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Males show their best side revisited – Effects of predation pressure on laterality in wild guppies

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Abstract

The ability to detect and avoid predators has been suggested as one of the main drivers for behavioral lateralization. This is supported by studies reporting that animals from high-predation environments are more lateralized than those from low-predation environments. Some studies, however, have shown no effect of predator regime on lateralization. Lateralized behavior can also be driven by phenotypic asymmetry, where the more attractive side of the body is preferentially displayed during male–female interaction and courtship. The importance of fluctuating asymmetry for mate choice has been highly debated for a number of reasons. Here, we revisited the concepts of predator-induced behavioral lateralization and phenotypic asymmetry using wild Trinidadian guppies *Poecilia reticulata* from three different stream systems. Using fish collected from both high- and low-predation environments, we quantified behavioral lateralization in terms of directional turning bias, measured right–left body color asymmetry, and investigated whether a male side-showing preference was present in male–female interaction trials. We found that guppies were, at best, moderately lateralized on average (across all populations), without any general effect of predator regime. There were some slight stream-dependent side biases in color asymmetry, but this did not translate into a side-showing preference in any of the populations in the interaction trials. Some significant observed effects align with previously published results, but these were dependent on stream-origin and were not repeated across different experiments, complicating interpretation. We conclude that when investigating the effects of predation regime in general, and such effects on behavioral lateralization or fluctuating asymmetry in particular, attention must be focused toward several factors such as experimental assay used and population origin, and broad generalizations from results stemming from experiments including only one population should be avoided.

KEYWORDS

color ornament, courtship behavior, fluctuating asymmetry, *Poecilia reticulata*, replication

1 | INTRODUCTION

Cerebral lateralization has long been studied in the context of human neuropsychology and experimental psychology, where the concept has predominantly stayed even after the phenomenon had been discovered in other vertebrate species (Güntürkün et al., 2020; Vallortigara & Rogers, 2005). By the early 2000s, studies showing that cerebral lateralization could influence animal behavior (so-called "behavioral lateralization") had started to frequent the scientific literature, and the occurrence of this behavior has now been described in a large number of taxa (Vallortigara & Rogers, 2005). The term refers to the asymmetric control of cognitive functions and relies on the assumption that behavioral left- or right biases reflect underlying asymmetries in the functioning of the nervous system (Bisazza & Brown, 2011; Rogers et al., 2013; Vallortigara & Rogers, 2005). Adaptive values of behavioral lateralization are often explained in terms of possible advantages of asymmetric cognitive control, such as benefits associated with enabling multiple stimuli to be processed simultaneously (Vallortigara & Rogers, 2005). For example, in a widespread number of taxa, such as birds, mammals, and fish, individuals that are more lateralized have been reported to show enhanced foraging/prey capture efficiency (Friedlaender et al., 2017; Güntürkün et al., 2000; Kurvers et al., 2017; Magat & Brown, 2009), improved abilities to manage feeding and predator avoidance simultaneously (Dadda & Bisazza, 2006; Rogers et al., 2004), improved spatial learning (Sovrano et al., 2005), and enhanced schooling performance (Bisazza & Dadda, 2005). While rapid directional escape decisions may be favored when attacked by a predator, asymmetric control of sensory input could also lead to disadvantages given that predators, food, competitors, or mates are likely to appear at random in the environment (Vallortigara & Rogers, 2005). Disadvantages of lateralization have indeed been reported, for example, interference with exploratory behavior in fish (Dadda et al., 2009). Given the potential for both positive and negative effects relating to individual fitness, it is perhaps not surprising that great variation in the degree of lateralization has been reported both between and within species, with some species not being detectably lateralized at all (Clark et al., 2020; Roche et al., 2020; Vallortigara et al., 1999).

The variation in lateralization can be affected by many factors, with one being variation in predation pressure. Predator detection and avoidance, and the escape response when faced with a threatening situation is of particular interest when it comes to the adaptive function of behavioral lateralization. A selection experiment using the goldbelly topminnow *Giradinus falcatus* showed that it is possible to artificially select for left- and rightward turning when faced with a dummy predator in a detour test (Bisazza et al., 2007). Individuals selected for either left- or right-turning bias also showed corresponding lateral biases when it came to rotational preference in their home tanks, direction of spontaneous swimming, and escape trajectories in response to an auditory stimulus, but not to a rapidly approaching visual stimulus (Bisazza et al., 2005). Hence, some, but not all, movement responses appear to have an underlying mechanism in common with other types of lateralization traits. Several studies have reported that individuals that are more lateralized show better performance in tasks related to predator avoidance (Dadda & Bisazza, 2006; Rogers et al., 2004). Given the importance of escaping predation attempts, one hypothesis is that behavioral lateralization could be selected for and, hence, be more strongly expressed in environments with higher predation pressure. Indeed, several studies report that fish from high-risk environments display stronger lateralization than individuals from low-risk environments (Brown et al., 2004, 2007; Hulthén et al., 2021). It has also been suggested that predator-induced strength in lateralization is heritable, as supported by the above-mentioned selection experiment (Bisazza et al., 2007), conservation of lateralization between generations (Brown et al., 2007), and more recently by a confirmed genetic basis (Hulthén et al., 2021). However, some studies have shown no difference in lateralization between high- and low-predation environments. For example, Broder and Angeloni (2014) experimentally manipulated perceived predation risk and found no resulting increase in lateralization. All in all, although not universal, the ability to detect and avoid predators might be one of the apparent drivers for lateralization (Dadda & Bisazza, 2006; Rogers et al., 2004).

In addition to the effects of predation, both the degree and direction of lateralization can be affected by a range of other variables. For example, parasite prevalence (Roche et al., 2013), sexual motivation (Bisazza et al., 1998), asymmetries in the signaling environment (Dakin & Montgomerie, 2009), the physical complexity of the rearing environment (Bibost et al., 2013), and time in captivity (Bisazza et al., 1997). It has also been reported that phenotypic asymmetries can induce lateralized behavior, where the more attractive side of the body is preferentially displayed during male–female interaction and courtship (Amcoff et al., 2009; Gross et al., 2007; Řežucha & Reichard, 2015). The latter finding is related to the theory of fluctuating asymmetry, where symmetry in a trait is proposedly linked to developmental stability (Livshits & Kobyliansky, 1991) and, subsequently, sexually selected for via mate choice (Møller & Pomiankowski, 1993). Fluctuating asymmetry is observable in many traits across species (Swaddle, 2003; Watson & Thornhill, 1994) and a number of papers report that females often prefer to mate with males that show a higher degree of symmetry in particular traits (Polak & Taylor, 2007, Rhodes & Simmons, 2007, Tomkins & Simmons, 2003). Some species have been shown to perform lateralized courtship behavior (Krakauer et al., 2016; Workman & Andrew, 1986), and males of some bilateral species appear to show their more attractive side during male–female interaction and courtship (Amcoff et al., 2009, Gross et al., 2007, Řežucha & Reichard, 2015). Thus, asymmetry is a concept of potential importance within the theory of behavioral lateralization. Its importance for mate choice has, however, been highly debated due to possible methodological flaws when analyzing asymmetries (Swaddle, 2003; Swaddle et al., 1994), publication bias inflating the apparent generality of the proposed effects (Palmer, 1999; Palmer & Strobeck, 2003), and retracted publications (Abbott, 2004; Palestis et al., 2014).

Here, we revisited the concepts of predator-induced behavioral lateralization and side-showing preference related to fluctuating asymmetry, using wild-caught Trinidadian guppies *Poecilia reticulata*. The fish were collected from both high- and low-predation environments in three stream systems, and behavioral lateralization was investigated for males presented with two different diversion objects (a shoal of females and a control object) in a detour test. We measured total area of black- and orange coloration on the males' left- and right side, to calculate symmetry, and tested whether a side-showing preference was present in male–female interaction trials. The same males were used in both tests, to enable us to investigate whether behavioral lateralization as determined in the detour test was related to male side-showing preference when proximate to a female. Based on previous published findings, as referred to above, we predicted that males from the high-predation environments would be more lateralized than males from the low-predation environments. We further predicted, if color asymmetry would be present, that the more colorful side would be preferentially displayed toward the female in interaction trials, and that side-showing preference would be correlated to side bias to the female object in the detour test. Since differences between Trinidadian river systems have previously been reported in life-history traits in guppies (El-Sabaawi et al., 2012; Neff et al., 2008), we also tested for possible stream-effects in our analyses. In addition, we provide analyses of overall lateralization for descriptive purposes, as well as descriptive data on total color area and body size, given the importance and usefulness of descriptive statistics (Murphy, 2021).

