**Supplementary material**

**A century later: adaptive plasticity and rapid evolution contribute to geographic variation in invasive mosquitofish**

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**Online Supplement 1**

**Sampling sites and environmental parameters**

**Table S1**. Overview of climatic and environmental factors used to characterise sites at which invasive *G. holbrooki* were sampled. For the mean, maximum, and minimum temperatures, values represent averages over 120 days (± SD) preceding sampling. In bold populations that were sampled in both 2013 and 2017.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site number |  | Population name and code | Year | Latitude | Longitude | Distance from sea [m] | Dissolved oxygen [mg L-1] | Conductivity [µS cm-1] | Water temp. [°C] | Average mean temp. [°C] | Average maximum temp. [°C] | Average minimum temp. [°C] | Rainfall [mm] (days) |
| 1 | Italy | Lago di Garda - LdG | 2013 | 45.4605 | 10.6113 | 136,892 | 1.54 | 560 | 17.0 | 10.33 ± 5.69 | 14.86 ± 6.56 | 6.39 ± 5.17 | 459 (58) |
| 2 | Italy | Comacchio – Com | 2013 | 44.6908 | 12.1866 | 5,188 | 8.24 | 8,970 | 20.7 | 10.56 ± 5.33 | 14.48 ± 5.83 | 7.06 ± 5.11 | 477 (54) |
| 3 | Italy | Lago di Bolsena – LdB | 2013 | 42.6314 | 11.9944 | 48,103 | 6.33 | 308 | 16.7 | 9.97 ± 4.58 | 13.73 ± 4.95 | 6.67 ± 4.56 | 329 (43) |
| 4 | Italy | Barletta – Blt | 2013 | 41.3025 | 16.3526 | 403 | 4.01 | 6,850 | 19.5 | 13.62 ± 4.22 | 16.61 ± 4.47 | 11.17 ± 4.21 | 261 (40) |
| 5 | **Italy** | **Torre Castiglione - TCa** | 2013 | 40.2890 | 17.8234 | 229 | 6.05 | 12,650 | 19.0 | 13.80 ± 4.23 | 16.89 ± 4.61 | 10.79 ± 4.07 | 217 (44) |
| 6 | **Italy** | **Porto Cesareo – PCe** | 2013 | 40.2752 | 17.8770 | 356 | 11.41 | 16,520 | 23.0 | 13.92 ± 4.29 | 17.07 ± 4.66 | 10.86 ± 4.07 | 218 (44) |
| 7 | France | Briere – Bri | 2013 | 47.3697 | -2.3133 | 12,310 | 9.00 | 430 | 18.9 | 18.04 ± 3.45 | 22.97 ± 4.29 | 13.25 ± 3.30 | 157 (38) |
| 8 | France | La Ligneron – LLi | 2013 | 46.7511 | -1.9166 | 7,664 | 9.56 | 1,196 | 20.7 | 18.41 ± 3.56 | 23.04 ± 4.38 | 13.94 ± 3.29 | 143 (35) |
| 9 | France | La Charente – LCh | 2013 | 45.9824 | -0.9242 | 10,135 | 9.63 | 611 | 19.6 | 18.86 ± 3.83 | 23.40 ± 4.84 | 14.53 ± 3.38 | 195 (30) |
| 10 | France | Garonne – Gar | 2013 | 45.0237 | -0.5028 | 54,674 | 6.98 | 1,849 | 20.4 | 19.63 ± 4.27 | 25.12 ± 5.53 | 14.45 ± 3.57 | 282 (38) |
| 11 | France | Avignon – Avi | 2013 | 43.7911 | 4.7547 | 42,719 | 3.28 | 458 | 20.0 | 21.48 ± 4.45 | 27.66 ± 5.25 | 15.38 ± 4.02 | 117 (17) |
| 12 | France | Arles – Arl | 2013 | 43.6378 | 4.5549 | 20,623 | 10.10 | 560 | 21.6 | 22.12 ± 4.32 | 27.97 ± 4.96 | 16.37 ± 4.00 | 94 (16) |
| 13 | France | Montpellier – Mon | 2013 | 43.5603 | 4.0298 | 1,731 | 8.10 | 47,100 | 25.0 | 21.79 ± 3.84 | 26.83 ± 4.24 | 16.80 ± 3.80 | 83 (14) |
| 14 | Spain | Zadorra – Zad | 2013 | 42.8337 | -2.7828 | 6,346 | 6.32 | 666 | 18.8 | 17.05 ± 4.70 | 24.13 ± 6.65 | 10.77 ± 3.96 | 180 (32) |
| 15 | Spain | Rio Ter – RTe | 2013 | 42.0450 | 3.1744 | 1,874 | 6.51 | 890 | 23.5 | 20.58 ± 3.80 | 26.63 ± 4.34 | 14.67 ± 3.41 | 129 (23) |
| 16 | Spain | Barcelona – Bcn | 2013 | 41.2628 | 1.6399 | 8,475 | 9.86 | 1,201 | 24.9 | 21.14 ± 3.80 | 26.70 ± 4.07 | 15.94 ± 3.75 | 114 (17) |
| 17 | Spain | Ebro Delta – EDe | 2013 | 40.7072 | 0.5943 | 10,257 | 13.33 | 1,380 | 25.4 | 22.37 ± 3.54 | 27.63 ± 3.89 | 17.56 ± 3.46 | 116 (12) |
| 18 | **Spain** | **Sagunt – Sag** | 2013 | 39.7286 | -0.2074 | 2281 | 4.21 | 1,884 | 23.9 | 23.11 ± 3.47 | 27.76 ± 3.51 | 18.52 ± 3.59 | 58 (11) |
