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

Francesco Santi, Emily Vella, Katherine Jeffress, Amy Deacon, Rüdiger Riesch

**Supplementary material**

***Environmental parameters measured during sampling***

During sampling, we recorded latitude and longitude of each habitat using a Garmin GPSMAP 64s (Garmin Ltd., Olathe, Kansas, USA) and documented the presence of other species in an opportunistic fashion. We also measured water temperature [ºC], dissolved oxygen [mg L-1], salinity [ppt] and pH using a Hach Rugged Field Kit (Hach, Loveland, Colorado, USA; Table S1). Preliminary screening did not reveal any significant influence of these abiotic factors on guppy phenotypes, so they were excluded from subsequent analyses.

***Extended methods for body-shape analyses***

We used geometric morphometrics (Zelditch et al. 2012) to analyse body-shape variation in 352 individuals (201 sexually mature males and 151 gravid females). We 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. All photos were collated into a .TPS file using tpsUtil32 V1.70 (Rohlf, 2016a), and 15 landmarks were added to each photo using tpsDig232 V2.26 (Rohlf, 2016b). Landmarks were as follows: (1) tip of the upper lip, (2) anterior, and (3) posterior insertion of the dorsal fin, (4) top, and (5) bottom of the caudal peduncle, (6) posterior, and (7) anterior insertion of the anal fin, (8) pelvic fin, (9) where the ventral end of the operculum meets the body, (10) dorsal end of the operculum, (11) anterior margin of the eye orbit, (12) centre of the eye, (13) posterior margin of the eye orbit, (14) dorsal, and (15) ventral insertion of the pectoral fin (following Santi et al., 2020). As some (*N* = 29) individuals became bent during fixation and preservation, we used the “unbend specimen” function in tpsUtil32, which employs quadratic regression to correct the bending effects. 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 from the final analysis (Santi et al., 2020).

We then performed a relative warp analysis (Zelditch et al., 2012) using tpsRelW32 V2.26 (Rohlf, 2016c). We obtained 5 relative warps (RWs) that described 90.95% of the total body-shape variation (Table S4). 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). We used these RWs as shape variables for all subsequent analyses, together with centroid size (i.e., the sum of the quadratic distances of each landmark from their centroid), which was used as a covariate in those analyses to control for body-size effects.

***Supplementary results and discussion: effect of oil pollution on female fecundity***

While we did not find an overall significant effect of habitat type on fecundity, visual inspection of the data suggested this might be largely driven by population 7, which shows very high fecundity (23.81 ± 1.26 developing embryos per female, Table S4). We do not know the reason for this (even though it could be linked to higher levels of predation, in particular by odonatan nymphs, experienced by population 7), nevertheless, after removing population 7 from the analysis, fish living in polluted environments had a significantly higher fecundity than those from non-polluted ones (ANOVA, *F*1, 116 = 26.57, *P* < 0.001). While that would agree with our *a priori* prediction 3, this is again surprising in the context of the classical life-history trade-off between offspring size and fecundity (Smith and Fretwell, 1974), given that guppies from oil polluted habitats also produced the largest offspring. The presence of “super” phenotypes, which are apparently counter to life-history theory, has been highlighted before, often in relation to further, previously undetected, selective pressures (Reznick et al., 2000). Similar to our study, Riesch et al. (2014) found both increased offspring size and increased fecundity in guppies living in toxic sulphide springs in Venezuela. Taking these two studies together, this suggests guppies are indeed able to decouple this trade-off under certain circumstances. In this context, the fact that guppies in oil-polluted habitats had deeper, rounder bodies, and that this effect was strongest in females, is also interesting. Hence, these patterns might be indicative of an attempt to increase available body cavity space in females, responding to increases in both offspring size and fecundity, rather than a response to an external environmental factor. Similar co-variation between body depth and fecundity/offspring size was recently also reported for *Gambusia hubbsi* from The Bahamas (Riesch et al., 2020). Future studies will be needed to further investigate these patterns.

