**Phenotypic responses to oil pollution in a poeciliid fish.**

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**Abstract**

Pollution damages ecosystems around the globe and some forms of pollution, like oil pollution, can be either man-made or derived from natural sources. Despite the pervasiveness of oil pollution, certain organisms are able to colonise polluted or toxic environments, yet we only have a limited understanding of how they are affected by it. Here, we analysed phenotypic responses to oil pollution in guppies (*Poecilia reticulata*) living in oil-polluted habitats across southern Trinidad. We analysed body-shape and life-history traits for 352 individuals from 11 independent populations, six living in oil-polluted environments (including the naturally oil-polluted Pitch Lake), and five stemming from non-polluted habitats. Based on theory of, and previous studies on, responses to environmental stressors, we predicted guppies from oil-polluted waters to have larger heads and shallower bodies, to be smaller, to invest more into reproduction, and to produce more but smaller offspring compared to guppies from non-polluted habitats. Contrary to most of our predictions, we uncovered strong population-specific variation regardless of the presence of oil pollution. Moreover, guppies from oil-polluted habitats were characterised by increased body size; rounder, deeper bodies with increased head size; and increased offspring size, when compared to their counterparts from non-polluted sites. This suggests that guppies in oil-polluted environments are not only subject to the direct negative effects of oil pollution, but might gain some (indirect) benefits from other concomitant environmental factors, such as reduced predation and reduced parasite load. Our results extend our knowledge of organismal responses to oil pollution and highlight the importance of anthropogenic pollution as a source of environmental variation. They also emphasise the understudied ecological heterogeneity of extreme environments.

***Key-words*:** *Poecilia reticulata*, pollution, phenotypic variation, Global Change, extremophiles

**Introduction**

Humans are a major source of environmental change (Folke et al., 2021) and impact ecosystems through climate change, habitat destruction, introduction of invasive species and pollution (Stockwell et al., 2003; Sih et al., 2011; Pelletier and Coltman, 2018), typically leading to a loss of biodiversity (Blowes et al., 2019). Pollution is among the most important anthropogenic sources of environmental change and contemporary human-induced evolution. The presence of pollutants in the environment has been associated with reduced fitness in affected organisms, reduced population sizes, depleted species richness and disrupted community integrity, but has also been shown to drive rapid evolution of tolerance in certain species (Freeman, 1995; Hendry et al., 2008, 2017; Palkovacs et al., 2012; Hamilton et al., 2017).

Among pollutants, crude oil is recognised as having widespread, negative effects on wildlife (Wake, 2005; Hamilton et al., 2017; Adzigbli and Yuewen, 2018). The effects of oil pollution on ecosystems have most often been studied in the context of large-scale disasters (e.g., Exxon Valdez: Peterson et al., 2003; Deep Water Horizon: Whitehead et al., 2011). However, crude oil can also enter the environment through human activities (Farrington, 2013), including spillages that occur during the exploitation of crude oil reserves in oil fields (Wake, 2005; Rolshausen et al., 2015). These smaller-scale spills can create localized yet highly toxic environments that impact only certain populations of a species, allowing for the study of organismal responses to crude oil (Rolshausen et al., 2015). While fish are usually highly susceptible to the negative effects of pollution (Harmon and Wiley, 2009), experiencing both lethal and sublethal effects (such as immunodepression and increased parasitism, Austin, 2010; or reduced reproduction, Aulsebrook et al., 2020), several species have been able to adapt to polluted and/or naturally toxic environments (e.g., *Fundulus heteroclitus*, Whitehead et al., 2012; *Gambusia* spp. and *Poecilia* spp., Riesch et al., 2016).

In the case of oil pollution, negative effects have been documented in multiple species and include high mortality rates (Saha and Konar, 1984a; Incardona et al., 2015), as well as sublethal effects of respiratory distress, hampered growth (Saha and Konar, 1984b), reduced fecundity (Rowe et al., 1983), and reduced exploratory activity (Aimon et al., 2021). Moreover, one of the main components of crude oil, polycyclic aromatic hydrocarbons, have well-established carcinogenic and mutagenic effects (Samanta et al., 2002). Even though some fish species seem more capable of coping with oil-based pollution than others (Irwin, 1965), we still do not properly understand why this is, which traits specifically might be affected, and which traits might be essential for survival in the presence of crude oil.

