most heavily-sampled river based on number of sampling instances was the Aripo with 90 sampling instances and 4183 fish sampled across 15 years, followed by Marianne, Guanapo, Turure, and Quare each having 38 - 42 sampling instances and 793 - 2138 fish sampled across 10 –13 years (Figure 1). The Paria River had 10 sampling instances and 840 fish sampled across 6 years. For the rivers we sampled, Aripo had an average of 278.87 (SD = 287.79) fish sampled per year whereas Paria had an average of 240.00 (SD = 136.66) fish sampled per year. For the specific sites that we sampled from, heavily sampled sites were confirmed to be much more heavily sampled than weakly-sampled sites in both rivers (Table 1).

Behavior

Across all fish tested, most emerged from the shelter (Aripo: 65.1%; Paria: 76.7%). On average, during the open field test Aripo fish travelled 989.99 cm (SD = 394.39 cm) and spent 60.98% (SD = 18.14%) of the trial along the outer edge whereas Paria fish travelled 849.67 cm (SD = 404.06 cm) and spent 57.26% (SD = 22.05%) of the trial along the outer edge. Aripo fish significantly preferred the outer edge ($V = 2475$, $n = 85$, $p = 0.002$), whereas Paria fish did not ($V = 2198$, $n = 86$, $p = 0.08$). Fish from both rivers significantly preferred the shoal over the empty container (Aripo: $t = 6.63$, $df = 78$, $p < 0.0001$; Paria: $t = 15.06$, $df = 83$, $p < 0.0001$).

We found no evidence that sampling intensity was associated with behavioral phenotypes (Figure 2; Table 2). Latency to emerge from the shelter was not significantly associated with sampling intensity (HR = 0.82, 95% CI: 0.47–1.45, $\chi^2 = 0.45$, $p = 0.50$, $n = 169$), sex (HR = 1.17, 95% CI: 0.77–1.79, $\chi^2 = 0.56$, $p = 0.45$), or body mass (HR per 0.1 g = 0.95, 95% CI: 0.74–1.23, $\chi^2 = 0.14$, $p = 0.71$). In the open field assay, neither distance travelled nor time spent in the outer edge were significantly associated with sampling, river, sex, or mass (Table 2). Very little variance in either trait was explained by sampling (semi-partial $R^2 = 0.002$ and 0.015 , respectively), and the fixed effects together explained 3.7% and 3.2% of the variance (marginal $R^2 = 0.071$; conditional $R^2 = 0.120$). Shoaling preference (the difference in time spent with the shoal versus an empty container) was also not significantly associated with sampling, river, sex, or mass, but was impacted by which side of the tank the shoal was on (Table 2).

Color and mass

We found that male guppies had higher relative black area in more heavily-sampled sites than in weakly-sampled sites (Figure 3; Table 2). Males from heavily-sampled sites were on average 15.8% black compared to 12.7% black for males from weakly-sampled sites, and sampling accounted for 8.4% of the variance in relative black area. Neither river nor extractor (the observer who scored coloration) were statistically significant. By contrast, no association was evident between sampling regime and relative orange area (Table 2). Rather, orange was most strongly explained by river (Table 2), with Paria males having over twice the relative orange area

as Aripo males. Extractor had no significant effect. Mass was not significantly associated with sampling or river (Figure 3; Table 2). Not surprisingly, the strongest factor impacting mass was sex (Table 2).

Discussion

In this study, we assessed the impact of differences in researcher sampling intensity on phenotypic traits in Trinidadian guppy populations, focusing on behavior, body mass and male coloration. We asked whether, by sampling individuals from the population, whether they later return them to their capture sites or remove them permanently, researchers could induce pressures similar to those of predators. We predicted that guppy traits known to be affected by natural predators (e.g., Endler, 1980; Harris et al. 2010; Magurran & Seghers, 1991) could be impacted by researchers in similar ways, but found no evidence that sampling intensity was associated with the behavioral phenotypes we measured (boldness, exploration, or shoaling), orange coloration, or body mass. These findings are reassuring for long-term guppy research focusing on these specific traits. However, guppies in more heavily-sampled sites had a higher relative black area (including black and fuzzy black) suggesting that researcher sampling could have influenced black pigmentation. We discuss the implications of these findings for guppy research and the broader community of researchers that carry out field sampling.