***Supplementary Tables and Figures***

**Table S1**. Overview of abiotic environmental parameters measured for guppy sampling sites at the time of fish collection, together with observed other species. 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).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Habitat type | Population | Latitude [N] | Longitude [W] | Water temperature [°C] | Dissolved oxygen [mg/L] | Salinity [ppt] | pH | Other species observed |
| Polluted | 1 – Pitch Lake | 10.235 | -61.625 | 27.7 | 3.29 | 0.83 | 6.69 | *Anablepsoides hartii, Polycentrus schomburgkii*, *Rhyncops niger*, *Sternula superciliaris* |
| 2 – Vance River E | 10.199 | -61.630 | 29.7 | 2.71 | 0.00 | 6.56 | *A. hartii* |
| 3 – Vance River S | 10.196 | -61.633 | 27.8 | 2.09 | 1.30 | 7.07 | Freshwater shrimp (*Macrobrachium* sp.) |
| 4 – Dehli Road | 10.190 | -61.569 | 26.8 | 1.04 | 3.81 | 7.76 | *A. hartii*, *Macrobrachium* sp. |
| 5 – Forest Reserve | 10.170 | -61.567 | 26.1 | 0.73 | 3.42 | 7.77 | *A. hartii* |
| 6 – Point Fortin | 10.171 | -61.681 | 29.7 | 1.00 | 5.05 | 7.47 | *A. hartii* |
| Non-polluted | 7 – La Brea | 10.237 | -61.614 | 32.2 | 3.20 | 0.11 | 6.97 | Odonata nymphs |
| 8 – Vance River N | 10.202 | -61.633 | 26.5 | 1.22 | 0.20 | 6.64 | Odonata nymphs |
| 9 – Roussillac | 10.199 | -61.597 | 28.7 | 1.42 | 0.56 | 7.46 | *A. hartii*, *Astyanax bimaculatus*, *Ardea herodias* |
| 10 – Parrylands | 10.173 | -61.681 | 29.7 | 1.60 | 0.19 | 7.73 | *A. hartii*, *A. bimaculatus*, *Hypostomus robinii*, *P. schomburgkii*, *Ardea herodias*, Odonata nymphs |
| 11 – Arima | 10.689 | -61.290 | 26.7 | 6.90 | 0.15 | 8.01 | *A. bimaculatus*, *H. robinii*, *Ardea herodias*, Odonata nymphs |

**Table S2.** Relative Warps (RWs) used in the body-shape analysis. Reported are Eigenvalues and % of variance explained by each RW.

|  |  |  |  |
| --- | --- | --- | --- |
| RW | Eigenvalue | % Variance explained | Cumulative % var. explained |
| RW1 | 1.328 | 79.21 | 79.21 |
| RW2 | 0.297 | 3.98 | 83.19 |
| RW3 | 0.262 | 3.08 | 86.27 |
| RW4 | 0.246 | 2.72 | 88.98 |
| RW5 | 0.209 | 1.97 | 90.95 |