| 19 | Spain | Rio Júcar S – RJS | 2013 | 39.1532 | -0.2448 | 491 | 7.75 | 1,443 | 25.1 | 23.46 ± 3.21 | 27.64 ± 3.25 | 19.28 ± 3.39 | 54 (11) |
| 20 | Spain | Rio Segura – RSe | 2013 | 38.1226 | -0.6965 | 5,299 | 6.26 | 2,400 | 24.0 | 23.64 ± 3.35 | 28.56 ± 3.58 | 19.10 ± 3.49 | 35 (5) |
| 21 | Spain | Almanzora – Alm | 2013 | 37.3138 | -1.8924 | 13,846 | 11.06 | 2,820 | 28.2 | 23.07 ± 3.82 | 28.47 ± 4.29 | 18.06 ± 3.43 | 32 (6) |
| 22 | **Spain** | **Guadalquivir – Gdq** | 2013 | 36.9378 | -6.0975 | 31,258 | 0.00 | 16,300 | 32.0 | 24.67 ± 4.02 | 32.54 ± 5.42 | 16.94 ± 3.22 | 12 (4) |
| 23 | Spain | Guadiaro – Gdr | 2013 | 36.3402 | -5.3148 | 6,604 | 8.45 | 734 | 23.7 | 22.51 ± 3.21 | 26.79 ± 3.54 | 18.15 ± 3.10 | 18 (5) |
| 24 | Italy | Lago di Fimon W – LFW | 2017 | 45.4708 | 11.5408 | 63,070 | 12.15 | 178 | 26.1 | 19.55 ± 5.17 | 25.29 ± 5.48 | 14.17 ± 4.74 | 391 (35) |
| 25 | Italy | Lago di Fimon S – LFS | 2017 | 45.4635 | 11.5427 | 62,750 | 5.77 | 275 | 23.8 | 19.55 ± 5.17 | 25.29 ± 5.48 | 14.17 ± 4.74 | 391 (35) |
| 26 | Italy | Marina Grosseto – MGr | 2017 | 42.7334 | 10.9652 | 136 | 7.26 | 4,020 | 24.5 | 21.30 ± 4.84 | 26.50 ± 5.00 | 16.23 ± 4.81 | 35 (8) |
| 27 | Italy | Grosseto – Gro | 2017 | 42.7335 | 11.0413 | 5,310 | 3.72 | 1,583 | 23.7 | 21.39 ± 4.85 | 26.63 ± 5.04 | 16.28 ± 4.82 | 30 (6) |
| 5 | **Italy** | **Torre Castiglione – TCa** | 2017 | 40.2888 | 17.8235 | 229 | 6.55 | 1,004 | 21.7 | 21.24 ± 5.06 | 24.79 ± 5.32 | 17.86 ± 4.86 | 73 (13) |
| 6 | **Italy** | **Porto Cesareo – PCe** | 2017 | 40.2752 | 17.8769 | 356 | 13.70 | 1,287 | 30.7 | 21.66 ± 5.04 | 25.26 ± 5.27 | 18.20 ± 4.83 | 64 (11) |
| 28 | Spain | Gualta – Glt | 2017 | 42.0311 | 3.1032 | 7,520 | 10.49 | 587 | 28.1 | 19.95 ± 4.63 | 25.98 ± 4.68 | 13.48 ± 5.08 | 79 (14) |
| 29 | Spain | Tortosa – Tor | 2017 | 40.8078 | 0.5172 | 15,390 | 5.83 | 1,276 | 27.1 | 21.63 ± 4.73 | 28.13 ± 4.87 | 15.77 ± 5.03 | 73 (12) |
| 30 | Spain | L'Estanyol – Est | 2017 | 39.7752 | -0.1522 | 235 | 5.95 | 4,050 | 24.8 | 22.40 ± 4.57 | 27.36 ± 4.57 | 17.60 ± 4.75 | 45 (6) |
| 18 | **Spain** | **Sagunt – Sag** | 2017 | 39.7308 | -0.2080 | 2,281 | 18.00 | 1,369 | 28.1 | 22.40 ± 4.57 | 27.36 ± 4.57 | 17.60 ± 4.75 | 45 (6) |
| 31 | Spain | El Palmar – EPa | 2017 | 39.3116 | -0.3205 | 2,150 | 2.53 | 1,628 | 28.0 | 22.66 ± 4.43 | 27.26 ± 4.44 | 18.10 ± 4.62 | 54 (10) |
| 32 | Spain | Rio Júcar N – RJN | 2017 | 39.1775 | -0.2692 | 2,620 | 17.48 | 1,082 | 29.3 | 22.62 ± 4.38 | 27.16 ± 4.33 | 18.17 ± 4.56 | 36 (6) |
| 33 | Spain | Rio Vaca – RVa | 2017 | 39.0606 | -0.2183 | 1,440 | 4.71 | 1,514 | 30.7 | 22.07 ± 4.44 | 26.74 ± 4.40 | 17.61 ± 4.57 | 36 (7) |
| 34 | Spain | Lebrija – Leb | 2017 | 36.9601 | -6.0645 | 33,830 | 3.07 | 7,260 | 22.6 | 24.13 ± 4.01 | 31.99 ± 5.46 | 16.36 ± 3.26 | 93 (8) |
| 22 | **Spain** | **Guadalquivir – Gdq** | 2017 | 36.9379 | -6.0974 | 31,258 | 7.64 | 5,630 | 21.3 | 24.13 ± 4.01 | 31.99 ± 5.46 | 16.36 ± 3.26 | 93 (8) |
| 35 | Spain | Doñana N – DoN | 2017 | 37.2260 | -6.1412 | 43,650 | 4.65 | 2,970 | 25.5 | 24.67 ± 4.20 | 32.22 ± 5.57 | 17.12 ± 3.33 | 86 (7) |
| 36 | Spain | Doñana W – DoW | 2017 | 37.2017 | -6.2618 | 34,110 | 6.28 | 2,650 | 26.7 | 24.51 ± 4.15 | 31.93 ± 5.48 | 17.03 ± 3.30 | 86 (7) |

**Table S2**. Principal component analysis (PCA) with Varimax rotation on latitude, longitude, temperature data and environmental parameters measured during sampling. For each principal component we present eigenvalues, % of variance explained and axis loadings for all variables included. Variables with axis loadings > 0.600 are highlighted in bold.