444 *Sampling regime was not associated with guppy behavior or mass*

445 We did not find evidence that researcher sampling was associated with any of the behavioral
446 traits we measured (boldness, exploration, or shoaling), nor were there strong associations
447 between sex, mass, or river and behavior. Some studies find impacts of sex or mass on our
448 measured behavioral traits in guppies (Piyapong et al., 2010; Jacquin et al. 2016), although
449 others show no or weak effects as in our study (Heckley et al., 2025; Fox et al., 2025). Similarly,
450 other studies have reported behavioral differences in low-predation sites between rivers (e.g.,
451 Jacquin et al. 2016), despite us finding no differences in the present study. The reasons for these
452 inconsistencies are unclear, though presumably reflect a combination of eco-evolutionary and
453 methodological factors. For instance, a strong shoal side bias in the shoaling test likely made it
454 hard to detect any significant impacts. Moreover, behaviors are highly plastic, shaped by factors
455 such as an individual's social environment, genetics, and past experiences (Webster & Rutz,
456 2020), and behaviors are also highly sensitive to methodological differences and contextual
457 factors (Hills et al. 2022; von Kortzfleisch et al. 2022). Compared to some other trait types,
458 behavior may therefore be especially difficult to accurately quantify in wild populations.
459 Repeated measurements of behavior would potentially be valuable.

460 We predicted that guppies from heavily sampled sites would shoal more, be less bold,
461 and be smaller as has been reported for high- versus low-predation guppies (e.g., Magurran &
462 Seghers, 1990, 1991; Harris et al. 2010). Although researcher effects could shape guppy
463 phenotypes through elevated overall mortality, researcher capture also differs from predation in
464 ways that complicate predictions for selection acting on specific traits. For instance, researchers
465 can often net whole shoals at a time, potentially leading to selection against shoaling.

Alternatively, by netting whole shoals, researchers could be capturing sufficient variation to not drive selection in either direction (i.e., if they are capturing both the tightest shoalers in the centre and the weakest shoalers near the periphery). Predictions are also unclear for exploration and boldness, as studies of predation effects show variable findings (Burns et al., 2016; Crane et al., 2022; Harris et al., 2010). In the context of researcher sampling, one could hypothesize that bolder or more active fish could be easier to spot but also could be harder to catch. There are often associations between size and behavior that could further complicate the impact of researchers on these traits. Therefore, researcher selection on these traits might not be so straightforward. An alternative explanation for the absence of significant effects is that researcher sampling might not be imposing strong impacts on those traits. For mass, the general consensus is that smaller guppies are favored by natural selection in high predation sites due to higher overall mortality, possibly selecting for lower ages at maturation (Reznick et al., 1996). However, researcher sampling may not be inducing strong enough mortality to result in similar effects. Other – potentially stronger – sources of selection likely shape behavior in the populations that we sampled, such as resource availability (Chapman et al., 2010), abiotic factors (Heckley et al., 2025), non-fish predation (e.g., from birds or bats; Magurran, 2005), parasitism (Croft et al., 2011; Reynolds et al., 2018), or human disturbances beyond research (e.g., recreation; Brusseau et al. 2025).