**Table S3**. Descriptive statistics (mean ± SE) of male *Poecilia reticulata* life-history traits.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Habitat type | Population | *N* | SL [mm] | Lean massa [g] | Fat contenta [%] | GSI [%] |
| Polluted | 1 – Pitch Lake | 21 | 17.0 ± 0.2 | 0.018 ± 0.001 | 2.67 ± 0.87 | 3.17 ± 0.23 |
| 2 – Vance River E | 14 | 14.9 ± 0.3 | 0.018 ± 0.001 | 1.11 ± 1.07 | 2.53 ± 0.12 |
| 3 – Vance River S | 24 | 16.9 ± 0.3 | 0.019 ± 0.001 | 2.80 ± 0.82 | 2.65 ± 0.11 |
| 4 – Dehli Road | 15 | 17.4 ± 0.3 | 0.021 ± 0.001 | 0.37 ± 1.05 | 2.49 ± 0.14 |
| 5 – Forest Reserve | 15 | 17.0 ± 0.3 | 0.023 ± 0.002 | 0.55 ± 1.03 | 2.19 ± 0.15 |
| 6 – Point Fortin | 18 | 15.0 ± 0.3 | 0.018 ± 0.001 | 4.66 ± 0.95 | 2.50 ± 0.20 |
| Total/Avg. | 107 | 16.4 ± 0.2 | 0.019 ± 0.001 | 2.03 ± 0.39 | 2.62 ± 0.07 |
| Non-polluted | 7 – La Brea | 24 | 16.0 ± 0.2 | 0.019 ± 0.001 | 4.73 ± 0.80 | 3.66 ± 0.20 |
| 8 – Vance River N | 26 | 15.9 ± 0.2 | 0.018 ± 0.001 | 3.26 ± 0.77 | 3.35 ± 0.20 |
| 9 – Roussillac | 15 | 14.9 ± 0.2 | 0.018 ± 0.001 | 3.33 ± 1.04 | 2.80 ± 0.12 |
| 10 – Parrylands | 15 | 13.9 ± 0.7 | 0.015 ± 0.001 | 5.23 ± 1.11 | 2.22 ± 0.17 |
| 11 – Arima | 14 | 17.3 ± 0.2 | 0.022 ± 0.001 | 0.46 ± 0.95 | 2.15 ± 0.13 |
| Total/Avg. | 94 | 15.6 ± 0.2 | 0.018 ± 0.001 | 3.38 ± 0.43 | 2.98 ± 0.10 |

a: estimated marginal means for SL = 16.1 mm.

**Table S4.** Descriptive statistics (mean ± SE) of female and offspring *Poecilia reticulata* life-history traits.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Habitat type | Population | *N* | SL [mm] | Lean mass [g]a | Fat content [%] | RA [%] | Fecunditya | Embryo lean mass [mg] | Estimated embryo mass at birth [mg]b | Embryo fat content [%] |
| Polluted | 1 – Pitch Lake | 15 | 21.4 ± 0.5 | 0.045 ± 0.002 | 4.15 ± 0.62 | 13.82 ± 1.12 | 10.50 ± 1.15 | 0.65 ± 0.04 | 0.56 | 7.91 ± 1.39 |
| 2 – Vance River E | 14 | 22.1 ± 0.3 | 0.053 ± 0.002 | 2.16 ± 0.44 | 14.72 ± 1.15 | 16.41 ± 1.16 | 0.54 ± 0.03 | 0.46 | 2.93 ± 0.55 |
| 3 – Vance River S | 14 | 24.5 ± 0.6 | 0.063 ± 0.002 | 5.03 ± 1.26 | 9.38 ± 0.77 | 7.95 ± 1.18 | 0.77 ± 0.05 | 0.57 | 6.22 ± 1.42 |
| 4 – Dehli Road | 11 | 25.3 ± 0.6 | 0.062 ± 0.003 | 4.27 ± 0.62 | 12.44 ± 1.12 | 9.05 ± 1.40 | 0.95 ± 0.05 | 0.87 | 2.50 ± 0.63 |
| 5 – Forest Reserve | 12 | 25.4 ± 0.4 | 0.068 ± 0.002 | 2.82 ± 0.37 | 9.57 ± 1.08 | 8.39 ± 1.30 | 0.83 ± 0.06 | 0.91 | 1.28 ± 0.46 |
| 6 – Point Fortin | 15 | 22.9 ± 0.4 | 0.065 ± 0.002 | 0.62 ± 0.26 | 13.92 ± 0.94 | 14.38 ± 1.13 | 0.74 ± 0.04 | 0.75 | 1.00 ± 0.38 |
| Total/Avg. | 81 | 23.4 ± 0.3 | 0.058 ± 0.001 | 3.12 ± 0.32 | 12.36 ± 0.47 | 11.19 ± 0.73 | 0.73 ± 0.02 | 0.69 | 4.45 ± 0.68 |
| Non-polluted | 7 – La Brea | 14 | 25.1 ± 0.6 | 0.061 ± 0.002 | 4.57 ± 1.12 | 17.84 ± 1.18 | 23.81 ± 1.26 | 0.63 ± 0.03 | 0.49 | 3.93 ± 1.35 |
| 8 – Vance River N | 15 | 21.1 ± 0.5 | 0.055 ± 0.002 | 4.65 ± 0.84 | 9.86 ± 0.85 | 8.55 ± 1.16 | 0.70 ± 0.03 | 0.72 | 9.20 ± 1.71 |
| 9 – Roussillac | 14 | 21.7 ± 0.5 | 0.056 ± 0.002 | 3.48 ± 0.61 | 11.88 ± 1.35 | 11.79 ± 1.17 | 0.68 ± 0.05 | 0.70 | 6.55 ± 1.65 |
| 10 – Parrylands | 13 | 18.3 ± 0.6 | 0.059 ± 0.003 | 2.23 ± 0.68 | 9.42 ± 0.75 | 12.07 ± 1.46 | 0.47 ± 0.02 | 0.54 | 1.83 ± 0.42 |
| 11 – Arima | 14 | 22.9 ± 0.4 | 0.061 ± 0.002 | 1.60 ± 0.42 | 7.18 ± 0.52 | 5.49 ± 1.16 | 0.82 ± 0.03 | 0.63 | 2.66 ± 0.65 |
| Total/Avg. | 70 | 21.9 ± 0.4 | 0.059 ± 0.001 | 3.34 ± 0.37 | 11.24 ± 0.61 | 12.42 ± 0.79 | 0.67 ± 0.02 | 0.62 | 4.92 ± 0.66 |

