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http://researchonline.ljmu.ac.uk/id/eprint/13049/

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**Gomes-Silva, G, Cyubahiro, E, Wronski, T, Riesch, R, Apio, A and Plath, M (2020) Water pollution affects fish community structure and alters evolutionary trajectories of invasive guppies (Poecilia reticulata). Science of the Total Environment. 730. ISSN 0048-9697**

# Water pollution affects fish community structure and alters evolutionary trajectories of invasive guppies (*Poecilia reticulata*)

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## Highlights

- Intense water pollution in overstocked Rwandan Mutara rangelands
- Pollution (from cattle dung, domestic wastewater, etc.) affects fish communities
- Invasive guppies were more likely to occur at highly polluted stream sections
- Population divergence in male nuptial color ornamentation was observed
- Pollution alters evolutionary trajectories of sexually selected traits

## Abstract

Anthropogenic habitat alterations have the potential to affect both, ecological dynamics of communities and populations, as well as evolutionary processes within populations. Invasive species may benefit from anthropogenic disturbance, such as water pollution, to which they sometimes seem more resistant than native ones. They also allow investigating evolutionary divergence among populations occurring along pollution gradients. We assessed fish communities at 55 sampling sites in the degraded and heavily overstocked Mutara Rangelands of north-eastern Rwanda (upper Nile drainage), which receive pollution from domestic wastewater and cattle dung. Diverse fish communities became apparent that included invasive guppies (*Poecilia reticulata*, Poeciliidae), and canonical correspondence analyses found significant differentiation of community structures along several environmental parameters (condensed into principal components), including pollution-effects. As predicted, generalized linear models found guppies to have a higher likelihood of occurrence at polluted sites. Local abundances of guppies, however, decreased at polluted sites. Since guppies are color-polymorphic, and color patterns have a heritable basis, they allow inferences regarding both pollution-induced suppression of male ornamentation (*e.g.*, through xenestrogens) and evolutionary population divergence. We thus quantified different ornament types (numbers and percent body surface cover). ANCOVAs uncovered several weak (based on effect strengths), but statistically significant pollution-effects and interactions with other environmental parameters. The direction of several interaction effects was similar for blue/black and red/orange ornaments, while white/iridescent ornaments responded dissimilarly. As responses differed between ornament types, they likely reflect evolutionary divergence due to site-specific alterations of selective regimes rather than developmental inhibition of male secondary sexual characters. We propose that pollution likely affects local fitness landscapes resulting, *e.g.*, from predation and mate competition (as a function of local abundances), altogether driving evolutionary divergence of sexually selected traits. This study highlights how human activities impact not only ecological dynamics, but—mediated by altered Eco-Evo dynamics—change the evolutionary trajectories of populations.

***Key words***: biological invasion, color polymorphism, predation, sexual selection, contemporary evolution

# 1 Introduction

Biological invasions are a major component of global change (Mack et al., 2000; Mazor et al., 2018). Invasive species represent a challenge to the functioning of resident ecological communities (Gallardo et al., 2016; Vitule et al., 2019) and can lead to the extinction of native taxa due to predatory (Koel et al., 2019), parasitic (Emde et al., 2016; Tavakol et al., 2017), or competitive interactions (Valero et al., 2008; Camacho-Cervantes et al., 2018). It has been suggested that human-induced habitat alterations—including environmental pollution—may pave the way for newly arriving invasive species to establish (and consolidate) local populations (Fine, 2002; Moyle and Marchetti, 2006), especially when invasive species are more resistant to pollution (Crooks et al., 2011; Piscart et al., 2011; Varó et al., 2015).

Livebearing fishes (Poeciliidae) are a prime example of a group of organisms harboring several highly invasive species; for example, guppies (*Poecilia reticulata*) invaded tropical freshwater ecosystems worldwide (Lindholm et al., 2005; Magurran, 2005; Deacon et al., 2011). Guppies have been released partly during campaigns to reduce vectors of malaria and dengue fever (*i.e.*, *Anopheles* and *Aedes* mosquito populations), with low to non-detectable success rates (El-Sabaawi et al., 2016; de Campos Júnior et al., 2017). Guppies also escaped from home aquaria—as they are widely used as ornamental pet fish—and formed feral populations, often closely resembling color-polymorphic 'wild-type' guppies (Magalhães and Jacobi, 2013; Oliveira et al., 2014). A previous study (Gomes-Silva et al., 2019), focusing on the effects of diffuse pollution from domestic and industrial wastewaters in a neotropical stream ecosystem (see also Araújo et al., 2009; Carvalho et al., 2019), found invasive guppies to replace the native poeciliid *Phalloceros* sp. in polluted urban stream sectors. This renders invasive guppies a potential focal species for the bioindicator-based assessment and monitoring of water quality (Gomes-Silva et al., 2019; Carvalho et al., 2019).

Our present study tested the generality of this pattern, but this time focusing on a paleotropical stream ecosystem in the upper Nile drainage (Swain, 2011). Streams in the heavily overpopulated and overstocked Mutara rangelands in northeastern Rwanda receive pollution chiefly from domestic wastewater and cattle dung (Dusabe et al., 2019). At the same time, the human population of Nyagatare district is critically dependent on clean surface water as a source of drinking water (Ministry of Infrastructure, 2010). We asked whether species compositions of teleost fishes in the studied stream ecosystem change in dependence of diffuse sources of pollution, making use of previously published data on water pollution levels collected at the same time as the data presented here (Dusabe et al., 2019). This part of the study was motivated by the idea that assessing fish communities could represent another cost-efficient, bioindicator-based approach for the assessment of water quality, augmenting the previously described macrozoobenthos-based approach (Dusabe et al., 2019). Given previous work, we predicted a higher likelihood of occurrence (considering presence/absence data, *prediction 1a*) and higher local abundances/densities of invasive guppies (count data) with increasing pollution levels (*prediction 1b*).

We also considered several phenotypic traits to unravel potential population differentiation along pollution gradients, starting with body size. Industrial pollution in Newark Bay does not seem to influence body size in Atlantic killifish (*Fundulus heteroclitus*; Bugel et al., 2010), and a similar lack of consistent body size responses was reported for roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*; Carru et al., 1996), as well as western mosquitofish (*Gambusia affinis*; Franssen, 2009), in relation to heavy metal pollution. However, a study on isopods (*Porcellio scaber*) found decreasing body size with increasing metal pollution (Jones and Hopkin, 1998), and body size was also reduced in guppies from the oil-polluted Pitch Lake on Trinidad (Santi et al., 2019). Based on the available literature, we predicted that, if pollution affects body size of invasive guppies, then individuals from polluted sites should be smaller-bodied (*prediction 2*). The underlying reasoning is that individuals need to invest relatively more resources into somatic maintenance (*i.e.*, physiological homeostasis) under stressful conditions, which can hamper somatic growth (Marchand et al. 2014; Passow et al. 2015).

Guppies are unique amongst most animal species as males show an unparalleled polymorphism in nuptial ornaments used to attract females (Endler, 1983; Reznick and Miles, 1989). This opens the possibility to use their nuptial coloration (*i.e.*, numbers of spots on the body surface and total surface area of a given ornament type) as another parameter to assess pollution (see Lifshitz and St. Clair, 2016 for review). Specifically, certain pollutants—*e.g.*, xenestrogenic substances (Baatrup and Junge, 2001; Toft and Baatrup, 2001; Kristensen et al., 2005; Shenoy, 2012)—can suppress the development of male sexual secondary characters, including color ornaments (Lifshitz and St. Clair, 2016). If interrupted hormonal balance during development (or other challenges to physiological homeostasis) played a role in our study, some (or all) of the examined male color ornament types should diverge in the same direction along the examined pollution gradient, with less colorful males occurring at polluted sites (*prediction 3a*).

At the same time, color patterns in guppies have a genetic basis (Winge, 1922, 1927; Haskins and Haskins, 1951), and a plethora of studies have demonstrated evolutionary population divergence of color ornamentation (Endler, 1978, 1980, 1989; Dick et al., 2018). In non-polluted streams (*e.g.*, in northern Trinidad), several environmental factors have been associated with evolutionary divergence of male nuptial color patterns; most prominently, selection from piscivorous fishes selects against colorful males (Godin and Briggs, 1996; Godin and Donough, 2003). In the Mutara Rangelands, pollution could affect local fitness landscapes arising from predation, for instance, if predatory fish are less tolerant to pollution (Gomes-Silva et al., 2019). If predation acts as a major selective agent, different color ornament types could respond differently to this selection (Endler, 1995). In particular, those ornament types that are most visible to predators should undergo the strongest divergence (Endler, 1991; Kwiatkowski and Guyer, 2003; Husak et al., 2006), while other ornament types could actually be more frequent in males from the same populations to compensate for the lack of other ornament types (*e.g.*, Rodd and Reznick, 1991). Specific predictions of how exactly different ornament types of guppies might diverge along pollution gradients depend on several details regarding the exact type(s) of predators present, as well as their visual ecology (Kemp et al., 2015; Endler and Mappes, 2017). Nevertheless, a general prediction can be formulated: if evolutionary divergence of male color ornaments along pollution gradients plays a role (mediated by predation and/or additional environmental selective agents correlated with pollution), then different ornament types could diverge in quite different directions, with some ornament types becoming less frequent, while others might actually increase as pollution levels increase (*prediction 3b*).