Guppies, *Poecilia reticulata*, are among the fish species that can tolerate high levels of water pollution (Araújo et al., 2009; Riesch et al., 2016; Gomes-Silva et al., 2019, 2020). They have also been the subject of decades of ecological and evolutionary research and their biology is well-understood (Magurran, 2005), making them an ideal model for the study of phenotypic responses to pollution in aquatic ecosystems. In southern parts of the island of Trinidad, West Indies, guppies occur in several environments that are highly polluted as a result of human exploitation of crude-oil reserves (Rolshausen et al., 2015), and also in the Pitch Lake, the largest natural asphalt lake in the world, where crude oil and bitumen naturally seep to the surface creating a highly toxic environment (Ponnamperuna and Pering, 1967; Schelkle et al., 2012; Kayukova et al., 2016; Santi et al., 2019).

Drawing upon these populations, we analysed morphological and life-history responses to oil pollution in guppies in Trinidad. A previous study showed that guppies living in oil-polluted environments of recent anthropogenic origin were characterised by shallower bodies and larger heads (and thus larger gills, which can reduce respiratory problems caused by oil pollution, Saha and Konar, 1984b) than those living in non-polluted environments (Rolshausen et al., 2015). Similarly, Pitch Lake guppies appeared to be characterised by *r*-selected life-histories, with increased reproductive investment and fecundity, and reduced body and offspring size compared to fish from a nearby, non-polluted site, pointing towards a scenario of increased mortality due to oil pollution (Santi et al., 2019). Both of these studies, therefore, suggest that oil-pollution can cause considerable physiological stress in guppies, and that phenotypic responses, even if only mediated by phenotypic plasticity, might be important in the colonization of polluted environments.

We made three specific predictions. Prediction 1: Guppies across polluted environments should be characterised by larger heads and shallower bodies than guppies from non-polluted sites. Prediction 2: Guppies from oil-polluted habitats should have reduced adult body size compared to their counterparts from non-polluted waters. Prediction 3: In oil-polluted environments, female guppies should be characterized by increased reproductive investment, with increased fecundity but smaller offspring size.

**Materials and Methods**

***Sampling sites***

This study took place on the Caribbean island of Trinidad, which forms part of the guppy’s native range and is famous for its natural asphalt seeps which are exploited for crude oil products. We sampled guppies from 11 populations across the island in May/June 2018 (Fig. 1*a*, *e*). Six populations originated from oil-polluted habitats (identified by the presence of an oil slick on the water surface; see Fig. 1*b*, *f* for a comparison between oil-polluted and non-polluted habitats) and five from non-polluted habitats. As polluted sampling sites we chose the Pitch Lake (population 1), where pitch and oil naturally seep to the surface (Ponnamperuna and Pering, 1967), and five other sites subject to crude oil spillage from the oil fields in southern Trinidad (Rolshausen et al., 2015). Four of these were located in streams flowing through the oil fields (populations 2, 3, 4, and 5), and the fifth was located next to an oil refinery in the city of Point Fortin (population 6). A previous study has shown that the rivers of Trinidad that are subject to crude-oil pollution from human exploitation of the oil fields are contaminated by high concentrations of multiple petrogenic hydrocarbons, including several toxic polycyclic aromatic hydrocarbons (PAH; Rolshausen et al., 2015), and these contaminants were absent in non-polluted tributaries of the same rivers. Similarly, PAH contamination has been found in proximity of the Pitch Lake (Agard et al., 1993; Hosein et al., 2018), and of oil-refining plants in the area (Agard et al., 1993). Unfortunately, we had to exclude previously established reference sites (Rolshausen et al., 2015; Santi et al., 2019) because they were inaccessible and/or polluted in 2018. We therefore chose four new non-polluted habitats that were as close as possible to the original ones. A population from a non-polluted habitat in the north of the island was included as a further comparison. All sampling sites were small natural or artificial aquatic habitats (e.g., ditches) with stagnant-to-slow-moving water. All fish were sampled with hand-held seines and dip nets, euthanised with clove oil immediately after collection, and then fixed and preserved in 90% ethanol for subsequent analyses.