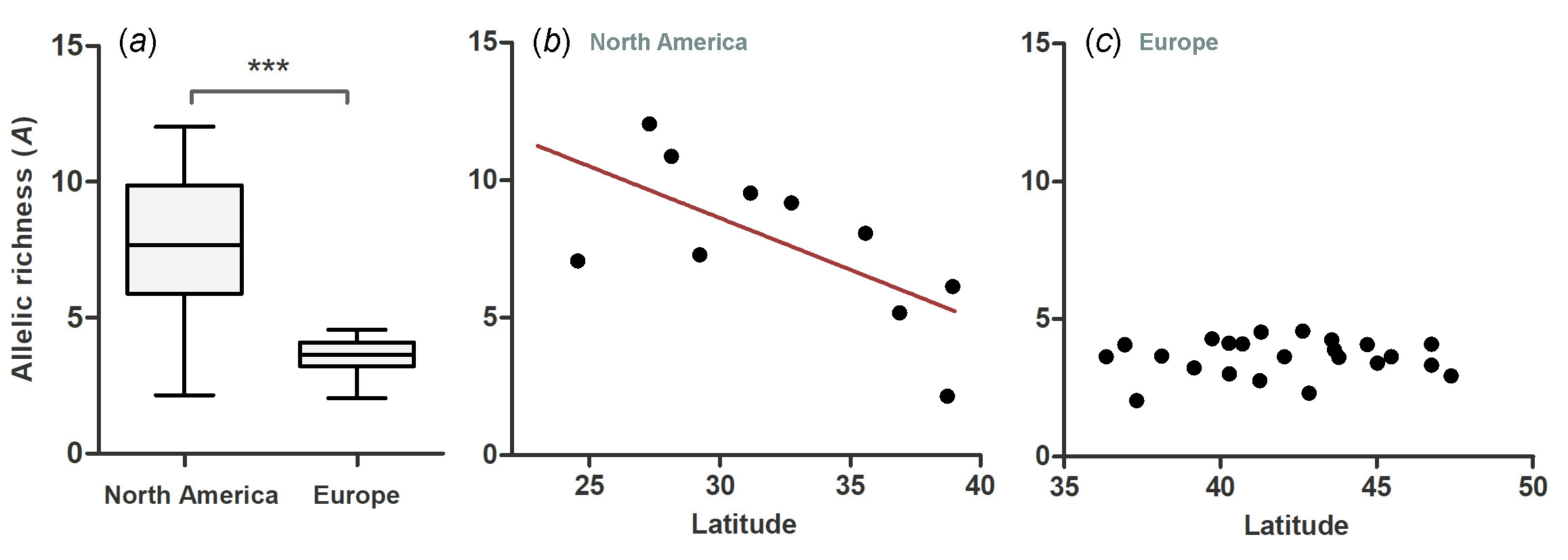
|  |  |  |  |
| --- | --- | --- | --- |
| Principal component | EPC1 | EPC2 | EPC3 |
| Eigenvalue | 5.048 | 1.569 | 1.183 |
| % var. explained | 48.76 | 15.69 | 11.83 |
| Cumulative % var. explained | 48.76 | 62.45 | 74.28 |
| Latitude | **-0.690** | 0.034 | 0.265 |
| Longitude | **-0.628** | 0.479 | -0.086 |
| Dissolved oxygen | 0.122 | **0.660** | 0.578 |
| Conductivity | -0.002 | 0.305 | **-0.823** |
| Water temperature | **0.741** | 0.223 | 0.026 |
| Distance from sea | -0.440 | **-0.715** | 0.105 |
| Mean temperature | **0.957** | -0.155 | 0.055 |
| Maximum temperature | **0.909** | -0.298 | 0.039 |
| Minimum temperature | **0.936** | 0.053 | 0.081 |
| Rainfall | **-0.897** | -0.150 | 0.090 |

**Online Supplement 2**

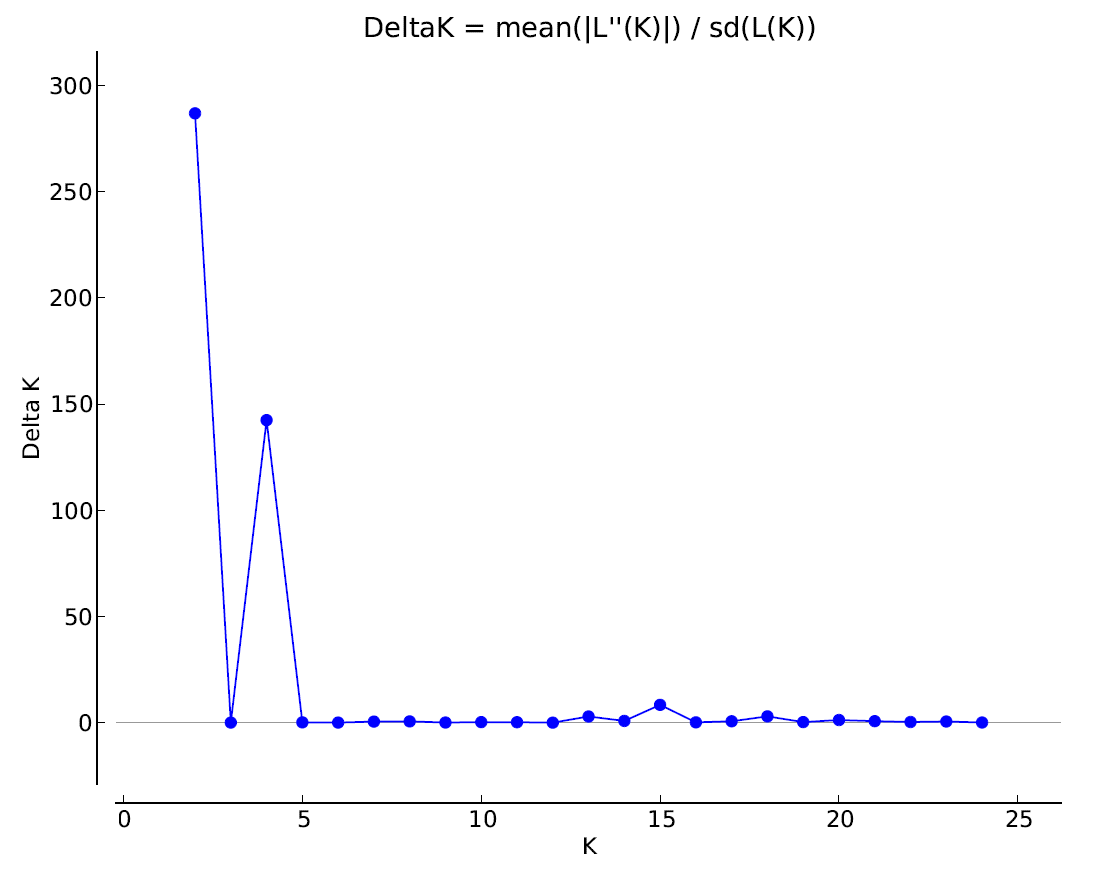
**Additional population genetic results**

We used Arlequin v 3.5 (Excoffier and Lischer, 2010) to calculate pairwise *F*ST-values between populations, as well as expected (*H*E) and observed heterozygosities (*H*O). FSTAT v 2.9.3 (Goudet, 2001) was used to calculate allelic richness (*A*). Estimated null allele frequencies (*R*) and estimation of FIS (inbreeding coefficient) values were assessed using Genepop software version 4.7.3 (Rousset, 2019). We compared genetic richness (*A*) between North American and European samples using a two-samples *t*-test. To explore potential footprints of latitude on the population genetic structure (Riesch et al., 2018), we tested for a correlation between allelic richness (*A*) and latitude by means of a Pearson correlation.

Allelic richness (*A*) was significantly reduced in European populations compared to populations in their native range (*t*31 = 6.614, *P* < 0.001; Fig. S1*a*). While populations in North America showed a strong decline in allelic richness (*A*) across latitude (Pearson’s *r* = -0.66, *P* = 0.038; reanalysed from Riesch et al., 2018; Fig. S1*b*), we could not find such a pattern in the genetically impoverished European populations (*r* = -0.58, *P* = 0.792; Fig. S1*c*). Descriptive statistics for site-specific means of standard indicators of genetic variability are provided in Table S4.



**Figure S1.** (*a*) Allelic richness (*A*) differed between native and invasive mosquitofish populations (two-samples *t*-test; *P* < 0.001), (*b*) varied with latitude in native (North American) populations (Pearson’s *r* = -0.66, *P* = 0.038), (*c*) but did not vary with latitude in invasive (European) ones (*r* = 0.06, *P* = 0.792).



**Figure S2**. Bayesian inference of the number of genetically distinct clusters (*K*) among the 33 genotyped populations using Δ*K* (Evanno et al., 2005).