a: estimated marginal means for SL = 22.7 mm, and embryonic stage of development = 25.5.

b: calculated using the slope of the regression between log-transformed embryonic dry mass and stage of development.



**Figure S1.** Thin-plate spline visualizations of body-shape variation along the 5 Relative Warps (RWs) used in the analysis of guppy body shape.

***Sex-specific body-shape and life-history analyses***

***Male body-shape analysis***

**Table S5**. Relative Warps (RWs) used in the analysis of male body shape. Reported are Eigenvalues and % of variance explained by each RW.

|  |  |  |  |
| --- | --- | --- | --- |
| RW | Eigenvalue | % variance explained | Cumulative % var. explained |
| RW1 | 0.317 | 29.24 | 29.24 |
| RW2 | 0.211 | 12.91 | 42.16 |
| RW3 | 0.192 | 10.75 | 52.91 |
| RW4 | 0.178 | 9.17 | 62.07 |
| RW5 | 0.148 | 6.34 | 68.41 |
| RW6 | 0.144 | 6.02 | 74.43 |

**Table S6**. Multivariate general linear model (GLM) on male body-shape variation. Significant effects are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | *F* | Degrees of freedom | *P* | Partial η2 |
| **Centroid size** | 2.898 | 6, 187 | **0.010** | **0.085** |
| **Population(habitat type)** | 4.213 | 54, 958 | **< 0.001** | **0.166** |
| **Habitat type** | 4.749 | 6, 187 | **< 0.001** | **0.132** |

**Table S7**. *Post-hoc* univariate GLMs on male body-shape variation. *α*-levels were corrected for multiple comparisons, with *α*’ = 0.008. Significant effects are highlighted in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Factor | Dependent variable | *F* | Degrees of freedom | *P* | Partial η2 |
| Centroid size | **RW1** | 8.473 | 1, 192 | **0.004** | **0.042** |
| RW2 | 2.258 | 1, 192 | 0.135 | 0.012 |
| RW3 | 0.899 | 1, 192 | 0.344 | 0.005 |
| RW4 | 1.152 | 1, 192 | 0.285 | 0.006 |
| RW5 | 0.925 | 1, 192 | 0.337 | 0.005 |
| RW6 | 0.279 | 1, 192 | 0.598 | 0.001 |
| Population(habitat type) | **RW1** | 4.566 | 9, 192 | **< 0.001** | **0.176** |
| **RW2** | 8.922 | 9, 192 | **< 0.001** | **0.295** |
| **RW3** | 3.646 | 9, 192 | **< 0.001** | **0.146** |
| RW4 | 1.748 | 9, 192 | 0.081 | 0.076 |
| **RW5** | 4.094 | 9, 192 | **< 0.001** | **0.161** |
| RW6 | 1.298 | 9, 192 | 0.240 | 0.057 |
| Habitat type | RW1 | 3.742 | 1, 192 | 0.055 | 0.019 |
| **RW2** | 22.710 | 1, 192 | **< 0.001** | **0.106** |
| RW3 | 0.072 | 1, 192 | 0.788 | < 0.001 |
| RW4 | 0.295 | 1, 192 | 0.588 | 0.002 |
| RW5 | 0.152 | 1, 192 | 0.697 | 0.001 |
| RW6 | 0.170 | 1, 192 | 0.680 | 0.001 |