***Body-shape analyses***

We analysed body shape variation among 352 individuals (201 sexually mature males and 151 gravid females) using geometric morphometrics (Zelditch et al., 2012). To facilitate collecting information on body-shape and life-history traits from the same set of individuals, as well as to eliminate unwanted noise and spurious results that might arise due to the changes in body shape following sexual maturation (Greven, 2011), we only used sexually mature males and gravid females. Sexual maturation and gravid status were established during subsequent life-history dissections (see below), on the basis of gonopodium morphology in males and the presence of developing embryos in females (Kelly et al., 2000). We first took standardised photographs of the left side of the body of each fish using a Canon EOS 400D DSLR camera with a 50 mm macro lens (Canon Inc., Tokyo, Japan) mounted on a copy stand. We then scored 15 landmarks for each individual (Fig. 1*g*; Santi et al., 2020; see also Supplementary Material for additional information). This step (and the subsequent life-history dissections) was performed blindly to habitat type in order to avoid bias. As some individuals (*N* = 29) became bent during fixation and preservation, we used the “unbend specimen” function in tpsUtil32. To that end, three temporary landmarks were added along the lateral line of the fish and in the middle of the caudal peduncle, and were subsequently removed again. From a relative warps analysis (Zelditch et al., 2012), we obtained 5 relative warps (RWs) that accounted for 90.95% of the cumulative variance (Table S2); these we used as shape variables for subsequent analyses. We also obtained the Centroid Size, or the square root of the sum of the squared distances of each landmark from their centroid, or centre of gravity, which was used to control for size-effects in all subsequent analyses. Visual representation using thin-plate splines showed that RW1 mainly described differences between males and females, caused by the different positioning of the anal fin, which is shifted to the posterior in females, and shifted to the anterior and modified into the gonopodium in males. RW2-5, on the other hand, described differences in the depth and roundness of the body, in the depth of the caudal peduncle, and in head size (Fig. S1).

***Life-history analyses***

We quantified life-history traits by following well-established dissection protocols (Reznick and Endler, 1982; Riesch et al., 2016). For males and females, we measured standard length (SL [mm]), dry mass [mg], lean dry mass [mg; mass after fat extraction], and fat content [% of dry mass]. For males, we also quantified the gonadosomatic index (GSI [%]) as the testis dry mass divided by the sum of testis and somatic dry mass. For females, we further quantified fecundity (number of developing embryos), offspring dry and lean mass [mg], offspring fat content [%], and reproductive allocation (RA [%]; total offspring dry mass divided by the sum of total offspring and somatic dry mass). The developmental stage of each embryo was assessed following Riesch et al. (2011), with embryonic stages ranging from 2 (fertilized oocyte with blastodisc present) to 50 (embryo ready to be born), and this metric was used as a covariate in order to control for developmental effects in subsequent analyses.

To meet statistical assumptions of normality of residuals, we log10-transformed (SL, adult lean mass and offspring lean mass), square root-transformed (fecundity), or arcsine(square root)-transformed (GSI, RA, adult and embryo fat content) all life-history variables. We subsequently *z*-transformed all variables to obtain unit-free variables with equal variance for all subsequent analyses.

***Statistical analyses***

Body-shape and adult life-history traits were analysed in both males and females together in order to investigate potential differences between the two sexes in their responses to oil pollution. Nonetheless, sex-specific analyses that had qualitatively similar results are shown in the Supplementary Material.

For body shape, we ran a nested multivariate general linear model (GLM) with RWs 1-5 as dependent variables, ‘sex’, ‘habitat type’ (i.e., polluted vs. non-polluted environments) and ‘population-nested-within-habitat type’ (henceforth ‘population(habitat type)’) as factors, and ‘centroid size’ as a covariate.

For life histories, we first investigated body-size differences by performing a nested univariate GLM with SL as the dependent variable and ‘sex’, ‘habitat type’, and ‘population(habitat type)’ as factors. Subsequently, we ran two separate multivariate GLMs. The first focused on adult life histories and included lean mass, fat content, and reproductive investment (GSI for males and RA for females) as dependent variables, ‘sex’, ‘habitat type’, and ‘population(habitat type)’ as factors, and ‘SL’ as a covariate. The second focused on offspring-related life histories and included fecundity, offspring lean mass, and offspring fat content as dependent variables, ‘habitat type’ and ‘population(habitat type)’ as factors, and ‘female SL’ and ‘embryonic stage of development’ as covariates.

All statistical analyses were performed with IBM® SPSS® Statistics 25 (2019; IBM Corp. Armonk, NY), and in multivariate analyses, *F*-values were approximated using Wilks’ *λ*. We initially entered all interaction terms in the models, but removed those with *P* > 0.1 from final models.

