**Online Supplementary Material:**

Riesch et al.: Two ecological gradients drive phenotypic differentiation of a cave fish over few hundred meters

**Environmental Parameters**

**Table A1.** H2S-toxicity and light gradients (means ± SD) in the Cueva del Azufre (CdA) system. The light gradient was estimated in October 2021, using a Wintact WT81 Digital Lux Meter (Shenzen Wintact Electronics Co., Ltd.) and by taking 10 different measurements at each sampling site (with sampling points at least 1.5 m apart). Data on H2S-toxicity were extracted from Tobler *et al.* (2008, 2011); values from non-toxic habitat are often slightly negative, but were adjusted to 0.0 (± 0.0) for the current study.

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| Sampling site | Light intensity [Lux] | H2S concentration [µmol] |
| Arroyo Bonita (AB) | 82,418.2 ± 1,201.5 | 0.0 ± 0.0 |
| Arroyo Mexiquito (AM) | 80,790.9 ± 1,027.1 | 0.0 ± 0.0 |
| El Azufre (EA) | 55,545.5 ± 20,309.1 | 18.3±17.4 |
| Cueva del Azufre, chamber II (CdA II) | 0.0 ± 0.0 | 24.0±21.6 |
| Cueva del Azufre, chamber V (CdA V) | 23.9 ± 6.5 | 28.4±15.3 |
| Cueva del Azufre, chamber VIII (CdA VIII) | 8.5 ± 9.9 | 60.0 ± 0.0\* |
| Cueva del Azufre, chamber X (CdA X) | 0.0 ± 0.0 | 91.5±32.3 |

\* No toxicity data was available for CdA VIII. However, given that this chamber contains no sulfidic springs and is downstream of CdA X but upstream of CdA V, we assigned this chamber a value that is based on half of the difference in toxicity between CdA X and CdA V.

**Preliminary GLMs**

**Supplementary Methods**

For analysis of body shape, we employed multivariate analyses of covariance (MANCOVA) using the four RWs as dependent variables, ‘sex’, ‘habitat type’ (three levels: sulphide-free surface vs. sulphidic/turbid surface vs. cave) and ‘population-nested-within-habitat-type’ [henceforth ‘population(habitat type)’] as factors, and ‘centroid size’ as a covariate. Our initial model also included the interaction ‘sex × habitat type’, but this term was removed from the final model in a stepwise-backwards approach (*P* = 0.23)*.*

With respect to life histories, we first tested for differences in SL by means of two sex-specific ANOVAs that included the factors ‘habitat type’ and ‘population(habitat type)’. We then ran two sex-specific MANCOVAs on all remaining life-history traits that additionally included SL as a covariate as well as the interaction ‘SL × habitat type’; and, for females, ‘embryonic stage of development’ (scored after Riesch et al. 2011) was also included as a covariate.

**Supplementary Results**

The MANCOVA on body shape divergence among *N* = 169 individuals revealed significant effects of the factors ‘sex’, ‘habitat type’ and ‘population(habitat type)’, while ‘centroid size’ was not significant (Table A1a). By far the strongest effects were due to ‘sex’ (partial *η*2 = 0.96) and ‘habitat type’ (0.73). *Post-hoc* univariate models showed that sexes differed in RW1 (*P* < 0.001) and RW2 (*P* = 0.002), and at a tendency in RW3 (*P* = 0.064). Fish from different habitat types showed pronounced differences in RW2 and RW3 (both *P* < 0.001), and to a weaker extent also differed in RW1 (*P* = 0.039), while differences between populations within a given habitat type [i.e., population(habitat type)] were identified for RW2 (*P* = 0.011) and RW3 (*P* < 0.001).

Body size (SL) differed significantly between habitat types, with fish from EA and CdA being smaller than their counterparts from AB and AM (ANOVA, males: *F*2,77 = 18.66, *P* < 0.001; females: *F*2,80 = 33.30, *P* < 0.001). We detected no significant differences between populations within a given habitat type [‘population(habitat)’, males: *F*4,77 = 1.59, *P* = 0.19; females: *F*4,80 = 1.38, *P* = 0.25].