Guppies were more black in heavily sampled sites

No association was found between researcher sampling intensity and orange coloration, but guppies from heavily-sampled sites had more relative black area than those from weakly-sampled sites. Male guppies from heavily-sampled sites had roughly 25% more relative black area than males from weakly-sampled sites. One possible explanation for this result is that darker individuals are less conspicuous to researchers when netting, especially against the typically dark litter background of streams. Interestingly, this result seemingly contrasts with the finding that guppies with more black suffer high mortality in the wild, even in low predation environments (e.g., Gordon et al. 2015). However, because humans and natural guppy predators have different visual systems, and “hunt” for guppies at different distances and angles, conspicuous colors could differ between “predator” types. Future experiments or visual modelling work could attempt to tease these effects apart. Previous studies have also assessed whether coloration affects guppy recapture rates in mark-recapture experiments (e.g., Weese et al. 2010). However, if color also affects initial catchability, then less detectable individuals would also be less likely to be captured, marked, and included in the first sampling period. Therefore, such studies would not directly capture the full effect of coloration on catch rates. Future studies on the impact of guppy color on catch rates would be useful to better predict the impacts of researcher sampling on guppy (and other species’) color.

It is also possible that the relationship between color and capture depends on interactions between different colors or other traits. For instance, structural colors are negatively correlated with fuzzy black area, black spot size, and the number of orange spots in guppies (Kemp et al. 2008). Structural coloration could be especially conspicuous to researchers, as it is highly visible over long distances (Endler, 1980), and increased relative black area reduces the proportion of

body area available to these other colors. Unfortunately, we were unable to consider other colors in the present study. However, this idea could be tested in future work. Additional studies could also consider other spectral properties (e.g., saturation or hue; Kemp et al. 2009; Yong et al. 2022). Genetics and plasticity contribute to these properties to varying extents (Houde et al. 2022), and we might therefore expect that the spectral properties respond along different trajectories or timelines to researcher effects.

Given that our study is correlational, the potential confounding roles of other ecological factors are important to consider. For instance, canopy openness is important for guppy color because light availability alters visual signalling, affecting conspicuousness to predators and mates, and primary productivity, affecting food availability (Endler 1991; Endler 1993; Schwartz & Hendry, 2010). In our study, the heavily-sampled sites always had more open canopies than the weakly-sampled sites. Several studies have reported that guppies are typically relatively *less* black in lower canopy cover habitats (Millar et al, 2006; Kemp et al. 2018). We contrastingly found that guppies are relatively *more* black in the low-cover (heavily-sampled) sites. This opposing pattern makes a stronger case for the possible role of researcher effects in driving the observed associations with relative black area. Of course, several other ecological factors could also contribute to these patterns, such as conspecific or heterospecific density, or other abiotic factors, as well as phenotypic covariates of black coloration, and we cannot rule-out any such effects.

Researcher sampling effects on black color could have implications for how guppy studies are interpreted. For instance, if darker guppies are harder to see for both researchers and predators, black could be favored in both high-predation sites and highly-sampled low-predation

sites, decreasing differences between these sites. Heckley et al. (2022) reported decreasing parallelism with increasing sample sizes across studies leveraging the high versus low predation dichotomy, and suggested that this finding could be driven by early studies (with smaller sample sizes) focusing on ‘low hanging fruit’ examples of parallelism. Although Heckley et al. (2022) examined several other guppy phenotypes alongside color, our findings suggest this trend is also consistent with researcher effects altering evolutionary trajectories. Similar impacts of experimenter effects could be relevant for other guppy traits or other heavily investigated systems in field biology; although the direction or magnitude of these effects might differ from what we observed and may be challenging to predict. Within the guppy system, researcher sampling may have similar effects to predation, but in other systems with other drivers of variation and methods of researcher sampling, assessing researcher impacts might be less straightforward. Nevertheless, our findings emphasize that these effects would be useful to consider.