***Female body-shape analysis***

**Table S8**. Relative Warps (RWs) used in the analysis of female body shape. Reported are Eigenvalues and % of variance explained by each RW.

|  |  |  |  |
| --- | --- | --- | --- |
| RW | Eigenvalue | % variance explained | Cumulative % var. explained |
| RW1 | 0.263 | 28.45 | 28.45 |
| RW2 | 0.222 | 20.31 | 48.76 |
| RW3 | 0.149 | 9.09 | 57.86 |
| RW4 | 0.141 | 8.22 | 66.07 |
| RW5 | 0.116 | 5.58 | 71.65 |

**Table S9**. Multivariate general linear model (GLM) on female body-shape variation. Significant effects are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | *F* | Degrees of freedom | *P* | Partial η2 |
| **Centroid size** | 17.630 | 5, 141 | **< 0.001** | **0.385** |
| **Population(habitat type)** | 4.754 | 45, 634 | **< 0.001** | **0.229** |
| **Habitat type** | 9.582 | 5, 141 | **< 0.001** | **0.254** |

**Table S10**. *Post-hoc* univariate GLMs on female body-shape variation. *α*-levels were corrected for multiple comparisons, with *α*’ = 0.010. Significant effects are highlighted in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Factor | Dependent variable | *F* | Degrees of freedom | *P* | Partial η2 |
| Centroid size | **RW1** | 48.590 | 1, 145 | **< 0.001** | **0.251** |
| **RW2** | 15.183 | 1, 145 | **< 0.001** | **0.095** |
| **RW3** | 9.262 | 1, 145 | **0.003** | **0.060** |
| RW4 | 2.304 | 1, 145 | 0.131 | 0.016 |
| RW5 | 0.040 | 1, 145 | 0.841 | < 0.001 |
| Population(habitat type) | **RW1** | 10.343 | 9, 145 | **< 0.001** | **0.391** |
| **RW2** | 4.555 | 9, 145 | **< 0.001** | **0.220** |
| **RW3** | 2.863 | 9, 145 | **0.004** | **0.151** |
| **RW4** | 3.938 | 9, 145 | **< 0.001** | **0.196** |
| RW5 | 2.105 | 9, 145 | 0.033 | 0.116 |
| Habitat type | **RW1** | 22.306 | 1, 145 | **< 0.001** | **0.133** |
| **RW2** | 7.276 | 1, 145 | **0.008** | **0.048** |
| **RW3** | 9.212 | 1, 145 | **0.003** | **0.060** |
| RW4 | 2.184 | 1, 145 | 0.142 | 0.015 |
| **RW5** | 7.357 | 1, 145 | **0.007** | **0.048** |

***Male life-history analysis***

**Table S11**. Univariate GLM on male Standard Length (SL) variation. *S*ignificant effects are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | *F* | Degrees of freedom | *P* | Partial η2 |
| **Population(habitat type)** | 13.622 | 9, 190 | **< 0.001** | **0.392** |
| **Habitat type** | 17.463 | 1, 190 | **< 0.001** | **0.084** |