**Table S3**. Combined Nei’s and pair-wise *F*ST distance matrix between native (North American; Riesch et al., 2018) and invasive (European) *G. holbrooki* populations. *F*ST-values are reported in the upper half of the table (grey background), whereas Nei’s distances are reported in the lower half of the table (white background).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site number | NJ | DE | VA | NC | SC | GA | FLDB | FLME | FLPL | FLZT | **LdG** | **Com** | **LdB** | **Blt** | **TCa** | **PCe** | **Bri** | **LLi** | **LCh** | **Gar** | **Avi** | **Arl** | **Mon** | **Zad** | **RTe** | **Bcn** | **EDe** | **Sag** | **RJS** | **RSe** | **Alm** | **Gdq** | **Gdr** |
| NJ | - | 0.329 | 0.172 | 0.122 | 0.216 | 0.21 | 0.242 | 0.168 | 0.191 | 0.325 | 0.296 | 0.218 | 0.227 | 0.210 | 0.294 | 0.257 | 0.306 | 0.300 | 0.231 | 0.316 | 0.234 | 0.287 | 0.257 | 0.354 | 0.242 | 0.281 | 0.217 | 0.209 | 0.287 | 0.258 | 0.441 | 0.258 | 0.257 |
| DE | 0.395 | - | 0.360 | 0.348 | 0.461 | 0.444 | 0.444 | 0.397 | 0.409 | 0.547 | 0.484 | 0.383 | 0.389 | 0.368 | 0.492 | 0.449 | 0.437 | 0.485 | 0.410 | 0.467 | 0.394 | 0.457 | 0.417 | 0.553 | 0.404 | 0.473 | 0.411 | 0.334 | 0.428 | 0.388 | 0.623 | 0.424 | 0.410 |
| VA | 0.412 | 0.429 | - | 0.125 | 0.252 | 0.250 | 0.263 | 0.224 | 0.230 | 0.355 | 0.317 | 0.213 | 0.219 | 0.194 | 0.306 | 0.269 | 0.279 | 0.313 | 0.225 | 0.261 | 0.199 | 0.278 | 0.251 | 0.377 | 0.233 | 0.279 | 0.233 | 0.189 | 0.239 | 0.202 | 0.443 | 0.239 | 0.235 |
| NC | 0.337 | 0.460 | 0.344 | - | 0.149 | 0.142 | 0.169 | 0.128 | 0.124 | 0.250 | 0.174 | 0.111 | 0.101 | 0.092 | 0.205 | 0.138 | 0.206 | 0.200 | 0.128 | 0.179 | 0.128 | 0.153 | 0.157 | 0.249 | 0.158 | 0.175 | 0.089 | 0.114 | 0.181 | 0.134 | 0.348 | 0.134 | 0.155 |
| SC | 0.526 | 0.701 | 0.565 | 0.438 | - | 0.065 | 0.126 | 0.120 | 0.069 | 0.202 | 0.244 | 0.218 | 0.206 | 0.199 | 0.281 | 0.235 | 0.278 | 0.255 | 0.224 | 0.274 | 0.229 | 0.243 | 0.236 | 0.309 | 0.257 | 0.290 | 0.208 | 0.254 | 0.319 | 0.290 | 0.415 | 0.233 | 0.267 |
| GA | 0.558 | 0.735 | 0.658 | 0.53 | 0.380 | - | 0.117 | 0.096 | 0.06 | 0.180 | 0.241 | 0.211 | 0.192 | 0.200 | 0.275 | 0.232 | 0.270 | 0.245 | 0.230 | 0.273 | 0.237 | 0.244 | 0.233 | 0.305 | 0.252 | 0.280 | 0.206 | 0.246 | 0.307 | 0.281 | 0.400 | 0.236 | 0.266 |
| FLDB | 0.554 | 0.701 | 0.615 | 0.50 | 0.456 | 0.447 | - | 0.08 | 0.056 | 0.162 | 0.263 | 0.227 | 0.216 | 0.209 | 0.279 | 0.242 | 0.277 | 0.266 | 0.230 | 0.264 | 0.242 | 0.238 | 0.203 | 0.332 | 0.259 | 0.297 | 0.214 | 0.250 | 0.306 | 0.276 | 0.400 | 0.231 | 0.261 |
| FLME | 0.450 | 0.655 | 0.563 | 0.439 | 0.425 | 0.404 | 0.372 | - | 0.035 | 0.149 | 0.244 | 0.206 | 0.198 | 0.190 | 0.260 | 0.222 | 0.272 | 0.261 | 0.204 | 0.252 | 0.224 | 0.228 | 0.195 | 0.332 | 0.248 | 0.288 | 0.180 | 0.222 | 0.266 | 0.246 | 0.385 | 0.226 | 0.245 |
| FLPL | 0.483 | 0.653 | 0.577 | 0.419 | 0.361 | 0.371 | 0.333 | 0.263 | - | 0.163 | 0.221 | 0.192 | 0.178 | 0.177 | 0.247 | 0.203 | 0.256 | 0.234 | 0.195 | 0.236 | 0.213 | 0.209 | 0.194 | 0.311 | 0.239 | 0.277 | 0.179 | 0.221 | 0.269 | 0.247 | 0.380 | 0.213 | 0.240 |
| FLZT | 0.678 | 0.831 | 0.725 | 0.620 | 0.516 | 0.493 | 0.502 | 0.462 | 0.511 | - | 0.349 | 0.321 | 0.309 | 0.319 | 0.397 | 0.353 | 0.389 | 0.371 | 0.338 | 0.369 | 0.349 | 0.360 | 0.303 | 0.416 | 0.369 | 0.393 | 0.318 | 0.356 | 0.410 | 0.383 | 0.503 | 0.341 | 0.372 |
| **LdG** | 0.523 | 0.510 | 0.501 | 0.345 | 0.547 | 0.608 | 0.576 | 0.543 | 0.544 | 0.699 | - | 0.088 | 0.083 | 0.074 | 0.227 | 0.094 | 0.250 | 0.143 | 0.133 | 0.191 | 0.149 | 0.120 | 0.145 | 0.254 | 0.210 | 0.258 | 0.159 | 0.174 | 0.245 | 0.241 | 0.393 | 0.230 | 0.214 |
| **Com** | 0.482 | 0.445 | 0.430 | 0.295 | 0.537 | 0.612 | 0.574 | 0.530 | 0.535 | 0.705 | 0.139 | - | 0.047 | 0.032 | 0.187 | 0.112 | 0.162 | 0.150 | 0.086 | 0.150 | 0.067 | 0.109 | 0.073 | 0.217 | 0.123 | 0.153 | 0.095 | 0.078 | 0.163 | 0.139 | 0.307 | 0.098 | 0.112 |