Where do we go from here? Conclusions and recommendations

Overall, our findings for body mass, behavior and orange color on males are encouraging from the perspective of long-term guppy research, as they suggest that researcher sampling has not noticeably altered these phenotypes in low-predation populations. Still, statistically significant associations between sampling intensity and black color on males highlights the importance of considering researcher effects when designing research projects in the field (and when

considering which research sites to select). Past guppy studies have reported that fish predation explains ~ 1% of variance in relative black area at the individual level (Millar et al. 2006) – a much weaker effect than the 8.4% of variance that we found could be attributed to researcher sampling. Our result for black coloration therefore suggests that researcher induced-selection could be quite strong in wild guppy populations. However, common garden experiments will be required to determine whether these effects are evolutionary or the result of within-lifetime changes. Still, several factors should make researcher effects hard to detect in nature, including the possibility of some gene flow between heavily- and weakly-sampled sites, high fecundity that should quickly replace sampled guppies, and short-term, varied and episodic researcher sampling (compared to presumably constant predation pressure). The fact that we detected any significant effects at all could suggest surprisingly strong effects of researcher sampling.

Mitigating researcher effects is not straightforward. Possible ways to reduce research impact include conducting studies within a field site (e.g., Magurran & Seghers 1994; Reader et al. 2003) or returning subjects to their site of capture (e.g., Potter et al. 2026). When this is not possible, researchers could sample away from highly-sampled sites; however, they then risk affecting phenotypes within those new environments. Still, choosing to reduce the pressure on any one site by spreading sampling across more sites could both mitigate researcher-induced effects and increase the generalizability of findings beyond a few specific sites (Webster & Rutz, 2020). Detailed and consistent reporting of sample sizes, study sites (including river name, access points, and GPS coordinates), capture techniques, and fate of sampled fish would greatly aid this endeavour (Magurran 2005). Frameworks such as ARRIVE 2.0 and STRANGE support the standardization of such reporting, yet implementation remains spotty (Sert et al., 2020;

Webster et al., 2026) . In addition to self-reporting in the literature and publication requirements, centralized records of all sampling and its impacts could help inform natural resource management decisions, as could reporting mechanisms for apparently oversampled sites. This would be especially important in systems studied by multiple research groups working independently. The meaningful involvement of local researchers has multiple benefits here, potentially including better oversight of sampling pressure on different populations if researchers at local institutions are aware of multiple projects, as well as reducing other problems of parachute or helicopter science (Barber et al. 2012).

Mitigating long-term researcher effects also requires that more hypothesis-driven research is conducted on this specific topic. As part of the fieldwork permissions process, researchers are often required to consider how their presence might impact the results of a given study (e.g., observer effects on behavior; Crofoot et al. 2010; Hoy & Brereton, 2022), or make practical decisions to mitigate lasting ecological effects irrespective of a scientific question (e.g., sanitizing equipment to limit parasite spread between populations). However, developing and testing hypotheses and predictions about researcher effects that last beyond the temporal scope of a given study remains extremely rare. Such studies, in combination with considerations of other human impacts (Magurran 2005), are critical for determining the scale of researcher effects within guppies and across diverse systems in order to inform efforts for minimizing their impacts, especially in heavily studied, unique, or at-risk populations or species.

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856 **Tables**

857 Table 1. Sampling Locations for the present study with details of previous sampling effort.

Site	River	GPS	# of fish collected	# of sampling instances	# of fish sampled	Site Description
AHS	Aripo	10.68555, -61.23259	31	13	464	Near road, open canopy
AWS1	Aripo	10.69041, -61.23697	52	3	73	Upstream from AHS, far from road, closed canopy
AWS2	Aripo	10.67805, -61.22506	33	0	0	Downstream from AHS, far from road, closed canopy
PHS	Paria	10.74915, -61.2649	43	6	242	Near road, open canopy
PWS1	Paria	10.750703, -61.26142	57	0	0	Upstream from PHS, far from road, closed canopy
PWS2	Paria	10.74661, -61.26240	57	3	59	Downstream from PHS, far from road, closed canopy

858

859 HS: heavily-sampled, WS: weakly-sampled, based on our literature survey. All sampling was
860 carried out between February 14th and 25th, 2024. Note: number of fish collected is the number
861 we collected but does not equal analysis sample sizes as extra fish were collected for stimuli in
862 the shoaling test. Number of sampling instances are the number of studies from the literature
863 review that gave GPS coordinates within 250 m of the site. Number of fish sampled is the total
864 number of fish sampled recorded in our literature survey across all sampling instances. Site
865 descriptions are based on personal observations at site.