Our MANCOVA on male life histories revealed a strong and significant effect of the covariate ‘SL’ (partial *η*2 = 0.86), but males from different habitat types and different populations within a given habitat type also exhibited significant differences, and the interaction of ‘SL × habitat type’ was also significant (Table A1b). *Post-hoc* univariate models revealed that ‘SL’ had a significant effect on lean mass (*P* < 0.001), fat content (*P* = 0.019) and GSI (*P* = 0.014). Males from different habitat types differed significantly in lean mass and GSI (*P* < 0.001 and *P* = 0.004, respectively) while males from different populations within a given habitat type differed significantly in fat content (*P* = 0.001) and GSI (*P* = 0.027).

In the MANCOVA on female life histories we found significant effects of both covariates (‘SL’ and ‘embryonic stage of development’), but females from different habitat types and different populations within a given habitat type also showed significant differences (Table A1c). Furthermore, we found a significant effect of the interaction ‘SL × habitat type’, suggesting different relationships between body size and life-history traits across habitat types (Table A1c). Again, the strongest effect was due to the covariate ‘SL’ (partial *η*2 = 0.95), and *post-hoc* univariate models revealed that female lean mass (*P* < 0.001), fecundity (*P* < 0.001), embryo lean mass (*P* = 0.019) and embryo fat content (*P* = 0.018) increased with female body size. ‘Embryonic stage of development’ significantly influenced embryo lean mass (*P* = 0.011) and embryo fat content (*P* < 0.001), and had a marginal, but non-significant, effect on RA (*P* = 0.059). Females from different habitat types differed significantly in female lean mass, fecundity and embryo lean mass (all *P* < 0.001), while the ‘population(habitat type)’-effect was significant for female lean mass (*P* = 0.010), female and embryo fat contents, as well as RA (all *P* < 0.001), and marginally non-significant for fecundity (*P* = 0.066). The interaction ‘SL × habitat type’ was significant for fecundity (*P* < 0.001) and embryo fat content (*P* = 0.030).

**References**

Riesch R, Schlupp I, Langerhans RB, Plath M (2011) Shared and unique patterns of embryo development in extremophile poeciliids. *PLoS ONE* **6**: e27377.

**Table A2.** Results of nested multivariate analyses of covariance (MANCOVA) examining body-shape and life-history variation of *Poecilia mexicana* from three different habitat types (non-sulfidic surface, sulfidic surface, and sulfidic cave). *F*-ratios were approximated using Wilks’ values, partial variance was estimated using Wilks’ partial *η*2, and terms with the highest partial variance as well as those at least half as strong as the strongest effect are given in bold for each model.

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| --- | --- | --- | --- | --- | --- |
| Effect | *F* | df | *P* | Partial variance | Relative variance |
| *(a) Body shape of wild-caught fish* |
| Covariate: Centroid size | 1.982 | 4, 157 | 0.100 | 0.048 | 0.050 |
| **Sex** | **839.206** | **4, 157** | **< 0.001** | **0.955** | **1.000** |
| **Habitat type** | **79.830** | **8, 314** | **< 0.001** | **0.670** | **0.702** |
| Population(Habitat type) | 4.154 | 16, 480 | < 0.001 | 0.094 | 0.098 |
|  |  |  |  |  |  |
| *(b) Male life histories* |
| **Covariate: SL** | **146.777** | **3, 72** | **< 0.001** | **0.859** | **1.000** |
| Habitat type | 13.312 | 6, 144 | < 0.001 | 0.357 | 0.416 |
| Population(Habitat type) | 3.284 | 12, 191 | < 0.001 | 0.153 | 0.178 |
| SL × Habitat type | 2.808 | 6, 144 | 0.013 | 0.105 | 0.122 |
|  |
| *(c) Female life histories* |
| **Covariate: SL**  | **213.143** | **6, 71** | **< 0.001** | **0.947** | **1.000** |
| **Covariate: Stage** | **14.065** | **6, 71** | **< 0.001** | **0.543** | **0.573** |
| **Habitat type** | **17.177** | **12, 142** | **< 0.001** | **0.592** | **0.625** |
| Population(Habitat type) | 5.198 | 24, 249 | < 0.001 | 0.298 | 0.315 |
| SL × Habitat type | 6.474 | 12, 142 | < 0.001 | 0.354 | 0.374 |
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