**Results**

***Variation of body shape***

Full results of the multivariate GLM are presented in Table 1*a*, while results of *post-hoc* univariate GLMs (*α*-level corrected for multiple comparisons to *α*’ = 0.010) are presented in Table 2. Guppies were sexually dimorphic in body shape (‘sex’-effect), even though the extent of sexual dimorphism varied between populations (‘sex × population(habitat type)’-effect). They also exhibited both significant population-specific variation within the same habitat type (‘population(habitat type)-effect’) and consistent responses to the presence of oil pollution (‘habitat type’-effect), with fish from polluted habitats being characterised by rounder, deeper bodies with relatively larger heads than fish from non-polluted sites (Tables 1*a*, 2; Fig. 2*a*). These responses were, however, much more pronounced in females than in males (‘sex × habitat type’-effect; Fig. 2*b*).

***Variation of adult life histories***

The univariate GLM on SL differences revealed that females were significantly bigger than males (‘sex’-effect; *F*1, 33 = 1429.10, *P* < 0.001, *η*p2 = 0.812), and in polluted habitats guppies were overall larger than in non-polluted ones (‘habitat type’-effect; *F*1, 330 = 53.29, *P* < 0.001, *η*p2 = 0.139; Fig. 2*c*). However, both body size (‘population(habitat type)’ effect, *F*9, 330 = 4.29, *P* < 0.001, *η*p2 = 0.408), and the extent of sexual dimorphism (‘sex × population(habitat type)’-effect; *F*9, 333 = 5.84, *P* < 0.001, *η*p2 = 0.150), varied significantly between populations within each habitat type.

In the multivariate analysis of the remaining adult life-history traits (Table 1*b*) and *post-hoc* univariate analyses (*α*-level corrected for multiple comparisons to *α*’ = 0.017; Table 3*a*), the effect of oil pollution was not significant (*P* = 0.070), even though there was a tendency towards heavier fish in oil-polluted habitats. Females were heavier than males (‘sex’-effect), in both sexes lean mass increased with increasing length (‘SL’-effect), and the effect of ‘population(habitat type)’ highlighted high levels of differentiation between populations within each habitat type. For instance, female size-corrected lean mass ranged from 0.068 ± 0.002 g (mean ± SE) to 0.045 ± 0.002 g in polluted habitats (populations 5 and 1, respectively), and from 0.061 ± 0.001 g to 0.055 ± 0.002 g in non-polluted sites (populations 7 and 8, respectively; Table S4).

***Variation of offspring-related life histories***

In our analysis of offspring-related life-history traits (Table 1*c*; *α*-level corrected for multiple comparisons to *α*’ = 0.017 for *post-hoc* univariate models: Table 3*b*), we found significant variation in offspring life histories between populations within habitat types [‘population(habitat type)’-effect]. For instance, size-corrected fecundity (number of developing embryos per female) in polluted habitats ranged from 16.41 ± 1.16 to 7.95 ± 1.18 (populations 2 and 3, respectively), and from 23.81 ± 1.26 to 5.49 ± 1.16 (populations 7 and 11, respectively) in non-polluted ones (Table S4). Also, bigger females produced more offspring than smaller ones (‘SL’-effect).

Nevertheless, guppies exhibited some consistent responses to the presence of oil pollution (‘habitat type’-effect), as females in oil-polluted environments produced heavier offspring than those in non-polluted ones (Fig. 2*d*).

**Discussion**

We analysed body shape and life-history traits in guppies from oil-polluted and non-polluted aquatic environments in Trinidad and detected consistent phenotypic differences between habitat types. We also uncovered high levels of population-specific phenotypic variation within each habitat type. While some results point towards direct negative effects of oil pollution on guppies, others suggest the presence of positive, indirect effects.

***Shared effects of oil pollution on guppy phenotypes***

Contrary to most of our predictions, our analyses revealed that guppies living in polluted environments were characterised by rounder, deeper bodies with bigger heads, larger adult body size and bigger embryos compared to fish from non-polluted habitats. This is partial support for our prediction 1, but also evidence against predictions 2 and 3, as well as other aspects of prediction 1.

In accordance with our prediction 1, guppies living in oil-polluted environments had larger heads. Increased head size is a common feature of fish inhabiting polluted waters (e.g., guppies in Trinidad: Rolshausen et al., 2015; tilapia, *Oreochromis* spp., in Taiwan: Sun et al., 2009), and is usually linked to the enlargement of the gills (Fracácio and Verani, 2003; Tkatcheva et al., 2004). Increased head size has also been demonstrated in fish that have been exposed to high levels of hydrogen sulphide (H2S; e.g., poeciliids: *Poecilia mexicana*, *Gambusia* spp., Riesch et al., 2016; anablepids: *Jenynsia sulfurica*, Aguilera et al., 2019). While we could not measure H2S levels during our sampling, its presence has been reported in the Pitch Lake (Kayukova et al., 2016), and it is commonly found alongside crude oil (Driver and Freedman, 1993). Gill enlargement can facilitate oxygen absorption efficiency in oxygen-depleted environments (Tobler et al., 2018), as well as decrease the uptake of pollutants by thickening the epithelium of the gills and increasing the production of mucus (Fracácio and Verani, 2003; Tkatcheva et al., 2004), thus limiting some of the negative effects of oil pollution, such as gill malformations (Sun et al., 2009) and respiratory distress (Saha and Konar, 1984b).