| **LdB** | 0.482 | 0.473 | 0.440 | 0.250 | 0.555 | 0.591 | 0.565 | 0.548 | 0.532 | 0.711 | 0.170 | 0.135 | - | 0.022 | 0.149 | 0.069 | 0.142 | 0.085 | 0.082 | 0.120 | 0.089 | 0.086 | 0.089 | 0.198 | 0.119 | 0.160 | 0.102 | 0.094 | 0.150 | 0.139 | 0.316 | 0.128 | 0.129 |
| **Blt** | 0.454 | 0.426 | 0.402 | 0.247 | 0.511 | 0.601 | 0.547 | 0.523 | 0.528 | 0.715 | 0.136 | 0.103 | 0.096 | - | 0.128 | 0.059 | 0.137 | 0.096 | 0.042 | 0.100 | 0.037 | 0.061 | 0.061 | 0.211 | 0.107 | 0.160 | 0.076 | 0.077 | 0.142 | 0.128 | 0.308 | 0.096 | 0.099 |
| **TCa** | 0.460 | 0.454 | 0.430 | 0.335 | 0.536 | 0.588 | 0.548 | 0.551 | 0.548 | 0.700 | 0.272 | 0.247 | 0.199 | 0.183 | - | 0.082 | 0.283 | 0.173 | 0.140 | 0.273 | 0.182 | 0.192 | 0.209 | 0.335 | 0.212 | 0.264 | 0.173 | 0.195 | 0.270 | 0.231 | 0.447 | 0.228 | 0.233 |
| **PCe** | 0.445 | 0.467 | 0.409 | 0.276 | 0.539 | 0.601 | 0.552 | 0.550 | 0.544 | 0.710 | 0.166 | 0.185 | 0.135 | 0.112 | 0.110 | - | 0.223 | 0.105 | 0.062 | 0.167 | 0.133 | 0.080 | 0.117 | 0.244 | 0.171 | 0.210 | 0.087 | 0.137 | 0.186 | 0.166 | 0.379 | 0.170 | 0.171 |
| **Bri** | 0.515 | 0.423 | 0.442 | 0.366 | 0.560 | 0.638 | 0.601 | 0.607 | 0.586 | 0.738 | 0.290 | 0.245 | 0.219 | 0.220 | 0.288 | 0.256 | - | 0.209 | 0.172 | 0.14 | 0.174 | 0.217 | 0.185 | 0.247 | 0.180 | 0.186 | 0.157 | 0.160 | 0.269 | 0.223 | 0.399 | 0.155 | 0.119 |
| **LLi** | 0.481 | 0.485 | 0.463 | 0.363 | 0.548 | 0.577 | 0.560 | 0.597 | 0.575 | 0.712 | 0.238 | 0.234 | 0.184 | 0.181 | 0.208 | 0.169 | 0.260 | - | 0.126 | 0.171 | 0.171 | 0.152 | 0.161 | 0.234 | 0.166 | 0.212 | 0.178 | 0.180 | 0.285 | 0.261 | 0.452 | 0.226 | 0.221 |
| **LCh** | 0.445 | 0.460 | 0.407 | 0.289 | 0.508 | 0.609 | 0.554 | 0.516 | 0.534 | 0.697 | 0.163 | 0.156 | 0.141 | 0.110 | 0.191 | 0.131 | 0.204 | 0.16 | - | 0.082 | 0.057 | 0.067 | 0.066 | 0.236 | 0.133 | 0.175 | 0.073 | 0.094 | 0.165 | 0.132 | 0.339 | 0.129 | 0.117 |
| **Gar** | 0.545 | 0.497 | 0.404 | 0.334 | 0.546 | 0.649 | 0.576 | 0.578 | 0.578 | 0.718 | 0.206 | 0.215 | 0.198 | 0.164 | 0.291 | 0.198 | 0.152 | 0.198 | 0.114 | - | 0.130 | 0.132 | 0.118 | 0.289 | 0.211 | 0.227 | 0.140 | 0.141 | 0.237 | 0.207 | 0.421 | 0.178 | 0.154 |
| **Avi** | 0.468 | 0.429 | 0.405 | 0.297 | 0.534 | 0.631 | 0.576 | 0.551 | 0.562 | 0.714 | 0.185 | 0.126 | 0.145 | 0.119 | 0.242 | 0.191 | 0.220 | 0.202 | 0.094 | 0.136 | - | 0.1 | 0.090 | 0.289 | 0.156 | 0.216 | 0.122 | 0.123 | 0.188 | 0.160 | 0.341 | 0.124 | 0.115 |
| **Arl** | 0.500 | 0.470 | 0.455 | 0.314 | 0.544 | 0.625 | 0.555 | 0.560 | 0.560 | 0.729 | 0.164 | 0.182 | 0.141 | 0.134 | 0.233 | 0.138 | 0.246 | 0.177 | 0.101 | 0.135 | 0.103 | - | 0.080 | 0.311 | 0.205 | 0.255 | 0.130 | 0.176 | 0.222 | 0.184 | 0.366 | 0.164 | 0.196 |
| **Mon** | 0.490 | 0.489 | 0.439 | 0.318 | 0.532 | 0.619 | 0.539 | 0.513 | 0.539 | 0.647 | 0.182 | 0.135 | 0.173 | 0.134 | 0.260 | 0.177 | 0.250 | 0.203 | 0.096 | 0.127 | 0.117 | 0.116 | - | 0.27 | 0.177 | 0.212 | 0.122 | 0.141 | 0.2 | 0.177 | 0.338 | 0.135 | 0.165 |
| **Zad** | 0.529 | 0.478 | 0.495 | 0.408 | 0.536 | 0.596 | 0.575 | 0.642 | 0.620 | 0.682 | 0.307 | 0.288 | 0.300 | 0.286 | 0.355 | 0.293 | 0.238 | 0.283 | 0.311 | 0.312 | 0.346 | 0.372 | 0.35 | - | 0.177 | 0.117 | 0.224 | 0.184 | 0.333 | 0.282 | 0.464 | 0.258 | 0.256 |
| **RTe** | 0.438 | 0.408 | 0.353 | 0.297 | 0.541 | 0.608 | 0.563 | 0.559 | 0.577 | 0.712 | 0.269 | 0.203 | 0.213 | 0.201 | 0.262 | 0.223 | 0.205 | 0.216 | 0.194 | 0.235 | 0.226 | 0.257 | 0.249 | 0.198 | - | 0.106 | 0.127 | 0.076 | 0.199 | 0.151 | 0.359 | 0.159 | 0.136 |
| **Bcn** | 0.475 | 0.462 | 0.393 | 0.309 | 0.558 | 0.618 | 0.582 | 0.613 | 0.606 | 0.716 | 0.335 | 0.233 | 0.258 | 0.242 | 0.289 | 0.260 | 0.200 | 0.272 | 0.243 | 0.239 | 0.279 | 0.305 | 0.276 | 0.159 | 0.131 | - | 0.167 | 0.112 | 0.275 | 0.198 | 0.441 | 0.177 | 0.179 |