In summary, this study was designed to answer three interrelated questions: (1) Are invasive guppies more frequent and more abundant at heavily polluted compared to less or non-polluted sites? (2) Do guppies occurring at heavily polluted sites grow to a smaller body size? (3) Finally, do we find differentiation of male color ornaments among guppy populations occurring along the examined pollution gradient, and is the pattern uncovered suggestive of developmental disruption of male secondary sexual characters (Lifshitz and St. Clair, 2016) or evolutionary population divergence (Endler, 1978, 1980; Dick et al., 2018)?

# 2 Materials and Methods

## 2.1 Study area and assessment of fish communities

We assessed fish communities in several small streams crossing the degraded and overgrazed Mutara rangelands in northeastern Rwanda (*i.e.*, Muvumba, Kizinga, Ngoma, Karangazi and Kakitumba Rivers) draining into the Akagera River (Fig. 1). The area was previously protected by the Akagera National Park and the Mutara Game Reserve, but in 1997 large parts were degazetted and populated by returning refugees and their livestock (Kanyamibwa, 1998). This led to a sharp increase in human population density (483.1 individuals/km$^2$; NISR, 2014) and severe overstocking with cattle (64.9 individuals/km$^2$; Wronski et al., 2017). Further details on the history, ecology and hydrography of our study area can be found in Dusabe et al. (2019).

Dusabe et al. (2019) reported on abiotic and biotic environmental parameters collected at our sampling sites, including altitude [m], stream width [m] and depth [m], flow velocity [m s$^{-1}$], water temperature [°C], dissolved oxygen [ppm], pH, conductivity [µS $m^{-1}$], and presence/absence of macrophytes. Furthermore, the study used a bioindicator approach, based on changes in the macrozoobenthos community (Knoben et al., 1995; Liess et al., 2008) to assess water quality. The score was derived from the Tanzania River Scoring System (TARISS; Kaaya et al., 2015), which is largely based on macrozoobenthos communities described for South African rivers (Day and de Moor, 2002; Stals and de Moor, 2007). Those data were collected roughly at the same time (July 2014 to August 2016). Depending on the accessibility of the water, sampling sites were set at approximately equal distances along the streams, *i.e.*, (mostly) 2 to (maximally) 6 km apart, covering app. 118 continuous river kilometers (Fig. 1). Here, we reanalyzed the aforementioned geo-physical, physico-chemical and biotic parameters and used them as predictor variables when analyzing fish communities and phenotypic divergence. Chemical analyses of water quality, however, could not be carried out in the course of our present study given the remoteness of our study area.

The same person (E.C.) sampled fishes between 1st March and 3rd October 2015 for 90 minutes at each sampling site (which we defined as the stream area along 50 m in up- and downstream directions from a marked point along the shoreline) using a 1.0 x 0.9 m seine net with a mesh width of 4 mm. In an attempt to cover different fish microhabitats equally, we sampled shallow and deeper portions of the river, open and heavily vegetated areas, benthic and pelagic parts, as well as lentic and lotic sectors. We euthanized voucher specimens of small-bodied and abundant taxa (*e.g.*, barbs) using clove oil and preserved them in 70% ethanol, but took digital photos of large-bodied and less abundant species for later identification. We released those individuals at the collection site after sampling was completed. Given the proximity of our study area to the Ugandan border, species determination followed an identification key developed for Ugandan fish species (Greenwood, 1966). Our assessment also provided quantitative (abundance) data of invasive guppies and other fish species by determining the catch-per-unit-effort (Millar et al., 2006; Millar and Hendry, 2011).

## 2.2 Additional sampling of guppies for body coloration analyses

During the assessment of fish communities (see above), we encountered invasive guppies at 26 sampling sites (Fig. 1) and collected 2–148 individuals (females and males) per site. To increase sampling sizes at sites with low population densities for our analyses of phenotypic divergence, we revisited several sites between 5th October 2015 and 15th June 2016 and collected additional specimens. Information on fish collected during those additional samplings was, however, not included in the analyses of fish communities and local abundances. In total, we collected 501 male guppies for the analysis of body coloration (9–45 individuals per site). To take photographs, males were anesthetized by adding few drops of clove oil to the water in the buckets in which they were collected. We laid each male flat on laminated millimeter paper and took digital photos of the left body side from centrally above (at approximately 30 cm distance) using the camera included in a Gionee E3 smartphone and saved them in *jpg format (Fig. 2). Photos were taken in the shade to avoid glare and reflection effects.

We processed the photos in the laboratory on a personal computer using the software tpsDig2 (version 2.31, 2017). To avoid inter-observer bias, all images were analyzed by the same person (G.G.-S.). We assessed each male's body size (standard length, SL), body surface area (excluding the fins), and—based on the observation that males varied drastically in the size and shape of their caudal fin (Fig. 2*a*–*g*)—caudal fin surface area (which we later expressed as relative area by dividing this value by the body surface area). We classified color ornaments as falling into the six color categories described by Endler (1978), *i.e*. red/orange, black (including 'fuzzy black': Ruell et al., 2013; Martínez et al., 2016), yellow, blue (which included 'purple': Millar et al., 2006), green, and white/iridescent (including 'blue-violet', 'greenbronze' and 'silver': Millar et al., 2006; Fig. 2*a*–*g*). For each color category we calculated the total number of spots on the left body side as well as the surface area covered by each spot, and we calculated the relative surface area covered by that color type (dividing the cumulative surface area for that color category by the total body surface area, Martínez et al., 2016).

## 2.3 Statistical analyses

Unless noted otherwise, all statistical analyses were conducted using SPSS v. 19 (IBM 2010, Chicago, USA). All percentages (relative surface areas of different color ornament types and relative caudal fin areas) were arcsine (square root)-transformed and length measurements were $\log_{10}$-transformed. Normality and homoscedasticity of model residuals were tested using Shapiro-Wilks' and Levene's tests, respectively. To reduce random noise, we subjected continuous (but not binary) environmental data to a smoothing procedure along river stretches (independently for each affluent) as follows: $y_i' = (0.05 \times y_{i-2}) + (0.15 \times y_{i-1}) + (0.6 \times y_i) + (0.15 \times y_{i+1}) + (0.05 \times y_{i+2})$. Note that the direction of statistically significant effects reported in this study did not change when models were calculated with non-smoothed data instead; nevertheless, model fit decreased throughout (results not shown).

### *2.3.1 Factor reduction of environmental parameters*

The 10-geo-physical, ecological and pollution-related predictor variables (Table S1) were condensed by subjecting them to principal component analysis (PCA), based on a correlation matrix, using the Varimax option. Four PCs were retained (eigenvalues > 1.0) and cumulatively explained 68.08 % of the total variance (Table S1).

Based on the observed variation in local abundances of guppies (Fig. 3*c*) and because population densities, by themselves, can alter selective trajectories mediated by competition for mates and resources (Kolluru and Grether, 2005; Torres-Dowdall et al., 2012; Bassar et al., 2016; Tadesse, 2018), we decided to include guppy abundance as an additional input variable in another PCA on ecological predictor variables (Table 1*a*), which we used for all analyses of phenotypic divergence. Note that, while guppy abundance indeed yielded moderate axis loadings on PC2 and PC4, major axis loadings did not change qualitatively (compare loadings > 0.6 in Table 1*a* and Table S1). We did, however, not include estimates of predation pressure in our second PCA, as numerous potential predators of guppies were encountered at low abundances in our sampling (Table S2), leaving doubt as to whether those data would provide a meaningful characterization of sites with respect to predation pressure (for studies focusing on a clearer 'low vs. high predation' dichotomy see Endler, 1978, 1980, 1982, 1983).

### *2.3.2 Fish community structure and local abundance of guppies*

We used canonical correspondence analyses (CCA), implemented in XLSTAT 2018, to analyze patterns of variation in fish assemblage compositions across sampling sites in relation to environmental characteristics and to evaluate whether and how environmental variables explain the occurrence of different species, including *P. reticulata*. Our first CCA used occurrence (presence/absence) data of all fish species encountered at our sampling sites (Table S2) as dependent data matrix, and the four environmental PCs (see above) as independent variables. The second CCA used smoothed abundance data as the dependent data matrix. In a second step, we considered only data on guppies. Using generalized linear models, we coded occurrence data (using a binary error structure, testing *prediction 1a*) or smoothed abundance data (Gaussian error structure, testing *prediction 1b*), as the dependent variables, while coding the four environmental PCs as independent variables (covariates). We initially included all two-way interactions, but removed interactions from the final models if $P > 0.1$.