2 | METHODS

2.1 | Fish collection and holding

The experiments were conducted at the University of the West Indies at St. Augustine, Trinidad, in March 2015. Fish collection and holding is only briefly presented, since it has been described in detail elsewhere (Herbert-Read et al., 2017). Wild adult female and male guppies were collected from three stream systems: Turure (Oropuche basin), Aripo (upper Caroni basin), and Tunapuna/Tacarigua (two neighboring tributaries in the lower Caroni basin), see Heckley et al. (2022) for a map of the stream systems. Within each stream system, we collected fish from one upstream site with low predation, and one downstream site with high predation. The spread of predators between low- and high-predation sites is hindered by natural barriers, such as waterfalls (Magurran, 2005). The high-predation sites were inhabited either by the Trinidadian pike cichlid *Crenicichla frenata*, a main predator of adult guppies, or other predatory fish species such as wolf fish *Hoplias malabaricus* or blue acara *Adinoacara pulcher* (Herbert-Read et al., 2017). The low-predation sites were inhabited by Hart's rivulus *Anablepsoides hartii* (Herbert-Read et al., 2017), a species which is not considered to be a major predator of adult guppies (Magurran, 2005). Effects of predation are considered as the main driving force for evolutionary divergence in life history, coloration, and behavioral traits among Trinidadian guppies, with other differences between streams and sites (e.g., water depth, canopy cover, and the spectral properties of the water) being argued to be less important (Grether et al., 2001; Millar et al., 2006; Reznick et al., 2001; Reznick & Endler, 1982) but see (Archard et al., 2009; Schwartz & Hendry, 2010). The fish were transported back to the aquarium facilities at the University of West Indies at St. Augustine and housed in glass tanks, each population was held separately, sexes were kept together (mixed-sex tanks). All fish were kept in aged tap water (i.e., without predator chemical cues), and were held for a minimum of 36 h before being used in any experiment. The water temperature in all holding and experimental tanks was held at 24°C. The fish were fed flake food to apparent satiation in the morning and noon of each day to maintain satiation levels. All fish used were adults, but the exact age was unknown.

2.2 | Behavioral lateralization – Detour test

To assess effects of predation environment on behavioral lateralization we used a detour test adapted from Bisazza et al. (1998). The lateralization arena consisted of a glass aquarium, covered with white opaque plastic sheets to prevent visual disturbance, which was divided into a double-sided T-maze, with a runway down the middle (dimensions: 90 cm long, 30 cm wide, with an 8 cm wide and 30 cm long middle runway, Figure S1). A diversion object was placed at both ends of the arena, at one end consisting of a shoal of three females enclosed in a cylinder, and at the other end, an empty cylinder representing a neutral object (to control for potential general turning direction preferences (Irving & Brown, 2013)) (Figure S1). At the commencement of a trial, a single male fish was carefully introduced into the middle runway of the lateralization arena and given 5 min to acclimate (during which time the ends of the runway were blocked). The fish was then gently encouraged to move forward (without touching the fish, using perforated plastic rods) until a left or right turning choice was made by the diversion objects, when the fish left the central channel and entered one of the perpendicular side channels (Figure S1). For each individual, a total of 12 consecutive runs (alternating which end of the arena the test began at) were performed and recorded individually through direct visual observation by one observer (FMG). Blind scoring was not possible since population history was known to the observer. We also noted start direction in order to account for any potential asymmetries in the setup, for example, caused by ambient cues (Roche et al., 2020). The start placement of the diversion objects was alternated between trials. When the trials were completed, the males were carefully netted from the lateralization arena and placed in individual net cages (13 × 12.5 × 16.5 cm) in an aquarium, in order to keep track of the individual males in-between the lateralization test and the interaction trials (see method description for interaction trials below). We tested 25–28 fish from each population; however, some fish appeared agitated, and could not be coerced to swim forward in the lateralization arena. It was not possible to complete all 12 runs for these fish and they were excluded from analyses. In total, 130 males (18–26 per population) were used in statistical analyses (Table 1).

TABLE 1 Sample size for the behavioral lateralization trials, side-showing preference – interaction trials, and male ornament analysis per river and predation regime (high- and low-predation)

River	Predation regime	*n* lateralization	*n* interaction and ornament
Aripo	Low	20	26
	High	23	20
Tacurigua/ Tunapuna	Low	26	28
	High	19	26
Turure	Low	24	11
	High	18	11

All males used in the lateralization test described above had been used in a previous lateralization test, which unfortunately had to be excluded due to circumstances undermining the intent of the test (see supporting information and Figure S2, for details). In-between the two lateralization tests, the males were kept in individual net cages (13 × 12.5 × 16.5 cm) in an aquarium for ~24 h.

2.3 | Behavioral lateralization – Interaction trials

To investigate whether males would preferentially display their left or right side during direct interaction with a female (from the same population), and to couple any such side-showing preference to behavioral lateralization side bias to the female object from the detour test, we ran interaction trials using the same males as in the lateralization test. Trials were conducted in glass tanks (46 × 23 × 23 cm), divided into two separate arenas (using opaque dividers, allowing us to run two trials simultaneously per tank, with one pair of fish per arena), with each arena measuring 23 × 23 × 23 cm. The sides of the aquariums were covered in white opaque plastic sheets to prevent visual disturbance (Figure S3). Sexually mature females were carefully hand netted from the holding tanks and introduced to the arena (one female per arena, new female for each trial) and given 10 min to acclimatize. A male was hand netted from the individual holding cages (see methods description for behavioral lateralization – detour test above) and introduced to each arena and allowed to freely interact with the female for 12 min. The trials were video recorded using a GoPro Hero 4 (GoPro, Inc., San Mateo) mounted above the aquariums. Four replicates were filmed simultaneously (i.e., 4 arenas in 2 tanks were used at a time, Figure S3). Prior to each trial, arenas were marked with a continuous ID number (not revealing collection site or predation pressure) to enable blind analysis of the videos. We ran a total of 123 trials, using 11–28 pairs of fish for each population (Table 1). In one trial (Aripo high predation), the female became ill, and the trial was therefore excluded, leaving a total of 122 trials for the analyses (Table 1). After trials, males were netted from the arenas, sedated in an ice slurry, and placed on a millimeter paper together with the ID note. A photo was then taken of the fish left and right side (using a Canon EOS 7D with a Canon EF-S lens, 18–55 mm f/3.5–5.6 IS II; Canon Inc., Tokyo). All males from the interaction trials were photographed (*N* = 122, Table 1). The females were sedated in an ice slurry and excess water was gently wiped off before weighing them to the nearest 0.01 g. All fish were then released into an isolated pond at the university area since it is prohibited to release experimental fish back into the wild.

2.4 | Video analysis of interaction trials

Videos of the interaction trials were analyzed using the software BORIS v 7.9.7 (Friard & Gamba, 2016). All videos (*N* = 122, Table 1) were analyzed blind with respect to treatment history and general experimental design (by WA). The first 3 min of the videos were regarded as an acclimation period for the males, leaving 9 min of each video to be analyzed. We quantified the time the males spent showing their left, right, or no side to the female when they were within proximity of each other. Proximity was defined as within 3 (male) body lengths (state events: proximity L; individuals in proximity and male showing left side, proximity R; individuals in proximity and male showing right side). The side the male was showing was determined using two 20° cones originating from the center and extending directly in front of and directly behind the male, respectively (Figure S4; also illustrated in Figure 4). If the female was outside the range of both cones (i.e., not directly in front of or directly behind the male), the side of the male facing the female was recorded. If the female was within the range of either cone, this was recorded as the male showing no side (state event: proximity N; individuals in proximity but male showing no clear side).