866

Table 2. Model results. Significant effects are in bold.

	F	df	p	Estimate [95% CI]	Semi-partial R ² [95% CI]
<i>Model: Distance travelled</i> (<i>n</i> = 171; <i>R</i> ² <i>m</i> = 0.037, <i>R</i> ² <i>c</i> = 0.071)					
Sampling	0.15	1, 3.5	0.72	-38.5 cm [-331.0, 253.9]	0.002 [0.000, 0.036]
River	3.00	1, 2.9	0.19	-157.7 cm [-453.4, 138.1]	0.035 [0.002, 0.106]
Mass	0.01	1, 163.2	0.91	5.2 cm [-80.8, 91.2]	0.000 [0.000, 0.030]
Sex	0.09	1, 165.5	0.77	21.0 cm [-120.6, 162.6]	0.000 [0.000, 0.032]
<i>Model: Edge time</i> (<i>n</i> = 171; <i>R</i> ² <i>m</i> = 0.032, <i>R</i> ² <i>c</i> = 0.120)					
Sampling	0.85	1, 3.3	0.42	18.0 s [-41.2, 77.2]	0.015 [0.000, 0.071]
River	0.46	1, 3.0	0.55	-12.3 s [-69.8, 45.2]	0.009 [0.000, 0.059]
Mass	2.95	1, 166.0	0.09	11.0 s [-1.7, 23.6]	0.017 [0.000, 0.075]
Sex	0.27	1, 164.6	0.60	5.5 s [-15.3, 26.4]	0.001 [0.000, 0.036]
<i>Model: Shoaling</i> (<i>n</i> = 163; <i>R</i> ² <i>m</i> = 0.071, <i>R</i> ² <i>c</i> = 0.156)					
Sampling	0.05	1, 3.3	0.84	10.1 s [-128.7, 148.9]	0.001 [0.000, 0.035]
River	0.78	1, 3.0	0.44	37.6 s [-96.8, 171.9]	0.016 [0.000, 0.075]
Mass	0.09	1, 157.0	0.76	-4.6 s [-34.1, 25.0]	0.001 [0.000, 0.033]
Sex	0.13	1, 155.7	0.72	8.8 s [-40.5, 58.2]	0.001 [0.000, 0.034]
Shoal side	7.68	1, 154.9	0.006	-61.0 s [-104.6, -17.5]	0.042 [0.003, 0.120]
<i>Model: Black</i> (<i>n</i> = 93; <i>R</i> ² <i>m</i> = 0.116)					
Sampling	8.33	1, 4.9	0.04	0.03 [0.003, 0.06]	0.084 [0.010, 0.215]
River	2.38	1, 2.7	0.23	0.01 [-0.02, 0.05]	0.029 [0.000, 0.129]
Extractor	0.78	2, 86.6	0.46	NA	0.017 [0.001, 0.119]

Model: Orange

($n = 93$; $R^2m = 0.353$, $R^2c = 0.410$)

Sampling	0.23	1, 3.6	0.66	0.016 [-0.08, 0.11]	0.005 [0.000, 0.073]
River	18.92	1, 3.0	0.02	0.13 [0.04, 0.23]	0.325 [0.187, 0.470]
Extractor	1.70	2, 85.8	0.19	NA	0.033 [0.003, 0.150]

Model: Mass

($n = 172$; $R^2m = 0.267$, $R^2c = 0.389$)

Sampling	0.91	1, 3.2	0.41	x0.85 [0.50, 1.45]	0.025 [0.000, 0.089]
River	0.50	1, 3.0	0.53	x1.12 [0.67, 1.87]	0.017 [0.000, 0.074]
Sex	65.66	1, 165.1	<0.001	x0.61 [0.54, 0.68]	0.243 [0.144, 0.351]