### *2.3.3 Male color ornaments, body size, and relative caudal fin area*

Numbers of spots of each of the six types of color ornaments, as well as relative surface areas of each color category, were treated in another PCA. Five PCs with eigenvalues > 1.0 that cumulatively explained 81.25 % of the total variance were obtained (Table 1*b*). We subjected those coloration-related PCs to MANCOVA (operated as multivariate general linear models, GLM), using the four environmental PCs (Table 1*a*) as well as body size (SL) as predictor variables (covariates). This was followed by five separate ANCOVAs of a similar model structure on single color PCs to identify the source(s) of variation. In each case, we first ran a model with all possible two-way interactions, but we removed the interactions from the final model if $P > 0.1$. We analyzed data on body size (SL) and relative caudal fin areas in similar ANCOVAs. The relative importance of each term in the final models was evaluated using Wilks' partial eta squared ($\eta_p^2$). We inspected the direction of statistically significant main and interaction effects of environmental PC4—which received axis loadings mainly from water quality/pollution (Table S1)—to address *predictions* *2*, *3a* and *3b*. We visualize significant effects via residuals, corrected for all other model terms.

# 3 Results

## 3.1 Fish community structure

### *3.1.1 Water pollution and other physico-chemical habitat parameters*

PC1 mainly captured altitudinal variation among sampling sites, as it received high axis loadings (|axis loading| > 0.6) from altitude, stream width and dissolved oxygen content (which was higher at several upstream-situated sites). PC2 received high axis loadings from pH and conductivity, whereby both variables showed an inverse relationship (see also Dusabe et al., 2019), while PC3 captured variation between fast- and slow-flowing stream sections and received high axis loadings from stream velocity and presence/absence of macrophytes. Finally, PC4 could be interpreted as chiefly water pollution-related, as it received high axis loadings from our macrozoobenthos-based assessment of water quality (TARISS score; Dusabe et al., 2019). Subsequently, we then tested whether environmental and especially pollution-related parameters—condensed into these four principal components (environmental PCs; Table 1*a*)—explain the distribution of teleost fish in general (*i.e.*, fish community structure) and that of invasive *P. reticulata* in particular.

### 3.1.2 Environmental effects on community compositions

Overall, we recorded 17 teleost fish species belonging to the families Amphiliidae, Clariidae, Cichlidae, Cyprinidae, Mastacembelidae, Mormyridae, and Poeciliidae, with a dominance of barbs (Cyprinidae, Barbinae; 6 species; Table S2). Based on those data, we conducted two canonical correspondence analyses using the four previously described environmental PCs as explanatory variables (Table S1). The first CCA used occurrence (presence/absence) data of all teleost species recorded per site (excluding seven sites at which no fish were recorded). The first two axes of the CCA ordination map explained 84.65% of the cumulative (constrained) variance (axis 1, eigenvalue = 0.44, 72.51% variance explained; axis 2, eigenvalue = 0.07, 12.14% variance explained). A permutation test detected a weak (*i.e.*, low pseudo-*F* value), albeit statistically significant effect of the four environmental PCs on the species distribution (pseudo-*F* = 0.91, *P* < 0.0001). The second CCA used abundance data of all fishes recorded per site (this time excluding only three sites due to the smoothing procedure). The first two axes of the CCA ordination map explained 87.88% of the cumulative variance (axis 1, eigenvalue = 0.38, 69.73% variance explained; axis 2, eigenvalue = 0.10, 18.15% variance explained). This analysis yielded a higher pseudo-$F$ value (1.76, $P < 0.0001$) when considering the separation of fish communities along the four environmental PCs (Fig. 3*a*). Despite this significant effect, visual inspection suggests that no single PC explained a large portion of the entire community composition (Fig. 3*a*). Moreover, visual inspection of community structures (Table S2) suggests no clear separation between invasive guppies and predatory fishes. For example, a *post hoc* Chi-square test considering 12 sites at which predatory catfish of the genus *Clarias* occurred (three of which did, while nine did not harbor guppies), found no significant deviation from random expectation ($\chi^2 = 1.60$, $P = 0.206$).

### 3.1.3 Likelihood of occurrence and abundances of invasive guppies

Generalized linear models using the occurrence (presence/absence) of *P. reticulata* as the dependent variable found none of the interaction terms to be significant (Wald's $\chi^2 < 2.07$, $df = 1$, $P > 0.15$ in all cases) such that all interactions were excluded in a step-wise elimination procedure. In the final model, all four environmental PCs (covariates) had significant effects (PC1: $\chi^2 = 9.59$, $df = 1$, $P = 0.002$; PC2: $\chi^2 = 5.59$, $df = 1$, $P = 0.018$; PC3: $\chi^2 = 6.10$, $df = 1$, $P = 0.014$; PC4: $\chi^2 = 6.62$, $df = 1$, $P = 0.010$). We focused on environmental PC4—the principal component previously identified to reflect water quality/pollution (Table S1). In support of *prediction 1a*, a *post hoc* Pearson correlation using residuals ($r = -0.394$, $P < 0.003$, $N = 55$) suggested a higher likelihood of guppy occurrence at polluted sites (towards negative values of PC4; Fig. 3*b*).

When we used the local abundance of *P. reticulata* instead as the dependent variable, we again found no significant interaction effects ($\chi^2 < 1.61$, $df = 1$, $P > 0.21$). After removing all interaction terms, only environmental PC2 ($\chi^2 = 11.68$, $df = 1$, $P = 0.001$) and PC4 ($\chi^2 = 4.48$, $df = 1$, $P = 0.034$) had significant effects, while the effects of PC1 ($\chi^2 = 2.07$, $df = 1$, $P = 0.15$) and PC3 ($\chi^2 = 0.79$, $df = 1$, $P = 0.38$) were not significant. Contrary to *prediction 1b*, however, a *post hoc* correlation analysis using residuals (Pearson correlation: $r = 0.28$, $P = 0.036$, $N = 52$) suggested that guppy abundances increased with increasing values of PC4, *i.e.*, towards non-polluted conditions (positive values of PC4; Fig. 3*c*). The effect of environmental PC2 ($r = 0.67$, $P = 0.006$, $N = 52$) is illustrated in the Online Supplementary Material (Fig. S1).

## 3.2 Population divergence of phenotypic traits

### *3.2.1 Pollution-effects on phenotypic traits other than nuptial coloration*

#### 3.2.1.1 Body size

Male standard length differed among sampling sites depending on PC4 (reflecting reduced body size at more polluted sites; Fig. 4*a*) and the interaction effects of PC2 × PC4 and PC3 × PC4, and effects strengths were higher than in our analyses of color patterns (Table 2*a*). The highest partial $\eta^2$-value of all analyses was indeed found in case of the interaction of PC2 × PC4; Table 2*a*)—also reflected by the highest $R^2$-values of partial linear regressions on residuals among the analyses of body size (Fig. 4*a*-*c*). In partial fulfillment of *prediction 2*, males became smaller with deteriorating water quality (towards negative values of PC4; Fig. 4*b*) at sites with a higher pH but lower conductivity (PC2 > mean) but *vice versa* at sites with a lower pH but higher conductivity. Both other effects yielded very low $R^2$-values of (partial) linear regressions on residuals (Fig. 4*c*). Additional (not pollution-related) significant effects are visualized in Supplementary Fig. S2*a*-*c*.

#### 3.2.1.2 Relative caudal fin area

Besides several other (not pollution-related) effects (Supplementary Fig. S3*a*-*d*), males' relative caudal fin area varied in dependence of the interaction effects of PC1 × PC4, PC2 × PC4, and PC3 × PC4 (Table 2*b*). Considering the first interaction term (PC1 × PC4), males had smaller caudal fins at polluted sites (towards negative values of PC4; Fig. 4*d*) in the cohort of fish from more downstream-situated river sections (PC1 > mean; for axis loadings see Table 2*b*), while males from more upstream-situated sites (PC 1 < mean) showed a weak tendency towards the opposite pattern.

Regarding the interaction of PC2 × PC4, males' caudal fins tended to become smaller with decreasing water quality (negative values of PC4; Fig. 4*e*) at sites with a higher pH but lower conductivity (PC2 > mean) but showed the opposite pattern at sites with a lower pH but higher conductivity (PC2 < mean). Exploring the interaction of PC3 × PC4, we uncovered exceedingly low $R^2$-values of partial linear regressions using residuals (Fig. 4*f*).

### *3.2.2 Factor reduction of different ornament types*

The six types of color ornaments encountered at our sampling sites were subjected to a factor reduction and condensed into five color PCs. Numbers of spots and relative areas covered by the respective ornament type on the male's body surface loaded on the same axis in four cases (white/iridescent, green, yellow and red/orange; Table 1*b*). Characteristics of black color ornaments, however, had moderate loadings on the same axis as blue ornaments (color PC1; Table 1*b*).

### *3.2.3 Environmental effects on general color pattern divergence*

The final MANCOVA model on male color characteristics yielded low effect strengths (partial $\eta^2$) for all factors and interactions retained in the final model (Table 2*c*); it included all interactions involving environmental PC1, as well as the interaction of 'PC2 × PC4'. No single 'most important' factor was identified—judging from relatively similar partial $\eta^2$-values for all model terms—but the interaction terms 'PC1 × PC2' and 'PC2 × PC4', as well as the main effect of PC4 had slightly stronger effects than other model terms.

We conducted *post hoc* ANCOVAs on single color PCs and again found low effect strengths throughout (partial $\eta^2 \leq 0.092$; Table 2*d*-*h*). This was due to considerable variation of all considered color traits within populations (reflecting the described color polymorphism in guppies), as compared to relatively small variation among populations/sampling sites (Fig. 5*a*-*h*; Fig. S4-8). In the following, we will focus on effects of environmental PC4 (*i.e.*, our pollution-related PC; Table 1*a*) and its interactions. Other significant effects are presented in the Online Supplementary Material (Fig. S4-8).