2.5 | Male color ornament

The digital photographs of the males used in the interaction trials (*N* = 122, Table 1) were analyzed using ImageJ v 1.52t (Schneider et al., 2012). The total area of black and orange on each male's left and right side was quantified (excluding the color of the caudal fin, since the fin is transparent and pigment spots are therefore visible from both sides and hence relatively symmetric (Figure S5) (Dick et al., 2018; Sheridan & Pomiankowski, 1997b)). Black and orange patches were identified by eye, and only clearly identifiable patches at 100% magnification were measured (at 100% magnification, the fish took up between 14% and 22% of the width of a 5184 × 3456 pixel image) similar to the methods used in (Ruell et al., 2013). Black was not measured if the general coloration of the male was very dark, as opposed to black being a specific patch of concentrated melanic pigment (Figure S5). Dark horizontal strips of color, located along the center of the rear half of some males were also left unmeasured since they varied markedly in color and were often difficult to define as black or dark purple/blue/green (Figure S5). Standard length of each male was also measured from the photos. All photos were analyzed blind regarding population history and general experimental design (by WA).

2.6 | Statistical analysis

2.6.1 | General notes

All analyses were conducted in R 3.6.3 (R Core Team, 2020). Models were run through the packages *lme4* (generalized linear models 'GLMM'; Bates et al., 2021) or *stats* (linear models 'LM' and generalized linear models 'GLM'; R Core Team, 2020), and pairwise contrasts investigated using *emmeans* (Lenth, 2021), unless otherwise stated. Dispersion of binomial models was evaluated through *DHARMa* (Hartig, 2021). Permutation tests (for absolute color asymmetry) were run through *coin* (Hothorn et al., 2021). Data handling and graphics were done using the *tidyverse* suite (Wickham et al., 2019) and *cowplot* (Wilke, 2020).

2.6.2 | Analysis: Behavioral lateralization – Detour test

Data were primarily analyzed as absolute lateralization score, that is, proportion of turns in the preferred direction. The absolute lateralization scores were analyzed using a binomial GLMM (logit link-function) on proportion data, based on the factors RIVER (catchment origin of the fish; categorical factor; 3 levels: Aripo, Tunapuna/Tacarigua, or Turure – Tunapuna and Tacarigua were combined as they belong to the same catchment), PRED (predation regime; categorical factor; 2 levels: high and low), OBJECT (diversion object within the trial; categorical factor; 2 levels: female group or neutral object), and ID (individual identity; random factor; 130 levels). All interactions of fixed effects were included in the model. Over−/underdispersion was tested using a two-sided DHARMa nonparametric dispersion test (Hartig, 2021), which indicated a significant underdispersion (dispersion = 0.20, $p < .001$), which means standard errors (and hence confidence intervals) are overestimated. To correct the model for the underdispersion, the summary table from the analysis was adjusted into a quasi-likelihood table (Bolker, 2021) by multiplying the standard error by the square root of the dispersion factor using the overdisp_fun() function from the *PsychHelperFunctions* package (Huff & Papenmeier, 2020), with 95% confidence intervals (t-adjusted) calculated based on the recalculated standard errors. "Preferred direction" was used as a grouping variable when constructing Figures 1 and 2, to investigate overall lateralization (it was not used as a response variable), where preferred direction across all runs (regardless of diversion object, assuming that turn direction in general is caused by a common neural lateralization mechanism, i.e., not context dependent) were classified as '1' and non-preferred direction as '0'; when equal number of turns were made in each direction, preferred direction (left or right) was assigned randomly.

In addition to the full factorial model, an intercept-only model (constant mean model) of overall absolute lateralization score was run, excluding all factors (i.e., only including the intercept; pooling objects, which eliminated the need for a mixed model). This GLM model was also underdispersed (dispersion = 0.06, $p < .001$), and therefore rerun using the quasibinomial distribution. Pairwise comparisons were based on GLMs/GLMMs on data subsets; pairs compared were (i) OBJECT|RIVER × PRED (i.e., comparing levels of OBJECT given levels of RIVER × PRED; GLMM; 6 comparisons), (ii) RIVER|OBJECT × PRED (GLM; 12 comparisons), and (iii) PRED|RIVER × OBJECT (GLM; 6 comparisons). For these comparisons, p-values were evaluated based on Benjamini-Hochberg tables (Benjamini & Hochberg, 1995) for each group of pairs, to take multiple testing into consideration.

Since overall absolute lateralization score data (proportion turned in the preferred direction) only ranges between 0.5 and 1.0 (since no fish can turn less than 50% in their preferred direction), the proper reference value representing randomly assigned data is not 0.5 (perfect symmetry), but slightly higher since values around 0.5 on the relative lateralization scale are added together, making these observations more common than perfect symmetry (as opposed to taking the absolute value of the relative lateralization score). To find a proper reference value that incorporates variation due to chance, we generated 20 random data sets (digital coin-toss datasets, simulating 'heads' or 'tails', i.e., 1 or 0) with a structure identical to our real data. Twelve values (1 or 0; representing right and left turns) for each of the 130 dummy individuals (matching the number of individuals in the trials), were randomly drawn from a binomial distribution ($p = .5$). For each random data set, the binary data were recalculated into absolute lateralization score, following the procedure used for our real data. To get the expected value for random data when absolute lateralization scores are divided based on diversion object (i.e., 6 observations for each diversion object; which also allows data for a certain object to drop below 0.5, depending on the relative number of turns into each direction for each diversion object type), we assigned half of the observations for each dummy individual to each of two categorical values ('A' and 'B'; representing dummy objects). Expected values for random data were derived by analyzing their simulated data using binomial GLMs, extracting the parameter estimates for the regressions as the values to compare against.

Data were also analyzed as relative lateralization score, that is, proportion of turns into one specific direction (left vs. right; data analyzed as proportion of turns to the right). Here, we assumed that individual fish would be lateralized into the same direction (left or right) regardless of which diversion object they faced in the trial. Hence, the model tests whether any of the (sub-)populations differ in their left−/right bias and only the factors RIVER, PRED, and their interaction were included in a GLM model. Based on binomial distribution, the model was underdispersed (dispersion = 0.152, $p < .001$) and, consequently, an additional quasibinomial GLM was run.

2.6.3 | Analysis: Male color ornament

Area of orange and black patches was square-root transformed prior to analysis, due to positive skew. To analyze patch size differences among (sub-)populations, we used linear models including the covariate standard length (SL) and the factors RIVER, PRED, and their interaction. The response variable was total patch area, with separate models run for black and orange coloration. Tukey-adjusted pairwise contrasts focused on PRED|RIVER and RIVER|PRED pairs (i.e., same as for body size analyses). Fluctuating asymmetry in black and orange color patches was calculated based on left-side bias where area on the left ($\text{Area}_{\text{Left}}$) was divided by total area ($\text{Area}_{\text{Left}} + \text{Area}_{\text{Right}}$) of each color. Based on left-side bias we calculated an asymmetry index:

$$\text{Assymetry index} = \left| 0.5 - \frac{\text{Area}_{\text{Left}}}{\text{Area}_{\text{Left}} + \text{Area}_{\text{Right}}} \right| \bullet 2$$

This index gives a value between 0 and 1, where 0 indicates perfect symmetry and 1 indicates that all patches of the color are located on one side only. To analyze if color asymmetry had an effect on side-showing behavior in males, left-side bias was analyzed using quasibinomial GLMs (binomial models underdispersed; black: dispersion = 0.08, $p < .001$; left: dispersion = 0.05, $p < .001$) including the factors RIVER, PRED, and their interaction. Tukey-adjusted pairwise contrasts focused on PRED|RIVER and RIVER|PRED pairs. Differences in asymmetry index were analyzed based on permutation-based independence tests, with the conditional null distribution of the test statistic approximated by Monte Carlo resampling (10,000 samples) (Hothorn et al., 2021). An initial test investigated the significance of the overall model's difference from the conditional null distribution, based on a model including the factors RIVER, PRED, and their interaction. If significant, this test was followed by separate tests for each stream system (analyzing levels of PRED) and for each predation regime (analyzing levels of RIVER). If the latter test was significant, we used subsets of data (i.e., systematically excluding one river) to investigate pairs of stream systems.

2.6.4 | Analysis: Behavioral lateralization and side-showing preference – interaction trials

To investigate whether male side-showing preference when proximate to a female was related to behavioral lateralization as determined in the detour test, we used Pearson correlation analyses (pooling data for all subpopulations). Lateralization was included as general relative lateralization score (proportion of turns to the right against diversion objects *females* and *neutral object* combined) and relative lateralization score against females only. We also correlated side-showing preference with respect to coloration asymmetry (black- and orange right-side bias; i.e., proportion of colored area present on the right side of the body, also see color analysis below).