In contrast with the second part of our prediction 1, however, guppies from polluted environments had rounder bodies than those living in non-polluted ones, with this effect being much more pronounced in females than in males. Guppies living in polluted environments were further characterised by larger body sizes than those from non-polluted habitats (contrary to our prediction 2), and they produced larger (not smaller) offspring (contrary to our prediction 3). These patterns contrast with what has previously been reported both for guppies in oil-polluted environments in Trinidad (Rolshausen et al., 2015; Santi et al., 2019), and for other poeciliids from H2S-toxic springs (Riesch et al., 2016). They also contradict studies in other fish species, including those that reported reduced body size and hampered growth in response to oil pollution (Saha and Konar, 1984a). Several factors could be behind this surprising pattern, with some of the more intriguing being the role of parasites and/or predators.

Water pollution has a direct toxic effect on the guppy parasite *Gyrodactylus turnbulli* (Gheorghiu et al., 2006), and a previous study on guppies in the Pitch Lake showed that this population lacks gyrodactylid ectoparasites, despite infections being nearly ubiquitous among other populations in Trinidad (Schelkle et al., 2012). The presence of internal parasites was also found to be lower in oil-polluted environments than in non-polluted ones (Jeffress et al., unpublished results). In guppies, larger individuals usually carry a higher parasite load and experience the highest mortality rates (Cable and van Oosterhout, 2007; van Oosterhout et al., 2007). This could result in larger (and usually older) individuals being removed from non-polluted populations at higher rates than from oil-polluted environments, as these individuals are more likely to succumb to parasite-related infections, and are also less able to escape predation.

Similarly, guppies appear to be more tolerant to water pollution than many of their predators (Rolshausen et al., 2015). Indeed, in most of our polluted habitats the only other species observed was the killifish *Anablepsoides hartii*, which, due to gape limitation, tends to prey predominantly on juvenile guppies (Reznick and Endler, 1982), and freshwater shrimps *Macrobrachium* sp., which have been described as infrequent guppy predators (Rodd and Reznick, 1991; Table S1). Meanwhile, we observed several potential guppy predators in non-polluted environments, including the two-spot sardine *Astyanax bimaculatus* (Magurran and Seghers, 1990), Guyana leaffish *Polycentrus schomburgkii*, dragonfly larvae (Reznick et al., 2001), and blue heron *Ardea herodias* (Table S1). In low-predation environments, guppies tend to be characterised by greater body size relative to individuals from high-predation habitats (Reznick and Endler, 1982), and this (in combination with reduced parasite numbers) could favour increased body size in guppy populations living in polluted environments (Reznick and Endler, 1982; Schelkle et al., 2012). Similarly, guppies in low-predation habitats are usually more rounded and less streamlined (Burns et al., 2009); this effect is expected to be stronger in females than in males, as the latter stages of pregnancy are associated with significant abdominal distension and this results in impairment of locomotor function (Fleuren et al., 2018).

Besides parasitism and predation, other factors could also be associated with the observed guppy body shape. Rounder bodies, due to their lower body surface-to-volume ratio, could help decrease the intake of pollutants through the skin (Jahn et al., 1997), but could also be associated with flow regime (Hendry et al., 2006) or food availability (Robinson and Wilson, 1995).

With respect to our finding that polluted populations produced larger offspring, a similar phenomenon has been documented in poeciliids inhabiting H2S-toxic habitats (Riesch et al., 2014, 2016; Tobler et al., 2018). Here, the lower body volume-to-surface area ratio of larger offspring is thought to help reduce toxin intake during the especially-vulnerable early life-stages (Cherr et al., 2017; Adzigbli and Yuewen, 2018; Serafin et al., 2019; see also the “bigger is better hypothesis”: Sogard, 1997). A similar mechanism might be at work in oil-polluted aquatic habitats (which contain at least traces of H2S: Driver and Freedman, 1993; Kayukova et al., 2016), but more research is needed on the exact mechanisms of when and how the other toxic compounds of oil enter the body of these fish.