**Table S12**. Multivariate general linear model (GLM) on male life-history variation. Significant effects are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | *F* | Degrees of freedom | *P* | Partial η2 |
| **SL** | 129.951 | 3, 177 | **< 0.001** | **0.688** |
| **Population(habitat type)** | 8.120 | 27, 518 | **< 0.001** | **0.291** |
| **Habitat type** | 7.127 | 3, 177 | **< 0.001** | **0.108** |
| **SL × population(habitat type)** | 3.316 | 30, 520 | **< 0.001** | **0.157** |

**Table S13**. *Post-hoc* univariate GLMs on male life-history variation. *α*-levels were corrected for multiple comparisons, with *α*’ = 0.017. Significant effects are highlighted in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Factor | Dependent variable | *F* | Degrees of freedom | *P* | Partial η2 |
| SL | **Lean Weight** | 392.552 | 1, 179 | **< 0.001** | **0.687** |
| Fat content | 3.332 | 1, 179 | 0.070 | 0.018 |
| GSI | 0.057 | 1, 179 | 0.812 | < 0.001 |
| Population(habitat type) | **Lean Weight** | 20.044 | 9, 179 | **< 0.001** | **0.502** |
| Fat content | 1.644 | 9, 179 | 0.106 | 0.076 |
| **GSI** | 5.929 | 9, 179 | **< 0.001** | **0.230** |
| Habitat type | **Lean Weight** | 15.288 | 1, 179 | **< 0.001** | **0.079** |
| Fat content | 1.038 | 1, 179 | 0.310 | 0.006 |
| GSI | 4.690 | 1, 179 | 0.032 | 0.026 |
| SL × population(habitat type) | **Lean Weight** | 8.547 | 10, 179 | **< 0.001** | **0.323** |
| Fat content | 0.341 | 10, 179 | 0.969 | 0.019 |
| GSI | 2.098 | 10, 179 | 0.027 | 0.105 |

***Female life-history analysis***

**Table S14**. Univariate GLM on female Standard Length (SL) variation. *S*ignificant effects are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | *F* | Degrees of freedom | *P* | Partial η2 |
| **Population(habitat type)** | 14.060 | 9, 140 | **< 0.001** | **0.475** |
| **Habitat type** | 27.921 | 1, 140 | **< 0.001** | **0.166** |

**Table S15**. Multivariate general linear model (GLM) on female life-history variation. Significant effects are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Factor | *F* | Degrees of freedom | *P* | Partial η2 |
| **SL** | 58.915 | 6, 123 | **< 0.001** | **0.742** |
| Embryo stage | 1.480 | 6, 123 | 0.191 | 0.067 |
| **Population(habitat type)** | 6.073 | 52, 632 | **< 0.001** | **0.299** |
| Habitat type | 2.012 | 6, 123 | 0.069 | 0.089 |
| **SL ×** **population(habitat type)** | 2.978 | 60, 650 | **< 0.001** | **0.191** |