2.6.5 | Analysis: Body size

To analyze body size of males (standard length; SL_{male}) and females (wet body mass; BM_{female}) from different (sub-)populations, we used linear models including the factors RIVER, PRED, and their interaction. Analyses were followed by Tukey-adjusted pairwise contrasts focusing on (i) PRED|RIVER (six comparisons) (i.e., comparing levels of PRED given levels of RIVER) and (ii) RIVER|PRED (six comparisons). Normality and heteroscedasticity were assessed graphically; SL_{male} was analyzed on its original scale and BM_{female} was $\log_e$-transformed due to slight positive skew.

3 | RESULTS

3.1 | Behavioral lateralization – Detour test

For absolute lateralization, based on the intercept-only model (GLMs for observed and randomly generated data) we found that the estimate for general laterality (i.e., $\beta_{\text{intercept}}$) was slightly higher than that of the randomly generated data (Figure 1). The 95% confidence interval for the observed data $\beta_{\text{intercept}}$ overlapped the average $\beta_{\text{intercept}}$ for randomized data when based on a binomial model but not when based on a quasibinomial model (Figure 1; model summaries in Table S1). Given the significant underdispersion (see Methods: Analysis: behavioral lateralization – detour test), we assume that the quasibinomial model results produce better estimates of the standard error (and thereby the confidence intervals) and, hence, conclude that the Trinidadian guppies are moderately lateralized on average (also assuming that diversion object did not affect turning direction). To validate this conclusion, we ran a suite of non-parametric pairwise comparisons between observed data and all randomly generated data sets, using

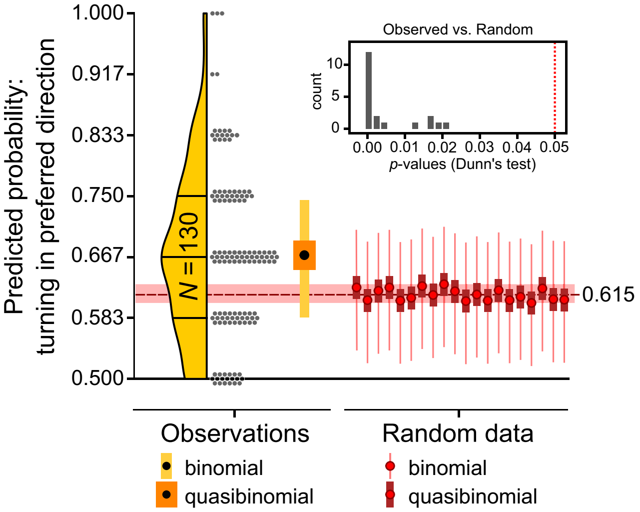

FIGURE 1 Absolute lateralization in guppy males was estimated using a binomial−/quasibinomial generalized linear model (GLM). Density plot (yellow) and jittered grey points show the distribution of the observed data. Black point with orange and yellow error bars (95% confidence intervals for binomial and quasibinomial models) show the overall estimated proportion of turns in the generally preferred direction (all populations and diversion objects pooled). Red points with error bars shows binomial GLM estimates for absolute lateralization in 20 completely randomized data sets; the dashed red line shows the average of the random data estimates (0.615) and the red shaded area around this line show the range of the random data estimates. Inlay shows p-values for Dunn's test comparisons between observed data and all random data sets (all $p < .03$).

Dunn's test with Benjamini–Hochberg correction (Benjamini & Hochberg, 1995, Dunn, 1964). All comparisons were significant, with $p < .03$ (Figure 1).

The more complex full factorial GLMM, where estimates are conditional on the factors river, pred, and object, revealed more details. Assuming that the quasibinomial model is more appropriate than the binomial model (both model summaries reported in Table S2), estimates indicate that only four groups have confidence intervals not overlapping the overall average of the randomized data (Figure 2). Three of these relate to females as diversion objects in the detour test (Tunapuna/Tacarigua fish from high- and low-predation regimes, and Turure fish from low-predation regime) and one relating to facing the neutral object (Turure fish from high-predation regime). All the three first groups have intervals overlapping at least one of the random data estimates, while the latter does not overlap any. Two groups have estimates with confidence intervals being lower than that of the random data (Tunapuna/Tacarigua fish facing the neutral object, both subpopulations; Figure 2). Hence, Tunapuna/Tacarigua fish were less lateralized when facing the neutral object than when facing females (Figure 2; pairwise comparisons with $p \leq .001$ for both subpopulations, Table S3). There was also a significantly stronger lateralization when facing females in Turure fish from low-predation regime ($p = .018$; Figure 2; Table S3). Overall, the more complex model provides a more complex picture of the general lateralization of Trinidadian guppies, with much less clear evidence for general lateralization in the species. However, the complex model also suffers from relatively low statistical power as compared to the intercept-only model evaluated above since the number of observations per fish and object is only six, and residual degrees of freedom are lower for the model.

For relative lateralization, the binomial model indicated no significant effects of the included factor terms (all terms with $p > .5$), but

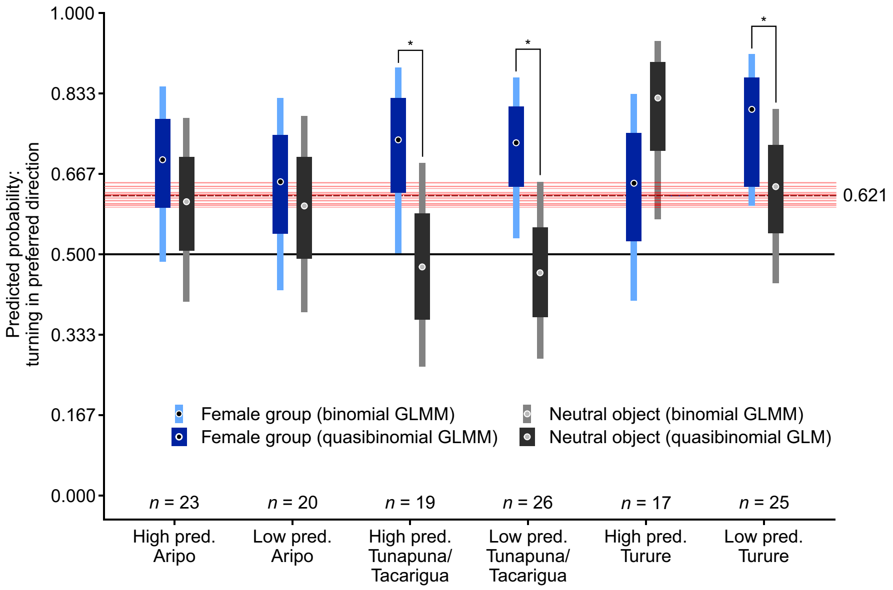

FIGURE 2 Absolute lateralization in guppy males. Point estimates show absolute lateralization with 95% confidence intervals (error bars) for binomial GLMM (narrow lighter-colored intervals) and quasibinomial GLMM (broad darker-colored intervals). Red lines show estimates derived from 20 randomized data sets, with the dashed line indicating their mean (0.62). Asterisk (*) indicate significant pairwise comparisons (investigated pairs: object|river × pred; river|object × pred; pred|river × object).