***Population-specific guppy phenotypes***

Even though the presence of oil pollution had a significant effect of some of the phenotypic traits considered, many adult life-history traits were not significantly affected by oil pollution. At the same time, every measured phenotypic trait was characterised by significant variation between populations, independent of habitat type. This suggests that, within each habitat type, population-specific variation in multiple environmental factors, such as predation pressure and parasite load, plays a role in driving the observed patterns.

For instance, while guppies living in polluted environments had an overall larger body and offspring size than those in non-polluted habitats, guppies living in the Pitch Lake appear to represent an exception to this pattern: females from the Pitch Lake were the smallest among fish from all polluted habitats (Table S4), and offspring of Pitch Lake guppies were small compared to those from other polluted habitats, and smaller than all but two of the populations from non-polluted habitats (Table S4). Despite being a polluted habitat, *A. hartii*, *P. schomburgkii* (Schelkle et al., 2012), and multiple predatory birds (e.g., yellow-billed tern, *Sternula superciliaris*; black skimmer, *Rhyncops niger*; Santi et al., 2019) are present in the Pitch Lake alongside guppies, likely increasing the predation pressure experienced by Pitch Lake guppies. Future studies will need to better quantify population-specific predation and parasite pressure, as well as other habitat characteristics, in order to properly tease apart the relative contributions of oil pollution and of other environmental factors on phenotypic variation.

One limitation of our study was that we could not characterise exactly what chemicals guppies were exposed to in the different sampling sites. While previous studies have identified multiple types of polycyclic aromatic hydrocarbons as major contaminant, both in the oil fields and in the area of the Pitch Lake (Agard et al., 1993; Rolshausen et al., 2015; Hosein et al., 2018), we cannot exclude that other chemicals might be present at some of these sites, contributing to the high levels of population-specific phenotypic variation. Similarly, we do not know when our guppy populations were first subject to crude oil pollution, nor if some non-polluted sites might have been previously polluted. We hope that future research on this topic – whether focused on the Pitch Lake study system or elsewhere – can incorporate more detailed chemical analyses alongside the pollution history of sites, ideally paired with some long-term controlled experiments, in order to fill these gaps.

**Conclusions**

Our study provides the first in-depth investigation into the phenotypic responses associated with the colonisation of oil-polluted environments by guppies. While we found some evidence for direct, negative effects of crude oil (such as increased head size in polluted environments), together our results point towards indirect, positive, effects of pollution, potentially mediated by a reduction in predation or parasite pressures.

On an ecosystem level, our results demonstrate the extraordinary ability of guppies to exploit polluted and/or degraded environments to their advantage. Indeed, in Brazilian streams subject to heavy-metal pollution (Gomes-Silva et al., 2019) and in rivers in Rwanda subject to contamination from animal and domestic waste (Gomes-Silva et al., 2020), it has been shown that guppy abundances increase while other fish populations decline. Our findings expand our understanding of how guppies, and other organisms thriving in polluted environments, may be responding to the direct and indirect effects of pollution, and highlights the need for future studies to accurately characterize the many ecological dimensions of polluted environments such as these. Only armed with that knowledge will we be able to identify the selective forces acting upon organisms in these environments, and how certain species might be able to exploit them to their advantage despite the negative effects of pollution, or other human-induced environmental changes.

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**Data accessibility statement**

Data supporting this paper can be found on Figshare (doi: 10.17637/rh.14191589).

Currently, the data can be found via <https://figshare.com/s/658ab969e269079c206c>.