**Table S16**. *Post-hoc* univariate GLMs on female life-history variation. *α*-levels were corrected for multiple comparisons, with *α*’ = 0.008. Significant effects are highlighted in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Factor | Dependent variable | *F* | Degrees of freedom | *P* | Partial η2 |
| SL | **Lean weight** | 346.188 | 1, 128 | **< 0.001** | **0.730** |
| Fat content | 0.354 | 1, 128 | 0.553 | 0.003 |
| **Fecundity** | 53.615 | 1, 128 | **< 0.001** | **0.295** |
| Embryo lean weight | 2.955 | 1, 128 | 0.088 | 0.023 |
| Embryo fat content | 0.007 | 1, 128 | 0.935 | < 0.001 |
| RA | 2.566 | 1, 128 | 0.112 | 0.020 |
| Embryo stage | Lean weight | 0.506 | 1, 128 | 0.478 | 0.004 |
| Fat content | 0.033 | 1, 128 | 0.855 | < 0.001 |
| Fecundity | 2.957 | 1, 128 | 0.088 | 0.023 |
| Embryo lean weight | 0.995 | 1, 128 | 0.320 | 0.008 |
| Embryo fat content | 4.026 | 1, 128 | 0.047 | 0.030 |
| RA | 4.036 | 1, 128 | 0.047 | 0.031 |
| Population(habitat type) | **Lean weight** | 12.006 | 9, 128 | **< 0.001** | **0.458** |
| **Fat content** | 5.168 | 9, 128 | **< 0.001** | **0.267** |
| **Fecundity** | 10.564 | 9, 128 | **< 0.001** | **0.426** |
| **Embryo lean weight** | 5.745 | 9, 128 | **< 0.001** | **0.288** |
| **Embryo fat content** | 4.934 | 9, 128 | **< 0.001** | **0.258** |
| **RA** | 5.78 | 9, 128 | **< 0.001** | **0.289** |
| Habitat type | Lean weight | 0.003 | 1, 128 | 0.959 | < 0.001 |
| Fat content | 0.08 | 1, 128 | 0.777 | 0.001 |
| Fecundity | 0.254 | 1, 128 | 0.615 | 0.002 |
| Embryo lean weight | 7.007 | 1, 128 | 0.009 | 0.052 |
| Embryo fat content | 2.006 | 1, 128 | 0.159 | 0.015 |
| RA | 2.713 | 1, 128 | 0.102 | 0.021 |
| SL × population(habitat type) | **Lean weight** | 6.081 | 10, 128 | **< 0.001** | **0.322** |
| Fat content | 1.097 | 10, 128 | 0.370 | 0.079 |
| Fecundity | 2.076 | 10, 128 | 0.031 | 0.140 |
| Embryo lean weight | 1.591 | 10, 128 | 0.116 | 0.111 |
| Embryo fat content | 0.729 | 10, 128 | 0.696 | 0.054 |
| RA | 0.961 | 10, 128 | 0.480 | 0.070 |

**References**

Reznick, D. N., Nunney, L., Tessier, A., 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol. Evol. 15, 421–425. https://doi.org/10.1016/S0169-5347(00)01941-8

Riesch, R., Martin, R. A., Langerhans, R. B., 2020. Multiple traits and multifarious environments: integrated divergence of morphology and life history. Oikos 129, 480–492. https://doi.org/10.1111/oik.06344

Riesch, R., Plath, M., Schlupp, I., Tobler, M., Langerhans, R. B., 2014. Colonization of toxic environments drives predictable life-history evolution in livebearing fishes (Poeciliidae). Ecol. Lett. 17, 65–71. https://doi.org/10.1111/ele.12209

Rohlf, F. J., 2016a. tpsUtil32 version 1.70. <http://life.bio.sunysb.edu/morph/>. Accessed 8 November 2016

Rohlf, F. J., 2016b. tpsDig232 version 2.26. <http://life.bio.sunysb.edu/morph/>. Accessed 8 November 2016

Rohlf, F. J., 2016c. tpsRelw32 version 1.65. <http://life.bio.sunysb.edu/morph/>. Accessed 8 November 2016

Rolshausen, G., Phillip, D. A., Beckles, D. M., Akbari, A., Ghoshal, S., Hamilton, P. M., Tyler, C. R., Scarlett, A. G., Ramnarine, I., Bentzen P., Hendry, A. P., 2015. Do stressful conditions make adaptation difficult? Guppies in the oil-polluted environments of southern Trinidad. Evol. Appl. 8, 854–870. https://doi.org/10.1111/eva.12289

Santi, F., Petry, A. C., Plath, M., Riesch, R., 2020. Phenotypic differentiation in a heterogeneous environment: morphological and life-history responses to ecological gradients in a livebearing fish. J. Zool. 310, 10–23. https://doi.org/10.1111/jzo.12720

Smith, C. C., Fretwell, S. D., 1974. The optimal balance between size and number of offspring. Am. Nat. 108, 499–506.

Zelditch, M. L., Swiderski, D. L., Sheets, H. D. 2012. Geometric Morphometrics for Biologists: A Primer. Academic Press.