### 3.2.4 Pollution effects on color pattern divergence

We found significant effects of environmental PC4, or interactions involving this covariate, on three color ornament types, namely blue/black, white/iridescent and red/orange color patterns (Table 2*d*, *e*, *h*). Green and yellow ornaments—while showing divergence along some other environmental PCs (Supplementary Fig. S6-S7)—were not significantly affected by PC4 (Table 2*f*, *g*).

#### 3.2.4.1 Blue/black color ornaments

Considering blue/black color ornaments, environmental PC4, as well as the interactions of 'PC1 × PC4' and 'PC2 × PC4', had statistically significant effects (Table 2*d*). Based on partial $\eta^2$ of model terms (Table 2*d*) and the degree of determination ($R^2$) of a linear regression using residuals corrected for other model terms, the main effect of PC4 (*i.e*., increasing blue/black coloration towards more polluted conditions, indicated by negative values of PC4; Fig. 5*a*) was exceedingly weak. The strongest effect—based on partial linear regressions using residuals—was due to males developing more blue/black ornamentation at polluted sites (negative values of PC4; Fig. 5*b*) particularly in the cohort of fish from more downstream-situated river sections (environmental PC1 > mean), reflecting the significant interaction effect of 'PC1 × PC4' (Table 2*d*). A trend towards the opposite pattern (less blue/black ornamentation at more polluted sites) was observed in males inhabiting more upstream-situated sites (PC1 < mean, Fig. 5*b*; see Table 1*a* for axis loadings).

Exceedingly low $R^2$-values of partial linear regressions on residuals (despite a comparatively high partial $\eta^2$-value; Table 2*d*) were uncovered when exploring the interaction effect of 'PC2 × PC4' (Fig. 5*c*). Males tended to develop fewer blue/black spots with deteriorating water quality (negative values of PC4; Fig. 5*c*) at sites with a higher pH but lower conductivity (PC1 > mean) and *vice versa* at sites with a lower pH but higher conductivity.

### 3.2.4.2 White/iridescent color ornaments

Likewise, white/iridescent color patterns showed a significant main effect of environmental PC 4, as well as significant interactions of 'PC1 × PC4' and 'PC2 × PC4' (Table 2*e*). Again, inspection of partial $\eta^2$-values of model terms (Table 2) and the degree of determination ($R^2$) of linear regressions using residuals (Fig. 5*d*-*f*) suggests that the main effect (decreased white/iridescent coloration towards polluted conditions; Fig. 5*d*) was exceedingly weak. Inspection of (partial) linear regressions using residuals again identified the strongest effect resulting from the interaction of 'PC1 × PC4' (Table 2*c*). In contrast to our previous analysis (see above), males developed less white/iridescent ornamentation at heavily polluted sites (towards negative values of PC4; Fig. 5*e*) in the cohort of fish from more downstream-situated river sections (PC1 >

mean; for axis loadings see Table 2*e*), while males from more upstream-situated sites (PC1 < mean) showed a weak tendency towards the opposite pattern (Fig. 5*e*).

Again, $R^2$-values of partial linear regressions on residuals were exceedingly weak when considering the interaction effect of ‘PC2 × PC4’ (Fig. 5*f*). Splitting the data by the mean of PC1 revealed that white/iridescent color patterns followed an opposing interaction pattern with respect to the response to increasing water quality (right portion in Fig. 5*e*) when compared to the pattern we had identified beforehand for blue/black ornaments. Hence, in line with *prediction 3b*, but counter to *prediction 3a*, both interaction effects reflected the exact opposite pattern of divergence along the pollution gradient as observed in the case of blue/black ornaments.

#### 3.2.4.3 Red/orange color ornaments

When we considered red/orange color ornaments, environmental PC4 and the interaction of ‘PC2 × PC4’ had significant effects (Table 2*h*). While the main effect was again weak (*i.e.*, more red/orange coloration towards polluted conditions; Fig. 5*g*), the interaction effect suggests that males developed more red/orange coloration particularly in populations exposed to high levels of pollution and characterized by a low pH, coupled with higher conductivity (PC2 < mean). A comparatively weak trend towards the opposite pattern was observed in populations occurring at sites with a higher pH and lower conductivity (PC2 > mean; Fig. 5*h*). This pattern matches the one previously identified for blue/black coloration (see above). Altogether, differential responses of the three color ornament types support *prediction 3b*, but not *prediction 3a*.

# 4 Discussion

We discovered diverse fish communities in the examined paleotropical stream ecosystem, with only weak (*i.e.*, low pseudo-*F* values), albeit statistically significant differentiation along the examined environmental gradients (including water pollution). In support of our *prediction 1a*, invasive guppies had a higher likelihood of occurrence, but counter to *prediction 1b*, local abundances decreased as pollution levels increased. In partial fulfillment of *prediction 2*, males became smaller with increasing pollution levels, but only at sites with a higher pH but lower conductivity, while the opposite pattern was observed at sites with a lower pH but higher conductivity. Regarding male nuptial color ornaments, males sported more blue/black ornaments as pollution intensified in populations from downstream- but not upstream-situated sites. Males also developed more red/orange ornamentation as pollution levels increased at sites with a relatively low pH and a high conductivity. However, males developed less white/iridescent ornamentation with increasing pollution levels in downstream- but not in up-stream situated populations. Altogether, our results are counter to our *prediction 3a* but support *prediction 3b*, as different ornament types showed unique patterns of divergence, sometimes in- and sometimes decreasing with increasing pollution levels.

## 4.1 Water pollution affects establishment of invasive guppy populations

Guppies are native to northern South America, including Venezuela and northern Brazil, and to several nearby islands like Trinidad and Tobago (Rosen and Bailey, 1963; Magurran, 2005), but guppies have become invasive in tropical freshwater systems worldwide, including the African continent (*e.g.*, Lawal et al., 2012; Tavakol et al., 2017). In the first part of our present study, we tested the hypothesis that human-induced habitat alterations—in our case water pollution—might provide invasive guppies with a competitive advantage over resident (native) species (Crooks et al., 2011).

How can the seemingly contradictory pattern be explained in which guppies were more likely to occur, but showed lower abundances (*i.e.*, population densities) at polluted sites? In their native distribution range, guppies occur in a wide range of habitat types, ranging from pristine headwaters towards lowland stream sections (Endler, 1995; Reznick et al., 1996) and also including hydrogen sulfide-containing streams (Riesch et al., 2015) as well as aquatic ecosystems exposed to high levels of polycyclic aromatic hydrocarbons as a result of oil pollution (Hermens et al., 1985; Rolshausen et al., 2015). Moreover, it remains unclear if invasive guppies in the upper Nile drainage might be descendants of domestic (ornamental) guppies, which are likely to have undergone prolonged selection for general 'hardiness' in captivity, such as an increased thermal tolerance (Jourdan et al., 2014). Hence, guppies likely show a high resistance to various forms of water pollution, as well as high evolvability/adaptability (see O'Steen et al., 2002) and/or ability to respond to pollution via adaptive phenotypic plasticity (see Rodd et al., 1997; Handelsman et al., 2014). However, abundances (*i.e.*, local population densities) decreased as pollution levels increased, suggesting that invasive guppies may show reduced population growth dynamics in polluted waters, and that they are merely forced into marginal habitats by competition and predation. We argue that diffuse pollution imposes challenges to physiological homeostasis (Welch and Lindell, 1992).

In support of the latter interpretation, males grew to a smaller body size at polluted sites (in partial fulfillment of *prediction 2*), suggesting that individuals needed to invest relatively more resources into somatic maintenance (*i.e.*, physiological homeostasis), resulting in an impaired body growth (Stearns, 1992; Roff, 2002). Notably, this effect was only seen at sites with a higher pH and lower conductivity, while a low pH, coupled with a higher conductivity, resulted in the opposite pattern. It has been argued beforehand that a low pH/higher conductivity in our study system reflects allochtonous input of organic material (Dusabe et al., 2019). Organic material represents a food source for the omnivorous guppies (Palkovacs et al., 2011; Zandona et al., 2011; Carvalho et al., 2019), likely allowing for an improved growth performance (in the face of intra-specific resource competition) despite the presence of pollutants.

What ecological mechanisms underlie the observed pattern of guppies being more likely to occur at polluted sites? The list of likely candidates includes, besides others, predation (Reznick and Endler, 1982; Johnson and Belk, 2001; Riesch et al., 2013) and interspecific competition (Borg et al., 2012; Camacho-Cervantes et al., 2018). Our assessment of predation risk was coarse, partly owing to the limited time spent at each site, while at the same time several large-bodied piscivorous fishes are not easy to detect. Moreover, it remains to be examined whether some of the cichlids we encountered (*i.e.*, *Pseudocrenilabrus multicolor* and *Astatotilapia burtoni*) might also prey on guppies (or their offspring: Torres-Dowdall et al., 2012). This prevented us from specifically addressing predation risk as an additional explanatory variable in our statistical analyses. However, the possibility remains that polluted sites harbor fewer large-bodied predatory species, *e.g.*, due to the somatic bio-accumulation of toxicants (Johnels et al., 1967; McClain et al., 2006). Likewise, inferences regarding competition and other interactions between invasive guppies and native fishes (Camacho-Cervantes et al., 2018; Gomes-Silva et al., 2019)—especially several small-bodied members of the genus *Barbus*—remain speculative, as little information is available regarding the trophic ecology of (and trophic niche differentiation between) those species (Greenwood, 1966).