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**Tables**

**Table 1**. Multivariate general linear models (GLMs) on guppy phenotypic variation. (*a*) Male and female body shape, (*b*) adult life-history traits, and (*c*) offspring life-history traits. Significant effects are highlighted in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Factor | *F* | Degrees of freedom | *P* | Partial *η*2 |
| (*a*) Body shape | **Centroid size** | 14.893 | 5, 325 | **< 0.001** | 0.186 |
| **Sex** | 407.341 | 5, 325 | **< 0.001** | 0.862 |
| **Habitat type** | 12.632 | 5, 325 | **< 0.001** | 0.163 |
| **Population(habitat type)** | 7.081 | 45, 1457 | **< 0.001** | 0.162 |
| **Sex × habitat type** | 3.963 | 5, 325 | **0.002** | 0.057 |
| **Sex × population(habitat type)** | 2.730 | 45, 1457 | **< 0.001** | 0.070 |
| (*b*) Adult life-history traits | **SL** | 297.580 | 3, 317 | **< 0.001** | 0.738 |
| **Sex** | 69.188 | 3, 317 | **< 0.001** | 0.396 |
| Habitat type | 2.376 | 3, 317 | 0.070 | 0.022 |
| **Population(habitat type)** | 8.666 | 27, 927 | **< 0.001** | 0.197 |
| **Sex × population(habitat type)** | 2.786 | 3, 317 | **< 0.001** | 0.081 |
| **SL × population(habitat type)** | 2.269 | 30, 931 | **< 0.001** | 0.067 |
| (*c*) Offspring life-history traits | **SL** | 20.386 | 3, 126 | **< 0.001** | 0.327 |
| Embryo stage | 2.434 | 3, 126 | 0.068 | 0.055 |
| **Habitat type** | 3.752 | 3, 126 | **0.013** | 0.082 |
| **Population(habitat type)** | 6.387 | 27, 369 | **< 0.001** | 0.312 |
| SL × population(habitat type) | 1.472 | 30, 371 | 0.055 | 0.104 |

**Table 2**. *Post-hoc* univariate GLMs on guppy body shape variation. *α*-levels have been corrected for multiple comparisons with *α*’ = 0.010. Significant effects are highlighted in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Factor | Dependent variable | *F* | Degrees of freedom | *P* | Partial *η*2 |
| Centroid size | **RW1** | 38.196 | 1, 329 | **< 0.001** | 0.093 |
| RW2 | 0.317 | 1, 329 | 0.563 | 0.001 |
| **RW3** | 23.989 | 1, 329 | **< 0.001** | 0.068 |
| RW4 | 0.056 | 1, 329 | 0.828 | < 0.001 |
| **RW5** | 12.914 | 1, 329 | **< 0.001** | 0.037 |
| Sex | **RW1** | 1652.450 | 1, 329 | **< 0.001** | 0.834 |
| RW2 | 0.340 | 1, 329 | 0.574 | 0.001 |
| **RW3** | 19.521 | 1, 329 | **< 0.001** | 0.056 |
| RW4 | 0.003 | 1, 329 | 0.813 | < 0.001 |
| **RW5** | 16.899 | 1, 329 | **< 0.001** | 0.049 |
| Habitat type | **RW1** | 11.997 | 1, 329 | **0.001** | 0.035 |
| **RW2** | 24.042 | 1, 329 | **< 0.001** | 0.068 |
| RW3 | 5.196 | 1, 329 | 0.023 | 0.016 |
| **RW4** | 11.786 | 1, 329 | **0.001** | 0.035 |
| RW5 | 0.174 | 1, 329 | 0.677 | 0.001 |
| Population(habitat type) | **RW1** | 6.227 | 9, 329 | **< 0.001** | 0.146 |
| **RW2** | 11.621 | 9, 329 | **< 0.001** | 0.241 |
| **RW3** | 11.668 | 9, 329 | **< 0.001** | 0.242 |
| RW4 | 1.968 | 9, 329 | 0.042 | 0.051 |
| RW5 | 2.425 | 9, 329 | 0.011 | 0.062 |
| Sex × habitat type | RW1 | 1.521 | 1, 329 | 0.218 | 0.005 |
| **RW2** | 7.603 | 1, 329 | **0.006** | 0.023 |
| **RW3** | 10.069 | 1, 329 | **0.002** | 0.030 |
| RW4 | 0.031 | 1, 329 | 0.860 | < 0.001 |
| RW5 | 1.066 | 1, 329 | 0.303 | 0.003 |
| Sex × population(habitat type) | **RW1** | 3.169 | 9, 329 | **0.001** | 0.080 |
| **RW2** | 3.932 | 9, 329 | **< 0.001** | 0.097 |
| **RW3** | 4.211 | 9, 329 | **< 0.001** | 0.103 |
| RW4 | 1328 | 9, 329 | 0.221 | 0.035 |
| RW5 | 1.576 | 9, 329 | 0.121 | 0.041 |