Models include site as a random effect. Extractor has three levels (JAF/AMH/both) so it has no single difference. Estimates are

differences between estimated marginal means (heavily - weakly sampled; Paria - Aripo; males - females; right - left shoal side), or

the change per 0.1 g for mass. Black estimates are in proportion of body area; orange estimates are on the square-root scale; mass

estimates are back-transformed ratios. Shoal side refers to the side the shoal was presented on (alternated between each subject), thus

the significant result suggests a bias to spend more time on one side of the tank. CI = Confidence interval; R^2m = marginal R^2 ; R^2c =

conditional R^2 .

Figures

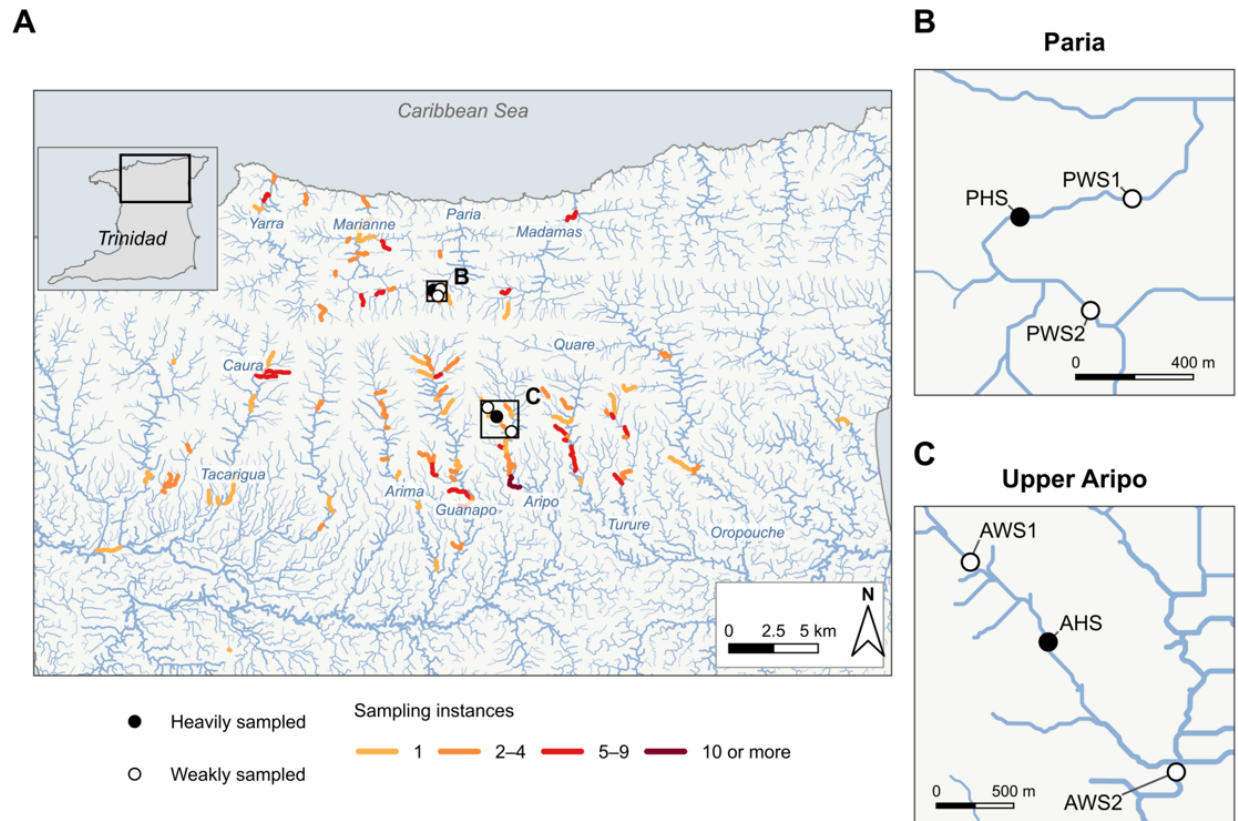


Figure 1. Sampling locations and researcher sampling intensity between 2003-2023 across the Northern Range of Trinidad. (A) Streams in the Northern Range with stream line width scaled by upstream drainage area. Colored areas show the number of sampling instances recorded in our literature review. Only sampling locations reported