| **EDe** | 0.478 | 0.484 | 0.437 | 0.269 | 0.535 | 0.634 | 0.564 | 0.545 | 0.555 | 0.710 | 0.247 | 0.183 | 0.200 | 0.183 | 0.243 | 0.165 | 0.201 | 0.275 | 0.167 | 0.205 | 0.211 | 0.249 | 0.214 | 0.274 | 0.180 | 0.209 | - | 0.071 | 0.161 | 0.131 | 0.337 | 0.076 | 0.096 |
| **Sag** | 0.427 | 0.380 | 0.364 | 0.258 | 0.542 | 0.612 | 0.559 | 0.541 | 0.550 | 0.713 | 0.236 | 0.161 | 0.189 | 0.167 | 0.235 | 0.196 | 0.213 | 0.274 | 0.181 | 0.223 | 0.213 | 0.268 | 0.236 | 0.203 | 0.122 | 0.150 | 0.102 | - | 0.102 | 0.070 | 0.307 | 0.116 | 0.084 |
| **RJS** | 0.511 | 0.464 | 0.422 | 0.344 | 0.634 | 0.656 | 0.599 | 0.548 | 0.571 | 0.734 | 0.314 | 0.251 | 0.226 | 0.244 | 0.335 | 0.263 | 0.307 | 0.342 | 0.281 | 0.288 | 0.286 | 0.304 | 0.292 | 0.347 | 0.261 | 0.321 | 0.240 | 0.182 | - | 0.055 | 0.328 | 0.195 | 0.151 |
| **RSe** | 0.477 | 0.404 | 0.368 | 0.286 | 0.586 | 0.638 | 0.578 | 0.572 | 0.567 | 0.710 | 0.321 | 0.227 | 0.216 | 0.236 | 0.293 | 0.248 | 0.268 | 0.344 | 0.247 | 0.269 | 0.244 | 0.268 | 0.285 | 0.284 | 0.201 | 0.227 | 0.206 | 0.123 | 0.111 | - | 0.299 | 0.145 | 0.120 |
| **Alm** | 0.575 | 0.505 | 0.543 | 0.467 | 0.644 | 0.670 | 0.597 | 0.624 | 0.638 | 0.766 | 0.379 | 0.331 | 0.343 | 0.362 | 0.419 | 0.392 | 0.362 | 0.462 | 0.364 | 0.405 | 0.365 | 0.386 | 0.395 | 0.345 | 0.330 | 0.379 | 0.350 | 0.272 | 0.281 | 0.244 | - | 0.359 | 0.287 |
| **Gdq** | 0.501 | 0.464 | 0.420 | 0.272 | 0.527 | 0.632 | 0.555 | 0.563 | 0.561 | 0.689 | 0.286 | 0.163 | 0.189 | 0.161 | 0.260 | 0.207 | 0.217 | 0.288 | 0.197 | 0.211 | 0.200 | 0.236 | 0.199 | 0.269 | 0.192 | 0.204 | 0.117 | 0.137 | 0.242 | 0.188 | 0.334 | - | 0.091 |
| **Gdr** | 0.491 | 0.451 | 0.421 | 0.304 | 0.571 | 0.656 | 0.585 | 0.590 | 0.584 | 0.741 | 0.287 | 0.183 | 0.197 | 0.193 | 0.279 | 0.219 | 0.171 | 0.290 | 0.209 | 0.204 | 0.210 | 0.272 | 0.259 | 0.242 | 0.190 | 0.196 | 0.138 | 0.135 | 0.204 | 0.164 | 0.260 | 0.116 | - |

**Table S4**. Genetic diversity in European invasive eastern mosquitofish (*Gambusia holbrooki*). For each population (*N* = 20 individuals each) and locus, we report the observed (*H*O) and expected heterozygosity (*H*E), allelic richness (*A*), estimated null allele frequencies (*R*) as well as the inbreeding coefficient (*F*IS). Zero-values indicate monomorphic loci. See Table S1 for population information.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Locus name** | | **N° of alleles** | **Size range** | **Test** | **LdG** | **Com** | **LdB** | **Blt** | **TCa** | **PCe** | **Bri** | **LLi** | **LCh** | **Gar** | **Avi** | **Arl** | **Mon** | **Zad** | **RTe** | **Bcn** | **EDe** | **Sag** | **RJS** | **RSe** | **Alm** | **Gdq** | **Gdr** | **Mean across populations** |
| **Gaaf10** | | 27 | 136 | *HO* | 0.35 | 0.55 | 0.45 | 0.60 | 0.05 | 0.30 | 0.50 | 0.45 | 0.55 | 0.70 | 0.20 | 0.40 | 0.35 | 0.50 | 0.45 | 0.40 | 0.70 | 0.30 | 0.65 | 0.75 | 0.35 | 0.50 | 0.95 | 0.48 |
| *HE* | 0.65 | 0.74 | 0.79 | 0.70 | 0.41 | 0.52 | 0.58 | 0.50 | 0.54 | 0.64 | 0.53 | 0.50 | 0.62 | 0.51 | 0.61 | 0.75 | 0.71 | 0.70 | 0.72 | 0.77 | 0.36 | 0.59 | 0.77 | 0.62 |
| *A* | 3.95 | 5.00 | 5.00 | 4.00 | 2.00 | 4.95 | 4.95 | 3.00 | 4.00 | 3.95 | 3.95 | 3.00 | 3.95 | 2.00 | 4.00 | 4.00 | 4.95 | 4.00 | 5.00 | 5.00 | 2.00 | 5.95 | 5.00 | 4.07 |
|  | |  |  | *R* | 0.18 | 0.07 | 0.18 | 0.09 | 0.26 | 0.16 | 0.15 | 0.00 | 0.01 | 0.00 | 0.22 | 0.06 | 0.16 | 0.00 | 0.12 | 0.19 | 0.00 | 0.23 | 0.02 | 0.00 | 0.00 | 0.06 | 0.00 | 0.08 |
|  | |  |  | *F*IS | 0.46 | 0.26 | 0.43 | 0.14 | 0.88 | 0.43 | 0.15 | 0.10 | -0.02 | -0.10 | 0.63 | 0.20 | 0.44 | 0.02 | 0.27 | 0.47 | 0.01 | 0.58 | 0.10 | 0.02 | 0.02 | 0.15 | -0.23 | 0.24 |
| **Gaaf11** | | 28 | 128 | *HO* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.10 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.05 | 0.15 | 0.05 | 0.00 | 0.00 | 0.00 | 0.15 | 0.00 | 0.04 |
| *HE* | 0.00 | 0.39 | 0.33 | 0.34 | 0.00 | 0.26 | 0.47 | 0.00 | 0.44 | 0.51 | 0.42 | 0.56 | 0.61 | 0.00 | 0.00 | 0.31 | 0.75 | 0.24 | 0.00 | 0.33 | 0.00 | 0.64 | 0.10 | 0.29 |