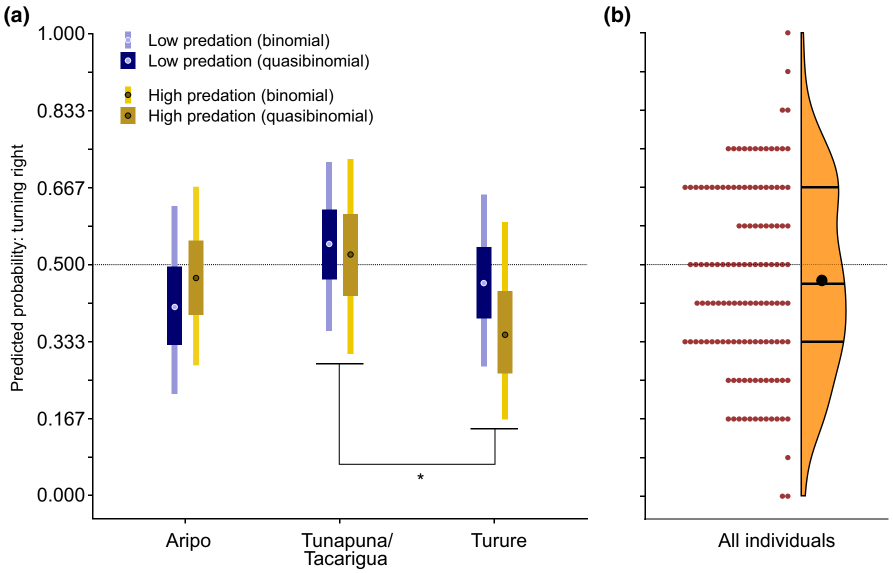

FIGURE 3 Relative lateralization in guppies conditional on river origin and predation regime. (a) Estimated probability of turning right, from binomial generalized linear model. Asterisk (*) indicate a significant river effect in the quasibinomial model. (b) Raincloud plot of all data, red points and density plot (including quantiles) show data distribution; global average indicated as a black point in the density plot. Grey dotted lines in panel A and B indicate perfect symmetry, that is, no side bias.

the quasibinomial model had a significant effect of RIVER (ANODEV: $\chi^2 = 7.37$, $p = .025$); model summaries and ANODEV-tables are found in Tables S4 and S5. Pairwise contrasts among levels of RIVER for the quasibinomial model indicated that Turure fish were more left-biased on average than Tunapuna/Tacarigua fish ($p = .008$), while no other contrasts were significant (Aripo vs. Tunapuna/Tacarigua: $p = .072$; Aripo vs. Turure: $p = .675$). Judging from the 95% confidence intervals from the quasibinomial model, only two subpopulations are significantly lateralized in a specific direction on average (Aripo:low-predation and Turure:high-predation; Figure 3a). Hence, the difference between Turure and Tunapuna/Tacarigua may be driven mainly by the high-predation subpopulation, although without a significant interaction effect in the ANODEV ($\chi^2 = 4.146$, $p = .126$). The low-predation subpopulation in this stream shows the same left-bias tendency (albeit with confidence intervals overlapping 0.5), contributing to the significant RIVER effect. Data for all individuals combined is slightly bimodal around 0.5 (Figure 3b), suggesting a possible weak (but statistically unsupported) absolute lateralization (as discussed above).

3.2 | Behavioral lateralization – Interaction trials

None of the correlations between behavioral lateralization, color ornament, and side-showing preference were significant, and all had low correlation coefficients (Figure 4a–d; see Table S6 for detailed statistics). The correlation between side-showing preference when proximate to a female (S-SP) and black-color right side bias (RSB) had a p-value below 0.1 (0.080), but this seems to be influenced by a few data points, with the majority of data being symmetrically distributed in the center of the correlation plot (Figure 4c). The general hypothesis of side-showing preference was assumed to relate to the species and not to any particular population. However, as an ad hoc analysis, we also ran separate correlations for each stream system and for predation regimes (Figure S6–S9). In these analyses, a significant positive correlation was found for males from high predation ($r = 0.264$, $p = .048$), although this too appears to be largely influenced by a few outlier data points at the extremes of the color asymmetry score (Figure S8). With Bonferroni correction for multiple testing, the significance is not maintained. No other correlation was significant.

3.3 | Male color ornament – Black coloration

The linear model for total area of black patches showed that the area increased with standard length ($F_{1,115} = 4.29$, $p = .041$; $\beta_{SL} = 0.089 \pm 0.043$ SE). The main effects of RIVER and PRED were non-significant (RIVER: $F_{2,115} = 0.67$, $p = .515$; PRED: $F_{1,115} = 0.24$; $p = .627$), but their interaction was close to significant ($F_{2,115} = 2.82$, $p = .064$). Parameter estimates from the model indicated a significant interaction component (Table S7); hence, we proceeded to interpret the pairwise contrasts. Contrasts indicated that high-predation males from Turure had less black color than the low-predation males from the same stream system ($p = .002$); non-overlapping 95% confidence intervals for estimated marginal means support this inference (Figure 5a). Contrasts for predation regimes in Tunapuna/Tacarigua were close to significant ($p = .079$) indicating a similar pattern as for Turure, but with substantially smaller difference (Figure 5a). No differences were indicated between subpopulations in Aripo ($p = .627$). Turure males from high-predation also had less black color than males from the high-predation regime in the other two stream systems (both $p < .002$) (Figure 5a). No other contrasts were significant for RIVER|PRED pairs (all $p > .4$). Worth noting is that Turure high-predation males still had a very positively skewed distribution after data transformation, with a median of 0 (= no black coloration). Due to this non-normality, p-values from the analyses should be interpreted with caution; however, raw data patterns align with the overall statistical results (see boxplots in Figure 5a).

3.4 | Male color ornament – Orange coloration

The linear model for total area of orange patches showed that the area increased with standard length ($F_{1,115} = 8.21$, $p = .004$; $\beta_{SL} = 0.158 \pm 0.055$ SE). The main effects of RIVER were significant (PRED: $F_{2,115} = 8.53$; $p < .001$) but PRED was non-significant ($F_{2,115} = 0.99$, $p = .321$), and so was the interaction term ($F_{2,115} = 0.40$, $p = .673$); see all parameter estimates in Table S8. Pairwise contrasts were limited to RIVER|PRED comparisons and indicated that males from Turure had less orange color than the other two stream systems, both for low-predation ($p < .02$) and high-predation ($p < .02$) males (Figure 5d). Differences between Aripo and Tunapuna/Tacarigua were non-significant ($p > .1$).

3.5 | Fluctuating asymmetry

No systematic side-bias differences were detected among streams or predation regimes for black coloration (ANODEV: all $p > .28$; Figure 5b; Table S9). For orange coloration, a significant interaction effect was detected (ANODEV: RIVER × PRED: $\chi^2 = 19.284$, $p < .001$; Table S10A). Pairwise contrasts focusing on PRED|RIVER pairs indicated that predation regimes differed in Turure ($p < .001$), while no differences were found within the other rivers ($p \geq .268$; Table S10B). Pairwise contrasts for RIVER|PRED pairs further indicated that Turure fish differed from the other streams in both high- and low-predation regimes (p: 0.009–0.042, Table S10B). High-predation fish in Turure had a slight left-side bias in orange coloration and the low-predation fish had a slight right-side bias; no other subpopulation showed any side bias (Figure 5e). Permutation tests of asymmetry index values corresponded with results from models of side-bias. The initial test for black-color asymmetry index