precisely enough to place within 100 m are shown and each was assigned to the nearest modelled stream reach within 150 m (325 of 391 sampling instances within the map area). Circles show our sampling, and boxes show the area of panels B and C. (B) Sampling sites in the Paria River: the heavily sampled site (PHS) and the weakly sampled sites upstream (PWS1) and downstream (PWS2). (C) Sites in the upper Aripo River: the heavily sampled site (AHS) and the weakly sampled sites upstream (AWS1) and downstream (AWS2).

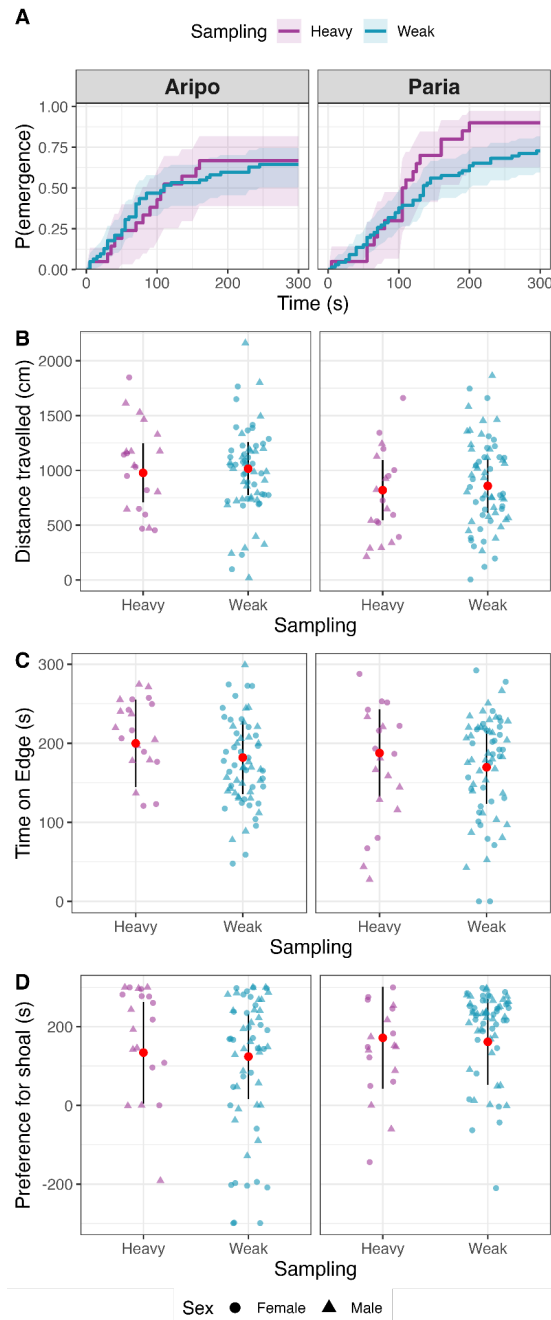


Figure 2. There was no detectable impact of sampling intensity on guppy behavior. (A) The cumulative probability of emerging from the shelter is plotted over time. (B - D) The red points are marginal means predicted by the model and the error bars represent 95% confidence intervals (both extracted using the *ggeffects* R package, Lüdtke, 2018). The blue and purple data points

represent the raw data and are jittered horizontally. The circular points represent females and the triangular points represent males.