As effects of environmental parameters (including pollution) on differentiation among local fish communities were weak, one could argue that pollution may have partly created barriers to up- or downstream fish movement. Whether or not such a scenario is likely remains currently unknown. It remains to be determined, for example, if highly contaminated waters in the studied stream sections are indeed acutely toxic to at least some of the occurring fish species. Assuming that this scenario plays a role in our study area, some headwaters with less polluted conditions could harbor truncated fish communities (resulting in weaker effects of pollution in the statistical analyses) because downstream pollution prevents the regular recolonization, *e.g.*, after extreme flooding events.

## 4.2 Water pollution alters evolutionary trajectories of invasive guppies

A major result of this study was that different ornament types of guppies exhibited differential responses, either in- or decreasing with increasing pollution. This aligns with our *prediction 3b* and suggests evolutionary divergence (Endler, 1980; Dick et al., 2018) rather than pollution-induced suppression of male ornamentation (*e.g.*, through xenestrogens; Baatrup and Junge, 2001; Toft and Baatrup, 2001; Kristensen et al., 2005; Shenoy, 2012; our *prediction 3a*).

Few studies have focused on pollution effects in guppies and reported, for example, that blue ornaments became more prevalent in a highly polluted Panamanian stream when compared with cleaner sites (Martínez et al., 2016). The magnitude of interaction effects in our present study suggests that multifarious selection affects phenotypic evolution. Likewise, population divergence in morphology and life histories of poeciliids has been shown to be driven by multiple concomitant environmental selective forces (*e.g.*, Riesch et al., 2015, 2018; Ouyang et al., 2018). While studies on the visual ecology of several potential guppy predators in our study system are missing (see Kemp et al., 2015; Endler and Mappes, 2017), piscine predators in natural guppy populations on Trinidad appear to select against red/orange ornamentation (Endler, 1980; Millar et al., 2006; for the same pattern in *Gambusia hubbsi* see Martin et al., 2014). Revisiting our previous assumption that predation risk might be reduced at heavily polluted sites (see above), this could partially explain why males in our study developed more red/orange ornaments as pollution levels increased. Following this interpretation, interaction effects with environmental PC1 (characterizing variation between upstream and downstream sites) would support the following scenario: headwaters tend to generally harbor fewer guppy predators, while guppies in lowland stream sections tend to experience strong piscine predation (Matthews, 1998; for Trinidadian guppies see Torres-Dowdall et al., 2012). Predator release due to pollution, therefore, becomes a force shaping and altering evolutionary trajectories only at downstream-situated stream sections. We can only speculate at this point that guppy predators in the streams examined here indeed target males sporting red/orange and blue/black ornaments more than males with other ornament types. Assuming that white/iridescent ornaments are less visible to the same predators, selection could indeed favor more white/iridescent but less red/orange and blue/black ornamentation at high-predation (less polluted) sites (Godin and McDonough, 2003).

Additional factors that we could not quantify in the course of our present study are known to affect color evolution in other poeciliids as well; for instance, besides predation, nuptial ornamentation in Bahamas mosquitofish (*G. hubbsi*) depends also on the visual background provided by water color (Martin et al., 2014). Co-variation of this and additional environmental factors with the environmental PCs considered here might explain several otherwise obscure interaction effects and could also help explain why the degree of determination ($R^2$) of significant model terms was low in most cases. Weak effects of population differentiation, however, were largely owing to the described pronounced color polymorphism typical of wild and feral guppy populations (Magurran, 2005). While we are lacking information about the invasion history of guppies in Rwanda (*i.e.*, active introduction, colonization from adjacent areas, or releases from home aquaria), invasive species are often characterized by reduced genetic diversity of founder populations (Allendorf and Lundquist, 2003). Feral guppies can regain their characteristic genetically-based color polymorphism (Tripathi et al., 2008, 2009; Kottler et al., 2013) because females have a mating preference for rare (*i.e.*, novel or emergent) male phenotypes, promoting the rapid spread of novel color phenotypes (*e.g.*, Hughes et al. 2013).

Finally, while several color patterns have a strong genetic (heritable) component (Winge, 1922, 1927; Haskins and Haskins, 1951), some degree of plasticity cannot be excluded. This might also apply to the development of caudal fins, the exaggeration/reduction of which was driven by interactions that are hard to explain based on the current empirical body of knowledge. Our study calls for future common-garden experiments to investigate if all components of color (and caudal fin) divergence determined here persist in +$F_2$ laboratory-reared individuals (*i.e*., to determine broad-sense heritability; Eifert et al., 2015).

## 4.3 Conclusions and applications

From an applied (human-oriented) perspective, a note of caution is required regarding the suitability of invasive guppies for bioindicator-based assessments of water pollution, as occurrence and abundance data may provide conflicting information. Trained persons (local university students or even laymen) assisting in water quality assessments ought to be taught that points with very low guppy abundances (but at which guppies nevertheless do occur) are of particular interest, as they may actually represent the most polluted sites, and strictly standardized sampling (in terms of the time spent seining, seining efficiency, and so forth) must be advised. Likewise, it needs to be considered that differentiation of fish community structures was weak.

From an evolutionary and conservation-oriented perspective, the present study adds to the growing body of literature demonstrating how anthropogenic habitat alterations (here: water pollution) not only alter local community structures but also facilitate the population establishment (and thus, further spread) of invasive species. Finally, the results demonstrate that water pollution has the potential to alter the evolutionary trajectories of populations. We call for future studies to disseminate how exactly different components of environmental variation associated with pollution levels—predation, interspecific competition, intraspecific (mate and resource) competition, and so forth—affect selective regimes acting on guppy color ornaments.

**Acknowledgements**

Financial support came from the University of Rwanda (Post-Doctoral Research Grant: UR—CRA/08/7/2014, cycle 2014), as well as from DFG-TWAS (*Vereinbarung zur Förderung eines Gastaufenthalts*; DFG-TWAS KL 2378/3-1; PL 470/4-1), Northwest A&F University (Z111021403), and from the Province of Shaanxi (A289021611).

## Authors' contributions

M.P., T.W. and A.A. designed the study. E.C. and G.G. collected the data and performed data analyses. G.G. and M.P. wrote the first manuscript draft with critical input from T.W. and R.R. All authors contributed to manuscript writing and approved the final version.

## Ethical statement

All experiments were performed in accordance with relevant regulations of the Republic of Rwanda (Law on environment N°48/2018 of 13/08/2018).

## Data availability

The datasets generated and/or analyzed for the current study will be uploaded to Dryad upon acceptance.

## Competing interests

The authors declare no competing interests.

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# Tables

## Table 1

Results of principal component analyses on (*a*) geo-physical, physico-chemical, and biotic predictor variables collected at all 55 sampling sites (66.04 % cumulative variance explained). (*b*) Numbers as well as arcsine (square root)-transformed relative surface areas of six types of color ornaments of 501 male *P. reticulata* collected at 18 sampling sites (81.25% cumulative variance explained). Principal components with an eigenvalue > 1.0 were retained; axis loadings > 0.6 are highlighted in bold typeface.

| Variable | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| **(*a*) Ecological predictor variables** | | | | | |
| Eigenvalue | 2.53 | 1.98 | 1.52 | 1.24 | — |
| Percent variance explained | 22.97 | 17.99 | 13.79 | 11.29 | — |
| Altitude [m] | **-0.820** | 0.287 | 0.083 | -0.200 | — |
| Width [m] | **0.862** | 0.263 | 0.073 | 0.062 | — |
| Depth [m] | 0.404 | 0.454 | -0.502 | 0.122 | — |
| Velocity [m $s^{-1}$] | 0.212 | 0.354 | **0.665** | 0.154 | — |
| Temperature [°C] | 0.464 | -0.201 | 0.501 | -0.323 | — |
| Oxygen [ppm] | **-0.618** | 0.024 | -0.168 | 0.123 | — |
| pH | -0.022 | **0.750** | 0.239 | -0.007 | — |
| Conductivity [µS $m^{-1}$] | 0.144 | **-0.746** | 0.047 | 0.097 | — |
| Water quality via bioindicators (ASPT) | -0.020 | -0.179 | 0.074 | **0.872** | — |
| Presence of macrophytes | -0.034 | -0.090 | **-0.783** | -0.102 | — |
| Guppy abundance | 0.117 | 0.551 | 0.099 | 0.595 | — |
| **(*b*) Male color patterns** | | | | | |
| Eigenvalue | 3.36 | 1.90 | 1.68 | 1.54 | 1.31 |
| Percent variance explained | 27.63 | 15.84 | 14.01 | 12.85 | 10.92 |
| No. of spots (black) | **0.696** | 0.187 | 0.073 | 0.048 | 0.229 |
| Rel. color area (black) | 0.519 | -0.266 | 0.056 | 0.030 | 0.038 |
| No. of spots (blue) | **0.848** | -0.162 | 0.068 | -0.206 | -0.054 |
| Rel. color area (blue) | **0.849** | -0.237 | 0.079 | -0.171 | -0.095 |
| No. of spots (white/iridescent) | -0.163 | **0.931** | -0.031 | 0.120 | 0.051 |
| Rel. color area (white/iridescent) | -0.162 | **0.927** | -0.020 | 0.076 | -0.011 |
| No. of spots (green) | 0.122 | -0.022 | **0.965** | -0.043 | 0.017 |
| Rel. color area (green) | 0.085 | -0.034 | **0.967** | -0.072 | 0.021 |
| No. of spots (yellow) | -0.080 | 0.120 | -0.051 | **0.937** | -0.004 |
| Rel. color area (yellow) | -0.110 | 0.058 | -0.063 | **0.942** | -0.070 |
| No. of spots (red/orange) | 0.095 | 0.104 | 0.036 | -0.015 | **0.906** |
| Rel. color area (red/orange) | -0.017 | -0.074 | -0.002 | -0.055 | **0.918** |