**Table 3**. *Post-hoc* univariate GLMs on (*a*) adult and (*b*) offspring-related life-history trait variation. *α*-levels have been corrected for multiple comparisons with *α*’ = 0.017. Significant effects are highlighted in bold.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Factor | Dependent variable | *F* | Degrees of freedom | *P* | Partial *η*2 |
| (*a*) adult life-history traits | SL | **Lean mass** | 882.810 | 1, 319 | **< 0.001** | 0.735 |
| Fat content | 3.884 | 1, 319 | 0.050 | 0.012 |
| GSI/RA | 1.842 | 1, 319 | 0.176 | 0.006 |
| Sex | **Lean mass** | 29.091 | 1, 319 | **< 0.001** | 0.084 |
| Fat content | < 0.001 | 1, 319 | 0.994 | < 0.001 |
| **GSI/RA** | 167.847 | 1, 319 | **< 0.001** | 0.345 |
| Habitat type | Lean mass | 5.549 | 1, 319 | 0.020 | 0.017 |
| Fat content | 1.501 | 1, 319 | 0.221 | 0.005 |
| GSI/RA | 0.037 | 1, 319 | 0.848 | < 0.001 |
| Population(habitat type) | **Lean mass** | 17.090 | 9, 319 | **< 0.001** | 0.325 |
| Fat content | 1.948 | 9, 319 | 0.045 | 0.052 |
| **GSI/RA** | 8.151 | 9, 319 | **< 0.001** | 0.187 |
| Sex × population (habitat type) | **Lean mass** | 6.753 | 10, 319 | **< 0.001** | 0.175 |
| Fat content | 0.962 | 10, 319 | 0.477 | 0.029 |
| GSI/RA | 0.956 | 10, 319 | 0.482 | 0.029 |
| SL × population (habitat type) | **Lean mass** | 5.383 | 10, 319 | **< 0.001** | 0.144 |
| Fat content | 0.371 | 10, 319 | 0.959 | 0.011 |
| GSI/RA | 1.283 | 10, 319 | 0.239 | 0.039 |
| (*b*) offspring life-history traits | SL | **Fecundity** | 53.615 | 1, 128 | **< 0.001** | 0.295 |
| Embryo lean mass | 2.955 | 1, 128 | 0.088 | 0.023 |
| Embryo fat content | 0.007 | 1, 128 | 0.935 | < 0.001 |
| Embryo stage | Fecundity | 2.957 | 1, 128 | 0.088 | 0.023 |
| Embryo lean mass | 0.995 | 1, 128 | 0.320 | 0.008 |
| Embryo fat content | 4.026 | 1, 128 | 0.047 | 0.030 |
| Habitat type | Fecundity | 0.254 | 1, 128 | 0.615 | 0.002 |
| **Embryo lean mass** | 7.007 | 1, 128 | **0.009** | 0.052 |
| Embryo fat content | 2.006 | 1, 128 | 0.159 | 0.015 |
| Population(habitat type) | **Fecundity** | 10.564 | 9,128 | **< 0.001** | 0.426 |
| **Embryo lean mass** | 5.745 | 9,128 | **< 0.001** | 0.288 |
| **Embryo fat content** | 4.934 | 9,128 | **< 0.001** | 0.256 |
| SL × population (habitat type) | Fecundity | 2.076 | 10, 128 | 0.031 | 0.140 |
| Embryo lean mass | 1.591 | 10, 128 | 0.116 | 0.111 |
| Embryo fat content | 0.729 | 10, 128 | 0.696 | 0.054 |

**Figure legends**

**Figure 1**. Overview of guppy sampling sites in Trinidad. (*a*), (*e*) maps of Trinidad with the location of the 11 sampling sites. Red dots: polluted environments; blue dots: non-polluted environments. The Pitch Lake (site number 1) is represented by a red star. Populations 2, 3, and 8 are from the Vance River drainage, while populations 4 and 5 are from the Morne River drainage (see Rolshausen et al., 2015). (*b*) Example of oil-polluted environment (population 6); (*f*) example of non-polluted environment (population 9). (*c*) Male and (*d*) female guppy from a polluted environment (population 3). (*g*) Female guppy with the 15 landmarks used for body-shape analysis. Maps (*a*, *e*) were created with the R package Maps v. 3.30 (Becker et al., 2018). Photos by F. Santi (*b*, *f*), E. Vella (*c*, *d*) and K. Jeffress (*g*).

**Figure 2**. Phenotypic variation (mean ± SE) between populations of guppies living in polluted and non-polluted habitats. (*a*) Body-shape (RW2) variation; (*b*) sex-specific body shape variation; (*c*) sex-specific body size variation; (*d*) offspring lean mass variation. Red: polluted environments; blue: non-polluted environments; squares: females; triangles: males. Filled symbols represent the overall average (Avg.) across polluted and non-polluted habitats.

**Figures**



**Figure 1**.



**Figure 2**.