| *A* | 1.00 | 2.00 | 2.00 | 3.00 | 1.00 | 2.00 | 2.00 | 1.00 | 3.00 | 2.95 | 3.00 | 3.00 | 4.00 | 1.00 | 1.00 | 3.00 | 4.95 | 3.95 | 1.00 | 2.00 | 1.00 | 3.00 | 2.00 | 2.30 |
|  | |  |  | *R* | - | 0.29 | 0.27 | 0.28 | - | 0.24 | 0.32 | - | 0.28 | 0.27 | 0.31 | 0.28 | 0.38 | - | - | 0.23 | 0.33 | 0.20 | - | 0.27 | - | 0.29 | 0.15 | 0.24 |
|  | |  |  | *F*IS | - | 1.00 | 1.00 | 1.00 | - | 1.00 | 1.00 | - | 0.89 | 0.81 | 1.00 | 0.82 | 1.00 | - | - | 0.84 | 0.80 | 0.79 | - | 1.00 | - | 0.77 | 1.00 | 0.92 |
| **Gaaf13** | | 35 | 196 | *HO* | 0.75 | 0.95 | 0.85 | 0.95 | 0.80 | 0.85 | 0.55 | 0.65 | 1.00 | 0.65 | 0.80 | 0.70 | 0.80 | 0.60 | 0.80 | 0.65 | 0.70 | 0.75 | 0.70 | 0.80 | 0.45 | 0.75 | 0.90 | 0.76 |
| *HE* | 0.77 | 0.87 | 0.87 | 0.88 | 0.69 | 0.87 | 0.67 | 0.85 | 0.87 | 0.72 | 0.83 | 0.85 | 0.84 | 0.60 | 0.69 | 0.76 | 0.88 | 0.87 | 0.72 | 0.81 | 0.43 | 0.86 | 0.82 | 0.78 |
| *A* | 6.70 | 10.90 | 8.90 | 10.90 | 5.85 | 10.00 | 4.95 | 7.00 | 8.90 | 5.85 | 6.95 | 8.00 | 7.90 | 4.90 | 5.95 | 5.90 | 9.85 | 11.80 | 5.90 | 9.80 | 3.90 | 9.85 | 7.95 | 7.77 |
|  | |  |  | *R* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.09 | 0.10 | 0.00 | 0.03 | 0.01 | 0.07 | 0.02 | 0.00 | 0.00 | 0.07 | 0.08 | 0.06 | 0.00 | 0.01 | 0.00 | 0.05 | 0.00 | 0.03 |
|  | |  |  | *F*IS | 0.03 | -0.10 | 0.03 | -0.08 | -0.16 | 0.02 | 0.18 | 0.24 | -0.16 | 0.10 | 0.04 | 0.18 | 0.04 | -0.01 | -0.17 | 0.15 | 0.20 | 0.14 | 0.03 | 0.01 | -0.04 | 0.13 | -0.11 | 0.03 |
| **Gafµ3** | | 39 | 138 | *HO* | 0.70 | 0.75 | 0.55 | 0.65 | 0.40 | 0.65 | 0.45 | 0.55 | 0.70 | 0.60 | 0.90 | 0.55 | 0.70 | 0.30 | 0.70 | 0.20 | 0.55 | 0.75 | 0.70 | 0.80 | 0.10 | 0.55 | 0.75 | 0.59 |
| *HE* | 0.85 | 0.70 | 0.81 | 0.83 | 0.57 | 0.78 | 0.73 | 0.77 | 0.83 | 0.74 | 0.83 | 0.86 | 0.82 | 0.45 | 0.67 | 0.26 | 0.60 | 0.65 | 0.70 | 0.77 | 0.10 | 0.61 | 0.74 | 0.68 |
| *A* | 6.95 | 6.90 | 7.95 | 6.00 | 4.90 | 5.00 | 4.95 | 5.00 | 7.90 | 6.90 | 7.90 | 7.95 | 7.00 | 2.95 | 3.95 | 2.00 | 4.90 | 4.00 | 3.95 | 5.00 | 2.00 | 4.00 | 5.95 | 5.39 |
|  | |  |  | *R* | 0.08 | 0.00 | 0.14 | 0.09 | 0.12 | 0.03 | 0.16 | 0.14 | 0.06 | 0.05 | 0.00 | 0.16 | 0.01 | 0.10 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.06 |
|  | |  |  | *F*IS | 0.18 | -0.07 | 0.33 | 0.22 | 0.30 | 0.17 | 0.39 | 0.29 | 0.16 | 0.20 | -0.09 | 0.36 | 0.15 | 0.33 | -0.05 | 0.24 | 0.09 | -0.15 | 0.00 | -0.05 | -0.03 | 0.10 | -0.02 | 0.13 |
| **Gaaf16** | | 23 | 136 | *HO* | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| *HE* | 0.33 | 0.26 | 0.30 | 0.19 | 0.26 | 0.19 | 0.10 | 0.26 | 0.19 | 0.00 | 0.10 | 0.10 | 0.19 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 |
| *A* | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 1.00 | 2.00 | 2.00 | 2.00 | 1.00 | 2.00 | 1.00 | 1.00 | 1.00 | 2.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.61 |
|  | |  |  | *R* | 0.27 | 0.24 | 0.22 | 0.20 | 0.24 | 0.20 | 0.15 | 0.24 | 0.20 | - | 0.15 | 0.15 | 0.20 | - | 0.13 | - | - | - | 0.15 | - | - | - | - | 0.14 |
|  | |  |  | *F*IS | 1.00 | 1.00 | 0.83 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | - | 1.00 | 1.00 | 1.00 | - | 0.65 | - | - | - | 1.00 | - | - | - | - | 0.96 |
| **Gaaf7** | | 25 | 120 | *HO* | 0.55 | 0.50 | 0.60 | 0.55 | 0.50 | 0.55 | 0.35 | 0.35 | 0.40 | 0.50 | 0.45 | 0.30 | 0.35 | 0.05 | 0.55 | 0.25 | 0.45 | 0.60 | 0.55 | 0.75 | 0.00 | 0.30 | 0.45 | 0.45 |
| *HE* | 0.52 | 0.51 | 0.55 | 0.55 | 0.59 | 0.69 | 0.41 | 0.30 | 0.43 | 0.39 | 0.48 | 0.33 | 0.41 | 0.05 | 0.71 | 0.30 | 0.61 | 0.66 | 0.56 | 0.59 | 0.00 | 0.58 | 0.55 | 0.47 |
| *A* | 2.95 | 2.00 | 3.00 | 3.00 | 3.00 | 4.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 1.95 | 4.95 | 2.00 | 3.95 | 4.00 | 3.00 | 3.00 | 1.00 | 3.00 | 3.00 | 2.69 |
|  | |  |  | *R* | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.06 | 0.04 | 0.00 | 0.02 | 0.00 | 0.01 | 0.02 | 0.04 | 0.00 | 0.08 | 0.04 | 0.10 | 0.00 | 0.00 | 0.00 | - | 0.17 | 0.03 | 0.05 |