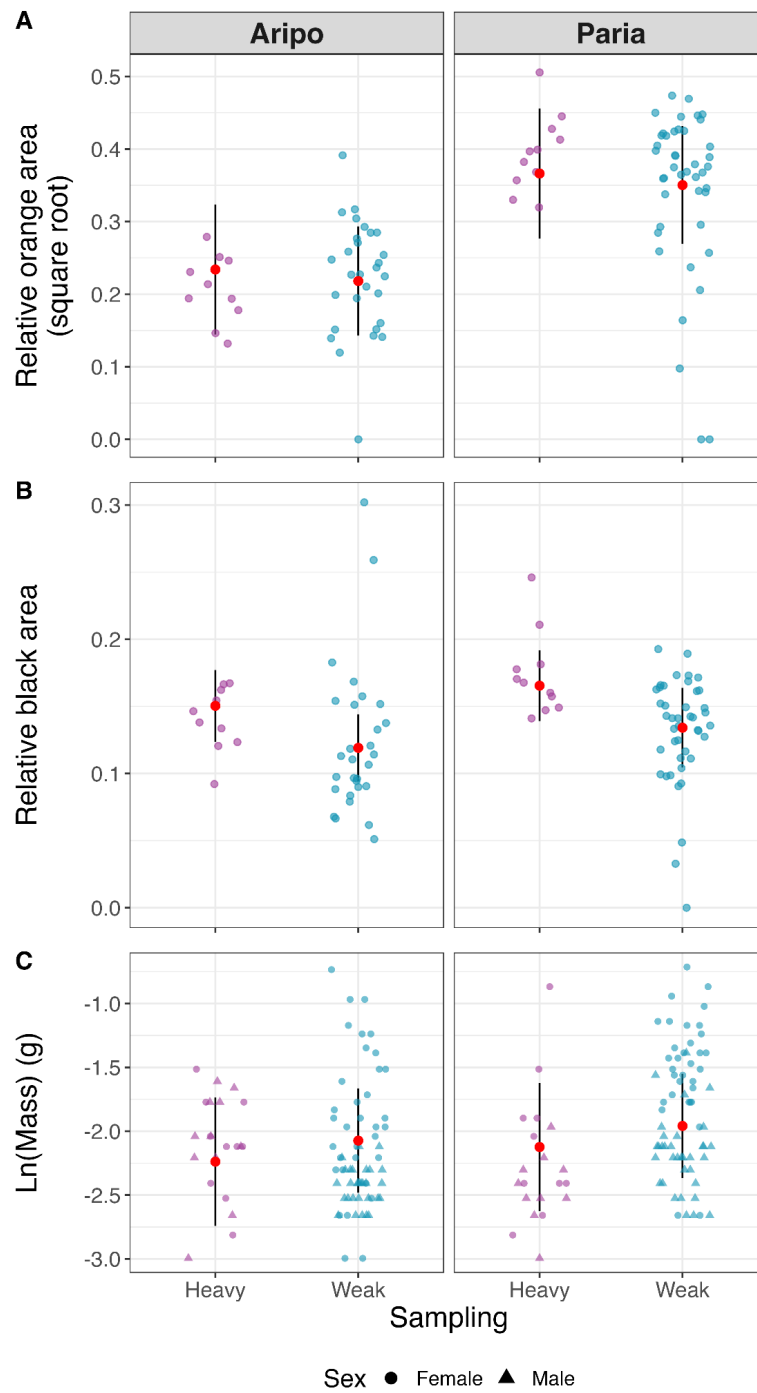


Figure 3. Male guppies in heavily-sampled sites had more black area than male guppies in weakly sampled sites (B), but there was no significant difference between heavily and weakly sampled sites for orange body colouration (A) or body mass (C). The red data points are marginal means predicted by the model and the error bars represent confidence intervals (both extracted using the *ggeffects* R package, Lüdecke, 2018). The blue and purple data points represent the raw data and are jittered horizontally. The circular points represent females and the triangular points represent males.