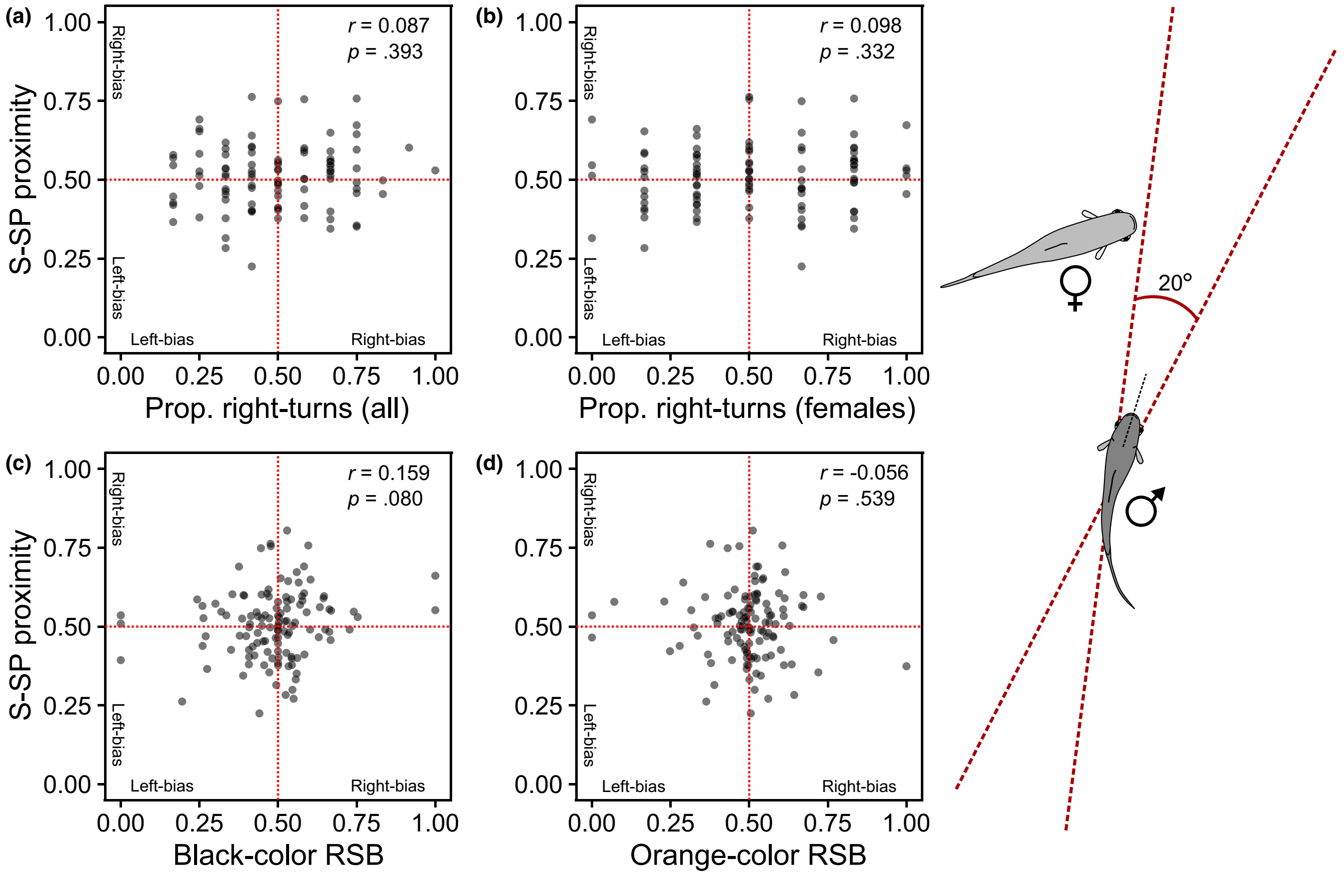

FIGURE 4 Correlation plots of side-showing preference (S-SP; proportion of time displaying right side when proximate to a female) of guppy males in relation to (a) proportion of right-turns in the detour test (against both the neutral object and female group as diversion objects), (b) proportion of right-turns in the detour test (against only female group as diversion objects), (c) proportion of black patch area on the right side of the body (RSB), and (d) proportion of orange patch area on the right side of the body. Red dotted lines in all panels indicate perfect symmetry for the respective variables. Illustration to the right shows the assessment criterion for when the male was considered to show a side to the female. The female in the illustration is outside of the two 20° "no-show" cones and the male would be considered to be showing his left side to the female.

(full model) indicated no difference from the conditional null distribution (maxT: 0.161, $p=.998$; Figure 5c). For orange color asymmetry index, the initial test was significant (maxT = 6.06, $p<.001$) and in pairwise tests, no PRED|RIVER pairs were significantly different, but all RIVER|PRED pairs involving fish from Turure were significant (all $p\leq.002$, with Turure fish having higher asymmetry index; Figure 5f).

3.6 | Body size

For male standard length, all included model terms were significant (ANOVA: RIVER: $F_{2,116}=41.44$, $p<.001$; PRED: $F_{1,116}=134.93$, $p<.001$; RIVER × PRED: $F_{2,116}=36.42$; $p<.001$). Pairwise contrasts comparing predation regimes within each stream system show that low-predation males in both Aripo and Turure were larger on average than the high-predation males ($p<.001$ for both contrasts; Figure 6a). No differences were detected between predation regimes in Tunapuna/Tacarigua ($p=.944$). Pairwise contrasts comparing low-predation males across streams showed that low-predation males in Tunapuna/Tacarigua were smaller than low-predation males in both Aripo and Turure ($p<.001$ for both contrasts), while no differences were detected between Aripo and Turure ($p=.806$) (Figure 6a). Contrasts for high-predation males across streams showed that Aripo had smaller males than both Tunapuna/Tacarigua and Turure ($p\leq.001$ for both contrasts), while no differences were detected between Tunapuna/Tacarigua and Turure ($p=.664$) (Figure 6a).

For female wet body mass, all included model terms were significant (ANOVA: RIVER: $F_{2,116}=8.10$, $p<.001$; PRED: $F_{1,116}=26.04$, $p<.001$; RIVER × PRED: $F_{2,116}=7.70$; $p<.001$). Pairwise contrasts comparing predation regimes within each river show that low-predation females in both Aripo and Tunapuna/Tacarigua were larger on average than the high-predation females ($p<.001$ for both contrasts), while no differences were detected within Turure ($p=.544$) (Figure 6b). Pairwise contrasts comparing low-predation females across streams showed that low-predation females in Aripo were larger than those in both Tunapuna/Tacarigua ($p=.008$) and Turure ($p=.001$), while no differences were detected between Tunapuna/Tacarigua and Turure ($p=.378$) (Figure 6b). Contrasts for high-predation females across streams showed that Tunapuna/Tacarigua had smaller females than