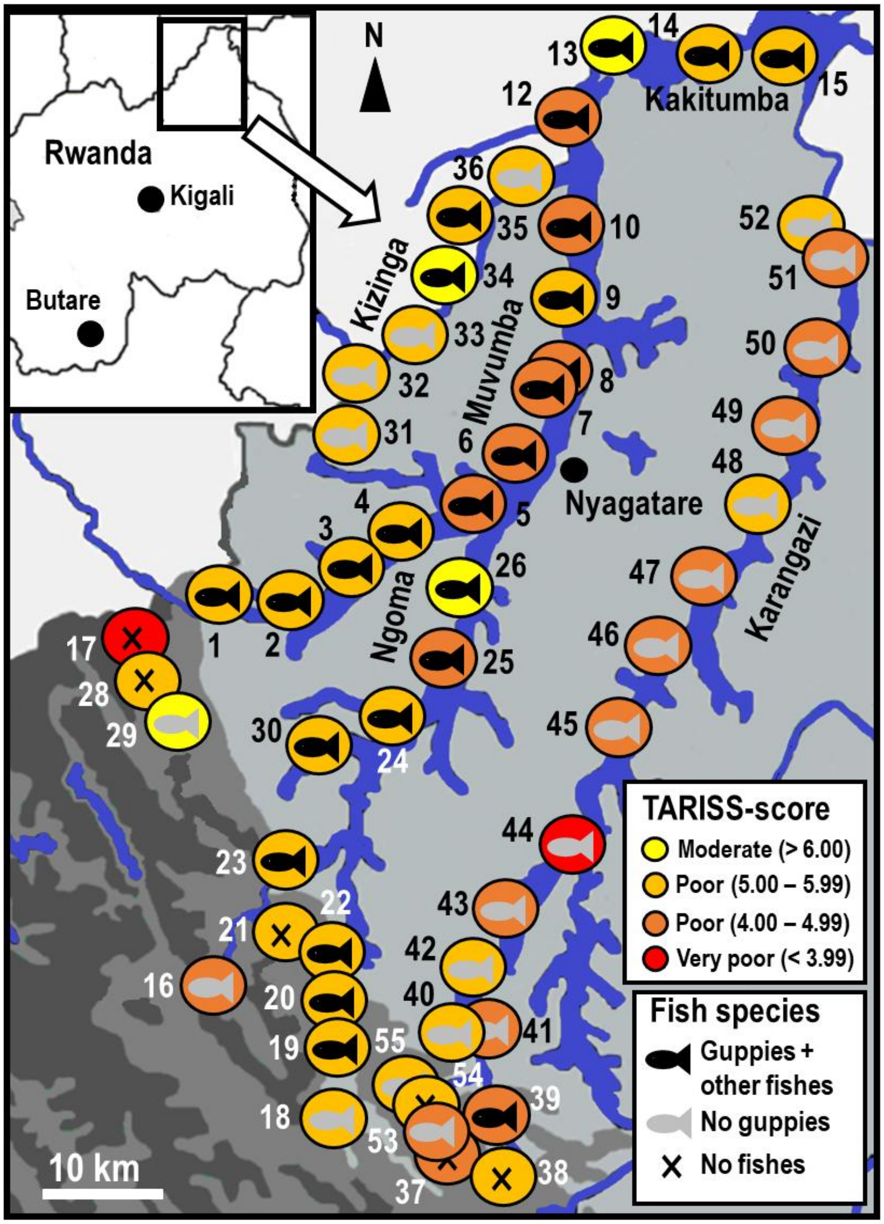
**Table 2**

Results of ANCOVAs on site-specific variation of phenotypic traits using (*a*) log$_{10}$-transformed body size (standard length, SL [mm]), and (*b*) arcsine (square root)-transformed relative caudal fin area (total caudal fin area [mm$^2$] divided by total body surface area [mm$^2$] as the dependent variable. (*c*) Results of MANCOVA using five body coloration-related principal components (PCs, Table 1*b*), obtained from the analysis of 501 male *P. reticulata*, as the dependent variables, and (*d*-*h*) separate ANCOVAs on all five color-related PCs. In all cases, four PCs capturing environmental variation between sampling sites (Table 1*a*) were included as covariates, but non-significant two-way interactions were excluded (all $F < 2.64$, $P > 0.11$). Significant effects are shown in bold.

| Factor | *df* | *F* | *P* | Wilks' partial $\eta_p^2$ |
|---|---|---|---|---|
| **(*a*) ANCOVA, body size (SL)** | | | | |
| **PC1** | **1** | **11.131** | **0.001** | **0.022** |
| **PC2** | **1** | **17.72** | **< 0.0001** | **0.035** |
| **PC3** | **1** | **5.407** | **0.020** | **0.011** |
| **PC4** | **1** | **40.618** | **< 0.0001** | **0.076** |
| **PC2 × PC4** | **1** | **66.633** | **< 0.0001** | **0.119** |
| **PC3 × PC4** | **1** | **9.541** | **0.002** | **0.019** |
| Error | 494 | | | |
| **(*b*) ANCOVA, relative area of caudal fin** | | | | |
| SL | 1 | 1.331 | 0.249 | 0.003 |
| **PC1** | **1** | **11.021** | **0.001** | **0.022** |
| **PC2** | **1** | **17.505** | **< 0.0001** | **0.034** |
| PC3 | 1 | 0.166 | 0.684 | < 0.0001 |
| PC4 | 1 | 0.138 | 0.710 | < 0.0001 |
| **PC1 × PC2** | **1** | **7.865** | **0.005** | **0.016** |
| **PC1 × PC4** | **1** | **14.531** | **< 0.0001** | **0.029** |
| **PC2 × PC3** | **1** | **7.259** | **0.007** | **0.015** |
| **PC2 × PC4** | **1** | **18.039** | **< 0.0001** | **0.036** |
| **PC3 × PC4** | **1** | **14.058** | **< 0.0001** | **0.028** |
| Error | 490 | | | |
| **(*c*) MANCOVA, color PCs** | | | | |
| **SL** | **5** | **4.417** | **0.001** | **0.043** |
| **PC1** | **5** | **5.672** | **< 0.0001** | **0.055** |
| **PC2** | **5** | **2.324** | **0.042** | **0.023** |
| **PC3** | **5** | **3.527** | **0.004** | **0.035** |
| **PC4** | **5** | **6.189** | **< 0.0001** | **0.060** |
| **PC1 × PC2** | **5** | **10.392** | **< 0.0001** | **0.096** |
| **PC1 × PC3** | **5** | **2.768** | **0.018** | **0.028** |
| **PC1× PC4** | **5** | **6.038** | **< 0.0001** | **0.058** |
| **PC2 × PC4** | **5** | **8.965** | **< 0.0001** | **0.087** |
| Error | 487 | | | |
| **(*d*) ANCOVA, color PC1 (blue/black)** | | | | |
| **SL** | **1** | **12.355** | **< 0.0001** | **0.025** |
| **PC1** | **1** | **9.189** | **0.003** | **0.018** |
| **PC2** | **1** | **6.387** | **0.012** | **0.013** |
| PC3 | 1 | 1.344 | 0.247 | 0.003 |
| **PC4** | **1** | **5.269** | **0.022** | **0.011** |