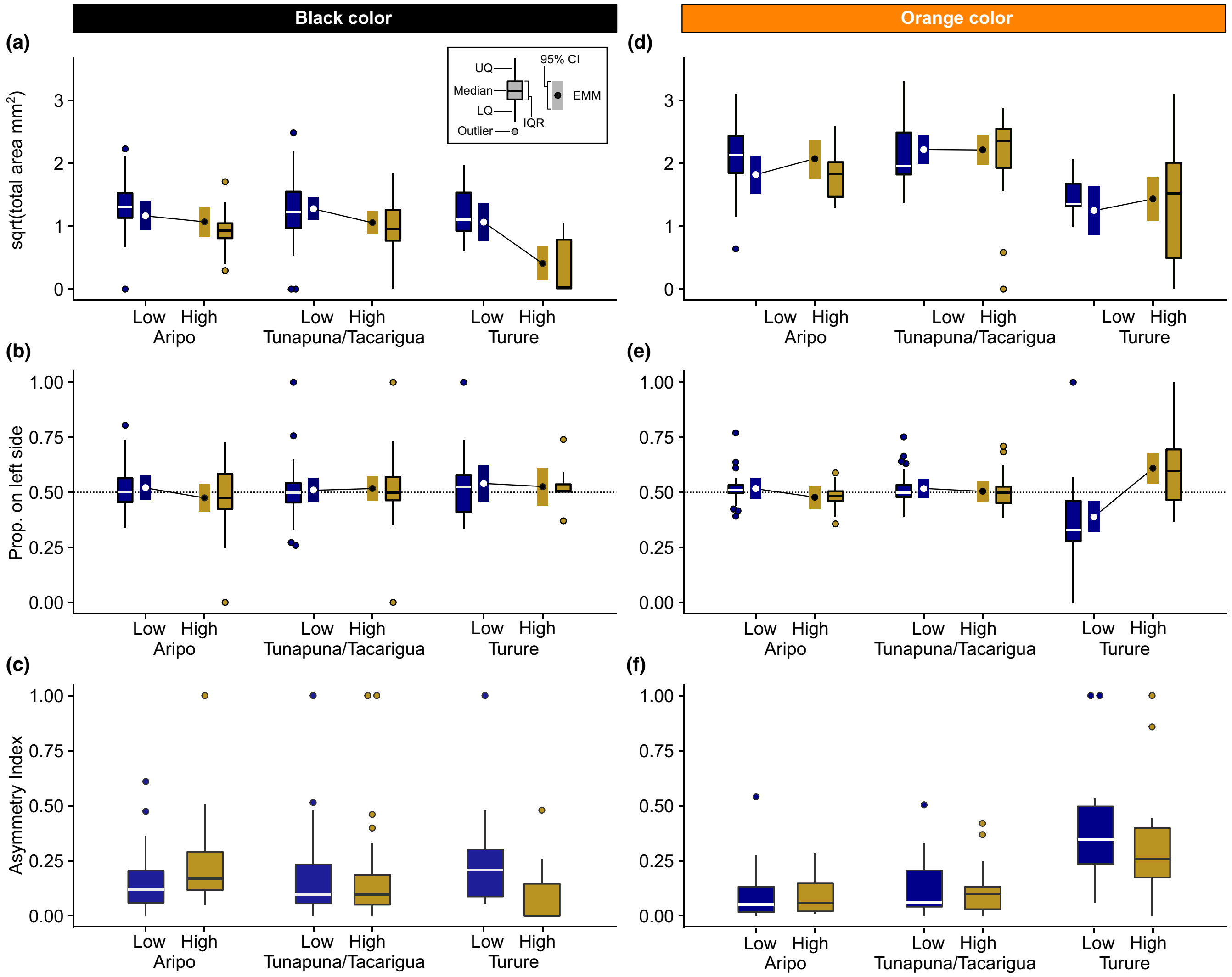

FIGURE 5 Male coloration in the low-predation (blue) and high-predation (orange) subpopulations. (a and d) Total area (left and right side combined) of black respectively orange patches on the body of male guppies. (b and e) Proportion of total black and orange coloration present on the left side of the body. (c and f) Asymmetry index for black and orange coloration. Lower values indicate more symmetrical distribution of the color (0 = left and right side have identical area coverage; 1 = one side has all color). All plots contain boxplots depicting the raw data distribution (not adjusted for body size); see legend in panel A: IQR = interquartile range, LQ = lower quartile, UQ = upper quartile, "Outlier" = values located >1.5 IQR away from the box hinges. On plots a, b, d and e, estimated marginal means (EMM; at the average body size for a and d) are presented as points with 95% confidence intervals (see legend in panel a).

both Aripo ($p = .005$) and Turure ($p < .001$), while no differences were detected between Aripo and Turure ($p = .418$) (Figure 6b).

4 | DISCUSSION

In this study, we found that wild Trinidadian guppies were moderately lateralized on average, given a non-critical generalized interpretation of all data from the different sampling sites being pooled. However, we found no general effect of high- or low-predation environment on lateralization in the more complex models. There were some slight side biases in color asymmetry, but this did not translate into a side-showing preference since no such effects were found for any of the populations in the interaction trials.

4.1 | Behavioral lateralization

Although there was no general effect of high- or low-predation environment on lateralization, we did observe some apparent population- and diversion object effects. Males from the low-predation regime in Turure were more lateralized when facing females compared to when facing the neutral object. Likewise, males from Tunapuna/Tacarigua were more lateralized when facing females compared to when facing the neutral object, albeit with no effect of predation regime. This limited and diversion object-dependent effect of predation did not support our prediction which was based on previous findings. For example, wild-caught adult bishop toothcarps *Brachyrhaphis episcopi* from high-risk rivers displayed stronger lateralization than did individuals from low-risk rivers (Brown et al., 2004), as did their

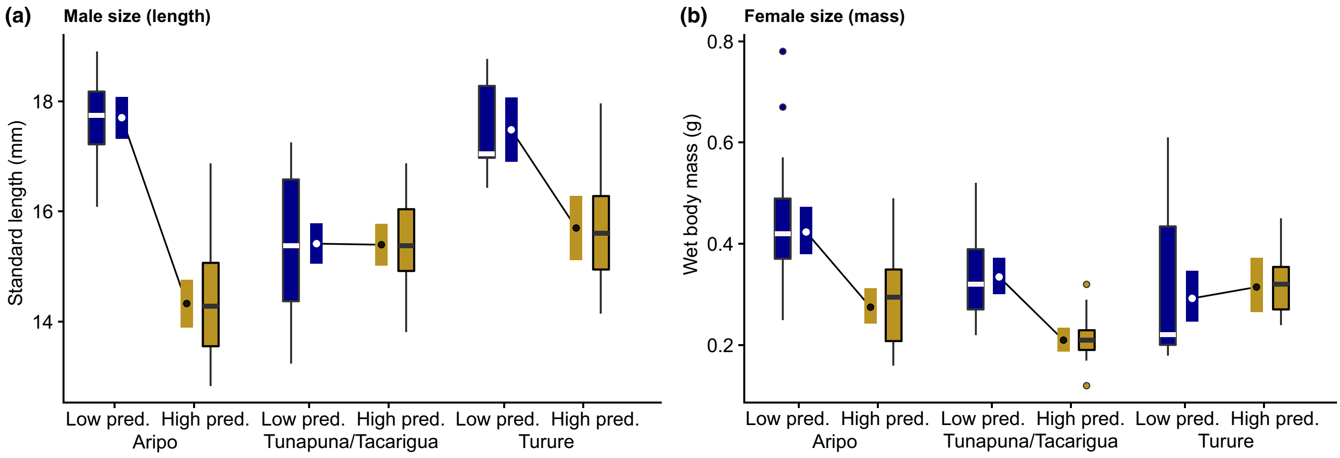

FIGURE 6 Estimated differences in average body size across (sub-)populations. (a) Estimated mean standard length (mm) for males. (b) Estimated $\log_e$-transformed wet body mass (g) for females. For both figures, parameter estimates are presented with white or black points, with 95% confidence intervals indicated as broad error bars extending from the point. Next to each estimate, boxplots are presented to show the data distribution; boxes show the interquartile range (IQR), with median as a horizontal bar; whiskers show upper and lower quartiles, and values located >1.5 IQR away from the box are shown as individual points.

laboratory-reared offspring (Brown et al., 2007). Similarly, both wild-caught adults and laboratory-reared descendants of Bahamas mosquitofish *Gambusia hubbsi* from high-risk environments were more lateralized than their conspecifics from low-risk environments (Hulthén et al., 2021). These studies suggest that effects of predation on lateralization can be a heritable trait and the genetic component makes it probable that we should have found the same result here. Another experiment using Trinidadian guppies found that second generation laboratory reared descendants from wild-caught low- and high-predation parents were more lateralized when kept in water containing predator chemical cues compared to guppies reared without predator cues, regardless of predation regime history (Broder & Angeloni, 2014). Since historical predation regime had no effect on lateralization, Broder and Angeloni (2014) suggested that predation risk experienced over evolutionary history does not shape laterality patterns to the same extent as acute perceived predation threat. When interpreting results from predation and/or fluctuating asymmetry experiments it can be important to know whether the test animal was wild-caught, or captive bred. If using captive bred fish, the number of generations in captivity should be given, since domestication can alter physiological traits (Morgan et al., 2019, 2022), as well as behavioral and genetic traits (Robison & Rowland, 2005; Uusi-Heikkilä et al., 2017). If using wild-caught fish, previous introductions or relocations could have an impact, wherefore detailed data of any such events is needed (Carvalho et al., 1996).

Relative lateralization, that is, left or right side turning preference, may also be altered by predation. We found that guppies from Turure were more left-biased on average than guppies from Tunapuna/Tacarigua, while no other contrasts were significant. Other studies have also reported effects of predation on relative lateralization when faced with a simulated predator (domesticated juvenile poeciliid, *Girardinus falcatus*, Cantalupo et al., 1995). In domesticated adult guppies, fish with prior experience of a predator showed a greater tendency to approach a live predator when their own mirror image was visible on their right side (De Santi et al., 2000). The observed effect on relative lateralization is suggested to be driven by cooperative predator inspection behavior (De Santi et al., 2000), which is important in guppies and several other species of fish and has a clear link to social interaction behavior (Dugatkin, 1988; Seghers, 1973). Interestingly, in a study using fish from the very same populations and collection events as used in the presented study, predator regime did have an effect on social interaction behavior, with fish from high-predation environments having an increased group cohesion compared to fish from low-predation environments (Herbert-Read et al., 2017). There was, however, no effect of predation on an individuals' alignment or turning responses, and the authors suggest that in this predator–prey system, predation has shaped the cohesion but not the directional alignment of individuals (Herbert-Read et al., 2017). This is in line with the general lack of predation effect on lateralization observed here.

As mentioned above, it can be difficult to make direct comparisons between studies to draw general conclusions, since the experimental designs often differ (e.g., differences in test arena, life stage, sex, holding time – rate of domestication). The reason our results differ from some previous publications could be due to such differences, or it could be due to the relatively small sample sizes from some of the river systems, or differences between studies in number of turns the fish were run in the lateralization arena. It has recently been shown that lateralization can be easily affected by experimental setup and it can have low repeatability (Clark et al., 2020; Penry-Williams et al., 2022; Roche et al., 2020; Sundin & Jutfelt, 2016). This was also found here, where the crack in the glass arena used in the first lateralization test (not included in the analyses) led to an unintended but strong preference for that side (Figure S2A). Within this experiment, we also intended to investigate whether using a predator model as diversion object would have an effect on lateralization.