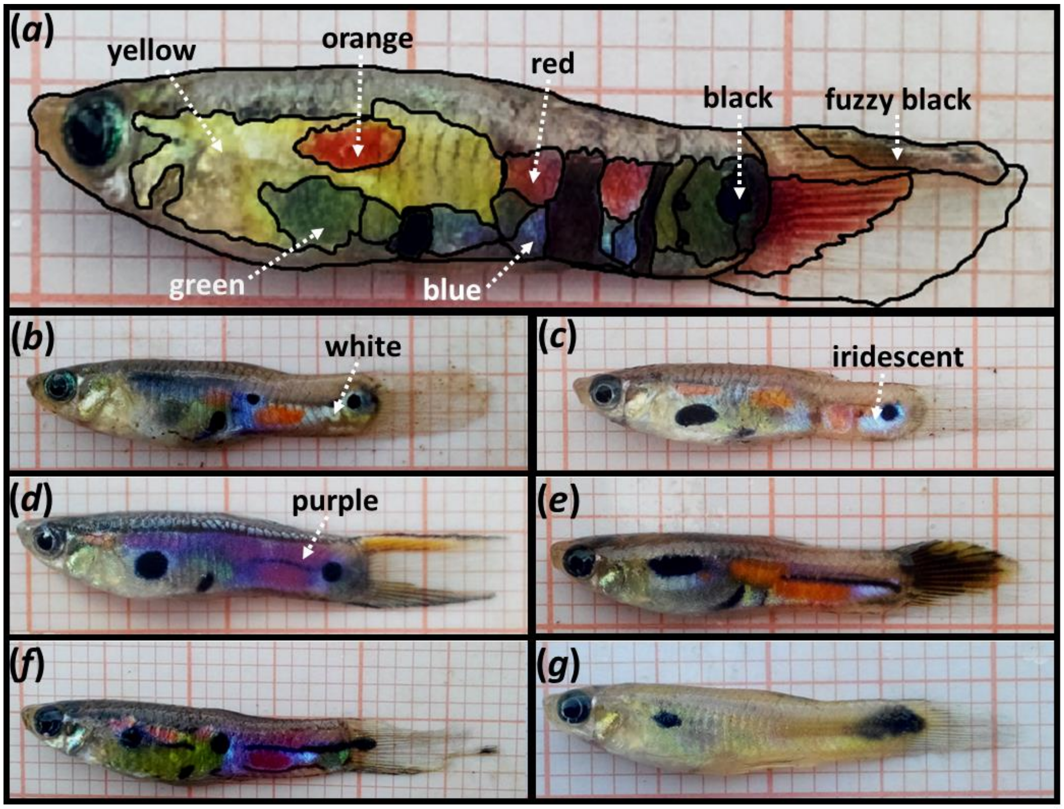
| | | | | |
|---|---|---|---|---|
| **PC1 × PC2** | **1** | **49.856** | **< 0.0001** | **0.092** |
| **PC1 × PC4** | **1** | **9.150** | **0.003** | **0.018** |
| **PC2 × PC3** | **1** | **4.823** | **0.029** | **0.010** |
| **PC2 × PC4** | **1** | **30.542** | **< 0.0001** | **0.050** |
| Error | 491 | | | |
| **(*e*) ANCOVA, color PC2 (white/iridescent)** | | | | |
| SL | 1 | 0.20 | 0.888 | < 0.0001 |
| PC1 | 1 | 0.106 | 0.745 | < 0.0001 |
| PC2 | 1 | 2.820 | 0.094 | 0.006 |
| **PC3** | **1** | **6.857** | **0.009** | **0.014** |
| **PC4** | **1** | **4.805** | **0.029** | **0.010** |
| **PC1 × PC3** | **1** | **7.344** | **0.007** | **0.015** |
| **PC1 × PC4** | **1** | **12.557** | **< 0.0001** | **0.025** |
| **PC2 × PC4** | **1** | **6.960** | **0.009** | **0.014** |
| Error | 491 | | | |
| **(*f*) ANCOVA, color PC3 (green)** | | | | |
| SL | 1 | 2.956 | 0.086 | 0.006 |
| PC1 | 1 | 0.804 | 0.370 | 0.002 |
| **PC2** | **1** | **22.816** | **< 0.0001** | **0.044** |
| PC3 | 1 | 0.163 | 0.686 | < 0.0001 |
| PC4 | 1 | 2.352 | 0.126 | 0.005 |
| Error | 495 | | | |
| **(*g*) ANCOVA, color PC4 (yellow)** | | | | |
| SL | 1 | 2.583 | 0.109 | 0.005 |
| **PC1** | **1** | **9.496** | **0.002** | **0.019** |
| PC2 | 1 | 0.040 | 0.841 | < 0.0001 |
| PC3 | 1 | 1.660 | 0.198 | 0.003 |
| PC4 | 1 | 1.136 | 0.287 | 0.002 |
| Error | 495 | | | |
| **(*h*) ANCOVA, color PC5 (red/orange)** | | | | |
| **SL** | **1** | **6.023** | **0.014** | **0.012** |
| **PC1** | **1** | **10.394** | **0.001** | **0.021** |
| **PC2** | **1** | **8.957** | **0.003** | **0.018** |
| **PC3** | **1** | **4.299** | **0.039** | **0.009** |
| **PC4** | **1** | **16.842** | **< 0.0001** | **0.033** |
| **PC2 × PC4** | **1** | **15.899** | **< 0.0001** | **0.031** |
| Error | 494 | | | |

# Figures



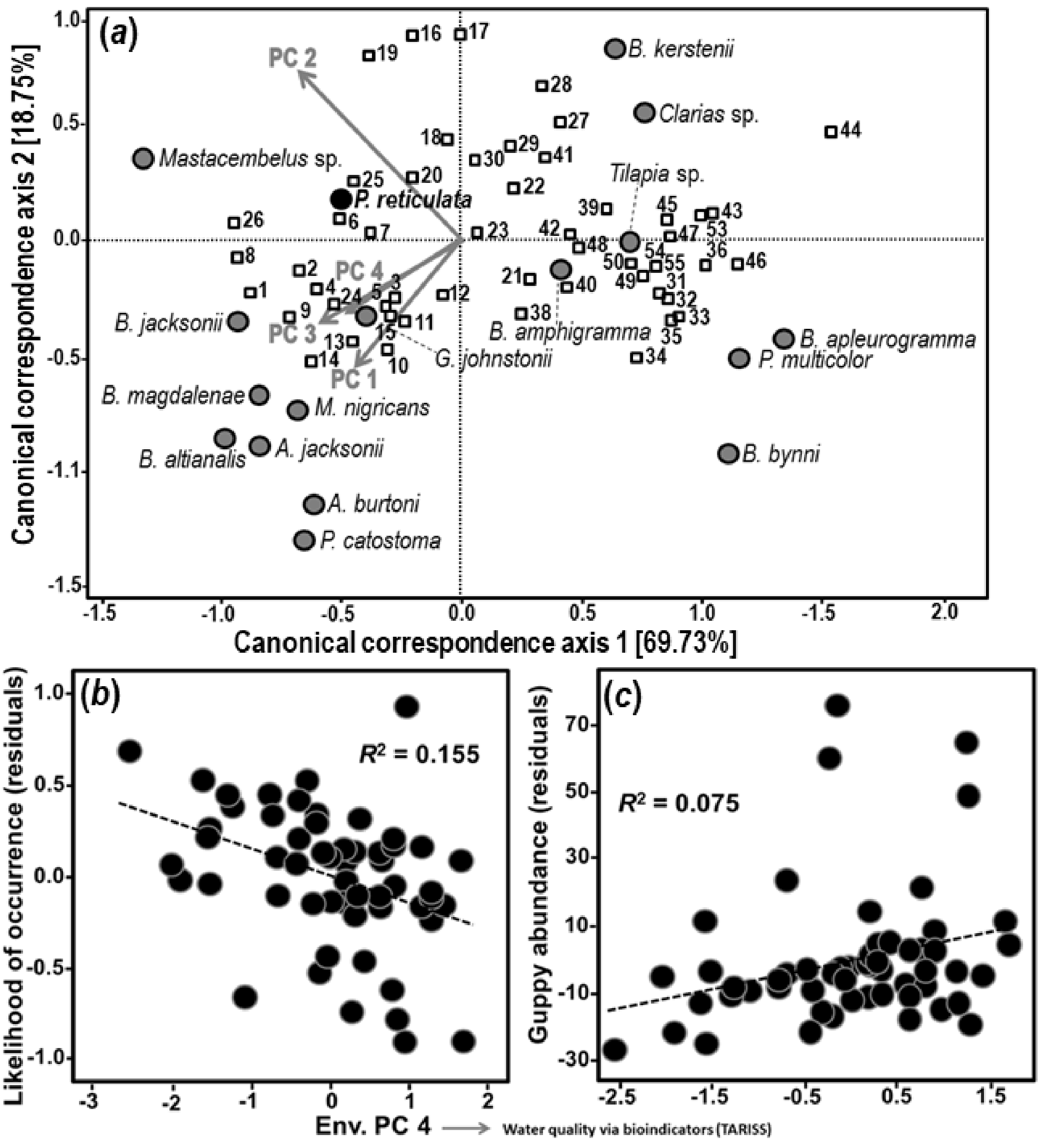
## Figure 1

Map showing the 55 sampling sites in the Rwandan Mutara Rangelands (site numbers are indicated) at which invasive guppies co-occurred with native fish species (black symbols), only native fishes occurred (gray symbols), or no fishes were collected (×). Water pollution was assessed using a macrozoobenthos-based approach (Dusabe et al., 2019), and the resulting TARISS-score (Kaaya et al., 2015) is depicted as falling into the categories 'moderate', 'poor', and 'very poor', following the classification of Aquilina (2013). To better depict variation in water quality, the category 'poor' is further subdivided (4.00–4.99 and 5.00–5.99). Note that the category 'good' was not found, suggesting strong overall pollution levels. Map modified from Kindt et al. (2014) depicting current land use forms (gray: degraded grasslands, dark grays: escarpment with agriculture). Rivers and the associated floodplains are shown in blue.