However, we observed that the predator model was apparently not viewed as a predatory threat, possibly due to being presented completely immobile (Näslund et al., 2016). Instead, the guppies were swimming toward the model, hiding underneath it and seemingly using it as shelter, and the test therefore had to be excluded from our analyses (see supporting information). This is in contrast to other studies (using captive-bred guppies) where an identical predator model did evoke an apparent predator response (van der Bijl et al., 2015). While the lack of response toward the predator model essentially resulted in a failed test, this knowledge is important when designing future experiments.

4.2 | Fluctuating asymmetry and side-showing preference

Overall, none of the tested variables (behavioral lateralization score in the detour test or color asymmetry) affected side-showing preference. We did find a weak significant correlation in line with our prediction in that males showed the side with more black when looking only at males from high-predation sites. This correlation, however, appeared spurious and dependent on a few extreme values; the statistical significance also did not survive correction for multiple testing. Hence, we do not consider this as clear evidence in favor of side-showing preference in males from high-predation sites. For the behavioral lateralization score, proportion of right turns in the detour test did not correlate with proportion of time displaying the right side to a female. This was true regardless of diversion object, or when using data from right turns when facing the female group only.

The measurements of male color ornament showed that males from the high-predation environment from Turure had a slight left-side bias in orange coloration, while the low-predation fish from the same stream had a slight right-side bias, on the population level. The males from Turure consequently also had an overall higher asymmetry index, regardless of predation regime, compared to the other streams. No other subpopulation showed any population-level side bias, although individual males could be substantially asymmetric. For black coloration there were no population-level side-bias differences among any of the stream systems and no effect of predation regime.

Color asymmetry did not translate to a corresponding side-showing preference in the interaction trials, since proportion of time displaying the right side when proximate to a female did not correlate to the proportion of orange or black patch area on the right side of the body. These results contrast with some previous studies (Amcoff et al., 2009; Gross et al., 2007; Řežucha & Reichard, 2015). Řežucha and Reichard (2015) found that the more attractive side was displayed by Endler's guppy *Poecilia wingei* males, but only with respect to orange coloration (black color- and iridescence asymmetry was also analyzed, without clear effects on display). In Gross et al. (2007), the correlation between color asymmetry and side-showing preference in Trinidadian guppy males was also only true for orange coloration; there was no correlation between black color asymmetry and side-showing preference (presented in the Supplementary material of Gross et al., 2007). The reason our results differ to these studies could be due to differences in how side-showing preference is defined. For example, some studies only measured side-showing preference when the male was performing the sigmoid courtship display, while we induced all data of side-showing preference when the male was following in close proximity to the female. We did not want to limit our data collection to sigmoid displays since male–female interaction and courtship behavior can be conspicuous to a human observer, and we did not want to risks excluding less obvious courtship behaviors.

When looking at total color, that is, combining color patches on the right and left side, high-predation guppies from Turure had less black color than low-predation males from the same stream. The same contrasts in Tunapuna/Tacarigua were close to significant, indicating a similar pattern as for Turure, but the difference was substantially smaller. No other effects were observed. We predicted that fish from high-predation environments would be less ornamented than fish from low-predation environments (Svensson & Wong, 2011), since increased conspicuousness may lead to increased predation risk (in birds: Götmark & Olsson, 1997), and more ornamented male guppies may be preferentially targeted and eaten by predators (Godin & McDonough, 2003; Weese et al., 2010). Studies have however reported that selection against male color can be high also in low-predation environments (Weese et al., 2010), which could lead to less difference in color between high- and low-predation sites, as was found here for some of the tested populations. It should be noted that we did not measure iridescent colors in this study, which is sometimes done when investigating fluctuating asymmetry (Sheridan & Pomiankowski, 1997b), and such color can indeed be important. The boundaries of iridescent color spots can however be difficult to define, thereby making it difficult to accurately measure the area of such colors (Sheridan & Pomiankowski, 1997a). Previous studies have found that iridescent spots are often symmetric (thereby not influencing measurements of asymmetry), while melanic and carotenoid spots are distinct and usually not symmetric (Sheridan & Pomiankowski, 1997a). While iridescent colors are indeed measured in some studies investigating fluctuating asymmetry (e.g., Sheridan & Pomiankowski, 1997b), this is not always the case, and asymmetry has been found in guppies when measuring orange and black color only (e.g., Gross et al., 2007).

4.3 | Differences among stream systems

Although we did not find any consistent effects of predation regime between the experiments in this study, the results from Turure stand out, albeit being rather scattered across the experiments. There is no connection between the Oropuche (Turure) and Caroni (Aripo and Tunapuna/Tacarigua) drainages, and differences in life-history traits in fish (guppies and Hart's rivulus) from these two drainages have been observed in other studies (El-Sabaawi et al., 2012; Neff et al., 2008). While this could potentially explain why males from Turure were different from the other two stream systems in some of the variables measured here, several studies have shown that guppies from Turure are genetically more similar to the Caroni drainage than the native Oropuche drainage (Becher & Magurran, 2000; Shaw et al., 1992; Willing et al., 2010). The reason for the genetic similarities is likely due to an experimental introduction of 200 guppies from the lower Arima River, belonging to the Caroni drainage, into the upper Turure in 1957 (by C. P. Haskins, Shaw et al., 1992). The introduction was highly successful and the upper, low predation, parts of Turure, previously free of guppies, is now inhabited by guppies in large numbers. Studies have also shown that guppies in the downstream, high predation, parts of Turure are genetically similar to fish in the Caroni drainage, although some of the native Oropuche drainage genes still exists (Becher & Magurran, 2000, Shaw et al., 1992, Willing et al., 2010). This means that the introduced guppies did not only establish themselves in the upper part of the river but also moved downstream of waterfall barriers and interbred with the indigenous lower, high predation, Turure population. Although the introduction may create scientific issues for researchers using fish from these populations in their experiments, they did occur close to 60 years ago (counting until 2015, the year of conducting the experiments presented here). Given the short generation time of guppies (Houde, 1997), this leaves room for local adaptation by natural selection during the time since introduction, and a recent study found little effect of time since colonization on predator-driven trait parallelism (Heckley et al., 2022). Indeed, low-predation Turure fish have been shown to differ in their behavior (schooling and inspection behavior) compared to high-predation counterparts in a manner that would be expected based on differences in predation pressure (Magurran et al., 1992), which was also observed to some extent in this study in males from Turure. Also, males from low-predation regimes from Turure were larger than high-predation males, which could be an effect of predation pressure (Reznick et al., 2001).

5 | CONCLUSIONS

There were no clear effects of predation regime on behavioral lateralization across experiments or stream systems, contradicting our predictions. The statistically significant effects we did observe mostly aligned with previous studies, but did not show a clear pattern of generality and the interpretations of our results were thereby far from straightforward. We conclude that when investigating effects of predation regime in general, and such effects on behavioral lateralization or fluctuating asymmetry in particular, attention must be focused toward several factors, such as experimental assay, definitions of side-showing preference, differences in measurements of color, sample size, and target population. Behavioral differences among fish populations, including poeciliids, from different watersheds are frequently observed (e.g., Archard & Braithwaite, 2011; Culumber, 2022; Magurran & Seghers, 1990), which could explain the variation in behavioral expression observed in the present study. Further investigations are needed to both replicate these effects and investigate differences in other types of behavioral expressions. If the observed differences are true and not due to statistical flukes, broad generalizations from results stemming from experiments including only one population should be avoided.

AUTHOR CONTRIBUTIONS

Josefin Sundin: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; writing – review and editing; supervision; project administration; validation. **Joacim Näslund:** Investigation; methodology; visualization; writing – review and editing; formal analysis; data curation; validation. **Fernando Mateos-González:** Conceptualization; investigation; methodology; writing – review and editing. **William Ashworth:** Writing – review and editing; validation; visualization; investigation. **Mirjam Amcoff:** Conceptualization; investigation; funding acquisition; writing – review and editing; methodology; supervision; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data and script used for analyses are archived in the figshare repository (https://doi.org/10.6084/m9.figshare.22360792), following best practices (Roche et al., 2015).

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SUPPORTING INFORMATION

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