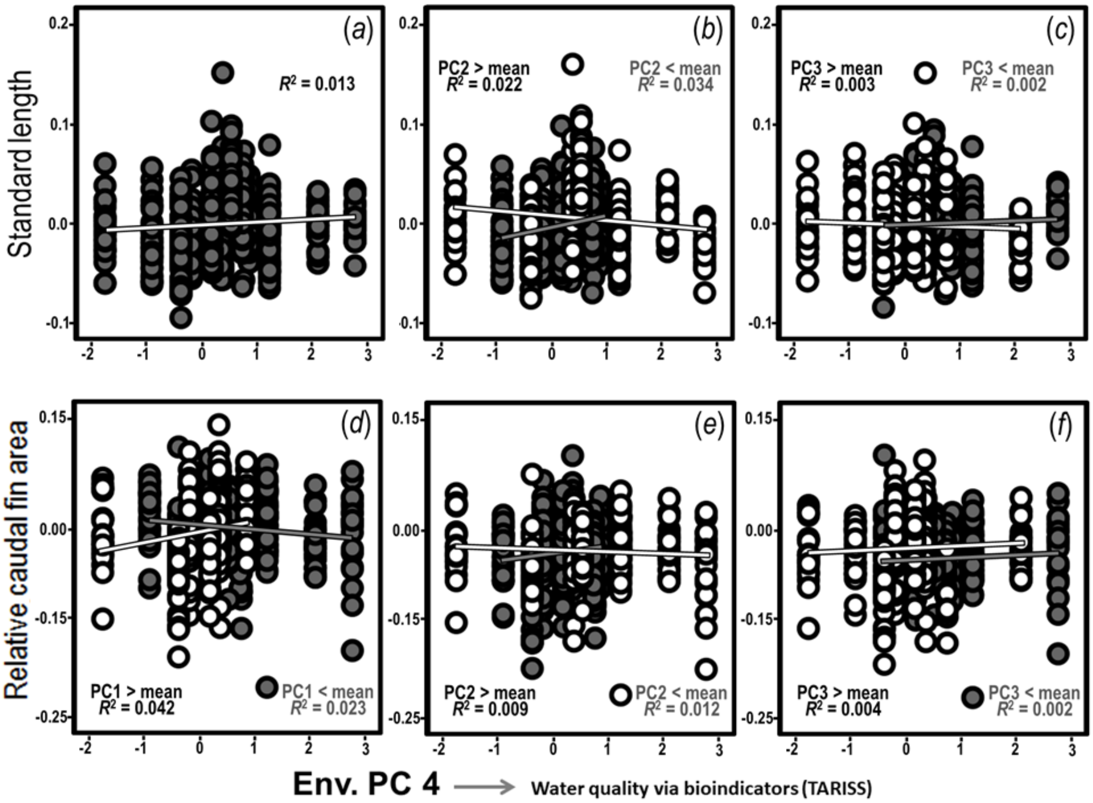
## Figure 2

(*a*) Example of a male used to quantify numbers and percent body cover of yellow, orange, red, black, fuzzy black, blue, and green nuptial color ornaments. (*b*)-(g) Representative photographs of male guppies from different sampling sites illustrating the observed color polymorphism. (*b*) Male sporting white, (*c*) iridescent, and (*d*) purple ornaments. Also note the pronounced variation of caudal fin areas.



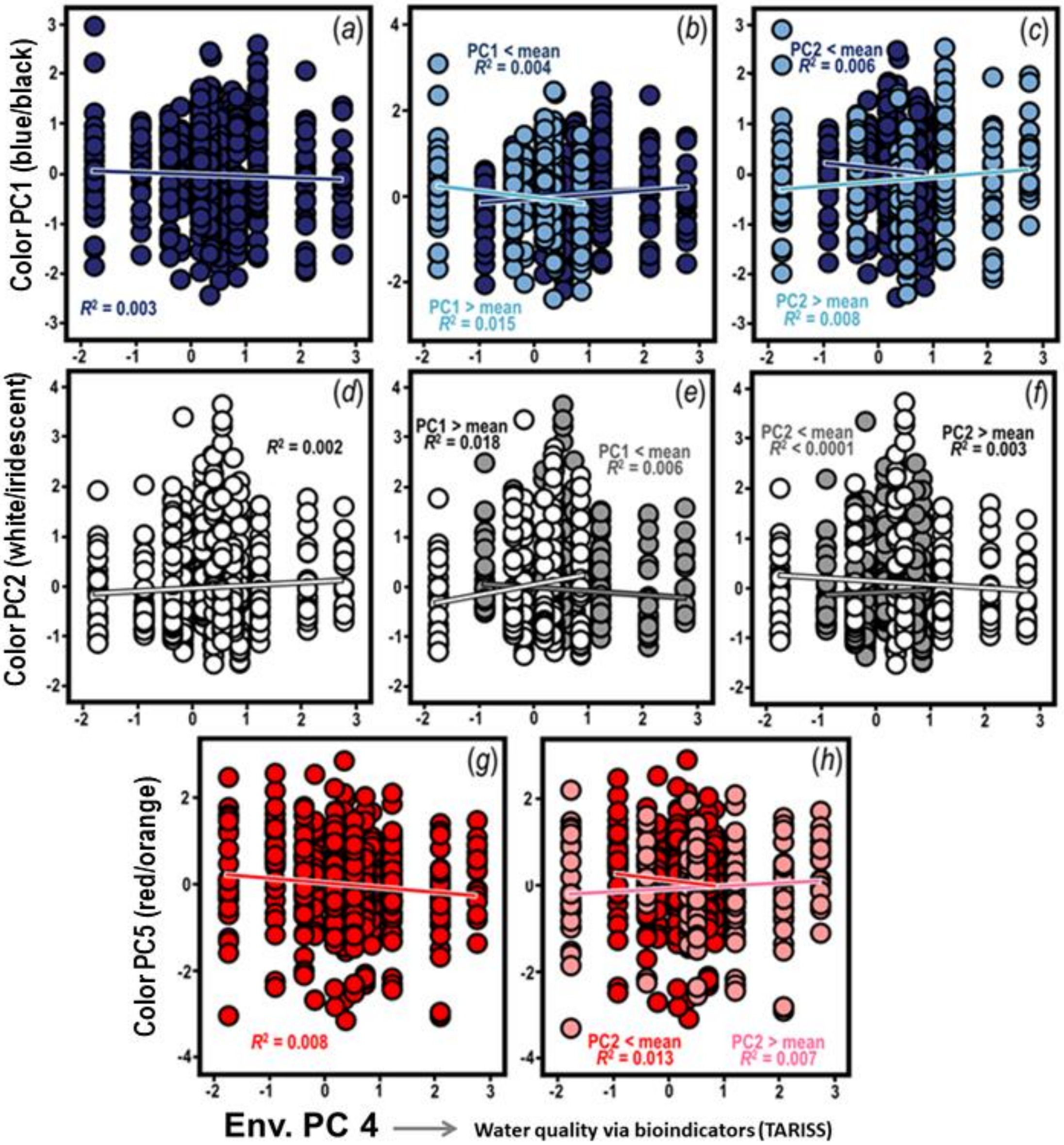
## Figure 3

Results of a canonical correspondence analysis (CCA) visualizing the effects of environmental variables ('environmental PC 1–4'; Table 1) on fish community structures based on abundance data. Species are marked by gray circles and our focal species *P. reticulata* by a black circle, while squares indicate the position of sampling sites. (*b*, *c*) Visualization of significant effects of PC4 on (*b*) the likelihood of occurrence of guppies (see Table S2 for fish community compositions), and (*c*) local abundances of guppies (residuals, corrected for other model terms). Note that the likelihood of occurrence increased while abundances decreased at sites with deteriorating water quality [negative values of PC4, which received a high axis loading from the macrozoobenthos-based assessment of water quality, the TARISS-score (Kaaya et al., 2015)].



## Figure 4

Visualization of significant effects detected in GLMs (Table 2*a*-*b*) on (*a*–*c*) body size (standard length) and (*d*–*f*) relative caudal fin areas (residuals, corrected for other model terms) of male guppies (*P. reticulata*). Scattergrams show the relationships with pollution-related environmental principal component 4 (PC4). To visualize interaction effects, data were split into cohorts with values of other PCs larger and smaller than the empirical mean. We present linear regression lines and the associated degree of determination ($R^2$).



## Figure 5

Visualization of significant effects detected in GLMs (Table 2*d*, *e*, *h*) on numbers and relative surface areas of male color ornaments (residuals, corrected for other model terms) of our focal species *P. reticulata.* Scattergrams show the relationships between environmental principal component 4 (PC4) and the extent of (*a*-*c*) blue/ black ornamentation, (*d*-*f*) white/iridescent ornamentation and (*g*-*h*) red/orange ornamentation. Heavily polluted sites are characterized by negative values of PC4 (left side) while less polluted sites show positive values of PC4 (right side). Shown are linear regression lines and the associated degree of determination ($R^2$).