|  | |  |  | *F*IS | -0.06 | 0.02 | -0.09 | 0.00 | 0.16 | 0.20 | 0.15 | -0.19 | 0.07 | -0.31 | 0.07 | 0.09 | 0.15 | 0.00 | 0.23 | 0.16 | 0.27 | 0.09 | 0.01 | -0.29 | - | 0.49 | 0.18 | 0.06 |
| **Gaaf22** | | 46 | 129 | *HO* | 0.60 | 0.75 | 0.55 | 0.75 | 0.30 | 0.65 | 0.50 | 0.60 | 0.75 | 0.55 | 0.55 | 0.65 | 0.65 | 0.35 | 0.85 | 0.40 | 0.65 | 0.95 | 0.60 | 0.40 | 0.30 | 0.90 | 0.45 | 0.60 |
| *HE* | 0.83 | 0.82 | 0.80 | 0.85 | 0.49 | 0.76 | 0.59 | 0.72 | 0.71 | 0.65 | 0.74 | 0.76 | 0.64 | 0.36 | 0.76 | 0.55 | 0.71 | 0.81 | 0.59 | 0.43 | 0.39 | 0.72 | 0.45 | 0.66 |
| *A* | 6.00 | 6.00 | 6.95 | 7.85 | 4.95 | 6.95 | 4.00 | 4.95 | 6.00 | 4.95 | 5.90 | 5.95 | 5.95 | 4.00 | 5.00 | 3.00 | 4.00 | 6.95 | 3.00 | 2.00 | 2.00 | 5.95 | 2.00 | 4.97 |
|  | |  |  | *R* | 0.11 | 0.04 | 0.13 | 0.03 | 0.15 | 0.01 | 0.06 | 0.07 | 0.00 | 0.07 | 0.08 | 0.04 | 0.00 | 0.00 | 0.00 | 0.11 | 0.01 | 0.00 | 0.00 | 0.02 | 0.06 | 0.00 | 0.00 | 0.04 |
|  | |  |  | *F*IS | 0.28 | 0.09 | 0.31 | 0.11 | 0.40 | 0.15 | 0.16 | 0.17 | -0.06 | 0.14 | 0.26 | 0.14 | -0.01 | 0.01 | -0.13 | 0.28 | 0.09 | -0.19 | -0.02 | 0.07 | 0.22 | -0.25 | 0.00 | 0.10 |
| **Gafµ2** | | 30 | 78 | *HO* | 0.00 | 0.6. | 0.50 | 0.45 | 0.35 | 0.55 | 0.55 | 0.35 | 0.35 | 0.55 | 0.50 | 0.45 | 0.45 | 0.70 | 0.40 | 0.55 | 0.60 | 0.35 | 0.15 | 0.05 | 0.20 | 0.30 | 0.30 | 0.42 |
| *HE* | 0.00 | 0.56 | 0.56 | 0.58 | 0.36 | 0.48 | 0.65 | 0.45 | 0.48 | 0.51 | 0.53 | 0.41 | 0.41 | 0.68 | 0.47 | 0.48 | 0.62 | 0.34 | 0.35 | 0.05 | 0.55 | 0.45 | 0.42 | 0.45 |
| *A* | 1.00 | 3.00 | 3.95 | 3.00 | 2.00 | 2.00 | 3.00 | 2.00 | 2.95 | 2.00 | 3.00 | 2.00 | 2.00 | 3.00 | 3.95 | 2.00 | 3.00 | 3.00 | 3.95 | 1.95 | 3.00 | 3.95 | 4.00 | 2.77 |
|  | |  |  | *R* | - | 0.00 | 0.02 | 0.09 | 0.00 | 0.00 | 0.07 | 0.07 | 0.08 | 0.00 | 0.07 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.04 | 0.00 | 0.16 | 0.00 | 0.23 | 0.11 | 0.13 | 0.04 |
|  | |  |  | *F*IS | - | -0.08 | 0.10 | 0.23 | 0.02 | -0.15 | 0.16 | 0.23 | 0.28 | -0.08 | 0.05 | -0.10 | -0.10 | -0.04 | 0.15 | -0.15 | 0.03 | -0.02 | 0.58 | 0.00 | 0.64 | 0.34 | 0.29 | 0.11 |
| **Gaaf9** | | 17 | 92 | *HO* | 0.30 | 0.25 | 0.25 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.05 | 0.55 | 0.05 | 0.25 | 0.00 | 0.20 | 0.00 | 0.35 | 0.05 | 0.00 | 0.05 | 0.00 | 0.40 | 0.30 | 0.22 |
| *HE* | 0.47 | 0.45 | 0.30 | 0.41 | 0.00 | 0.00 | 0.10 | 0.00 | 0.27 | 0.05 | 0.51 | 0.05 | 0.23 | 0.00 | 0.27 | 0.00 | 0.34 | 0.05 | 0.00 | 0.05 | 0.00 | 0.43 | 0.43 | 0.19 |
| *A* | 2.00 | 2.00 | 2.00 | 2.00 | 1.00 | 1.00 | 2.00 | 1.00 | 2.95 | 1.95 | 2.00 | 1.95 | 2.95 | 1.00 | 3.00 | 1.00 | 2.95 | 1.95 | 1.00 | 1.95 | 1.00 | 2.00 | 2.00 | 1.85 |
|  | |  |  | *R* | 0.11 | 0.14 | 0.04 | 0.12 | - | - | 0.15 | - | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | - | 0.10 | - | 0.02 | 0.00 | - | 0.00 | - | 0.02 | 0.09 | 0.05 |
|  | |  |  | *F*IS | 0.36 | 0.45 | 0.16 | 0.39 | - | - | 1.00 | - | 0.07 | 0.00 | -0.08 | 0.00 | -0.09 | - | 0.27 | - | -0.04 | 0.00 | - | 0.00 | - | 0.07 | 0.31 | 0.18 |
| **Gafµ6** | | 20 | 120 | *HO* | 0.35 | 0.15 | 0.05 | 0.20 | 0.05 | 0.20 | 0.00 | 0.20 | 0.10 | 0.05 | 0.10 | 0.00 | 0.20 | 0.00 | 0.15 | 0.00 | 0.25 | 0.10 | 0.50 | 0.30 | 0.00 | 0.10 | 0.25 | 0.14 |
| *HE* | 0.38 | 0.55 | 0.56 | 0.59 | 0.55 | 0.34 | 0.19 | 0.66 | 0.69 | 0.36 | 0.47 | 0.49 | 0.41 | 0.49 | 0.54 | 0.51 | 0.63 | 0.57 | 0.54 | 0.51 | 0.33 | 0.43 | 0.47 | 0.49 |
| *A* | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 2.00 | 4.00 | 4.00 | 2.00 | 2.00 | 2.00 | 3.00 | 2.00 | 3.00 | 2.00 | 3.00 | 3.95 | 2.95 | 2.00 | 2.00 | 2.00 | 3.00 | 2.73 |
|  | |  |  | *R* | 0.02 | 0.25 | 0.32 | 0.25 | 0.33 | 0.13 | 0.20 | 0.27 | 0.34 | 0.24 | 0.25 | 0.33 | 0.17 | 0.33 | 0.25 | 0.33 | 0.24 | 0.29 | 0.01 | 0.13 | 0.27 | 0.24 | 0.15 | 0.19 |
|  | |  |  | *F*IS | 0.07 | 0.73 | 0.91 | 0.67 | 0.91 | 0.42 | 1.00 | 0.70 | 0.86 | 0.86 | 0.79 | 1.00 | 0.52 | 1.00 | 0.73 | 1.00 | 0.61 | 0.83 | 0.07 | 0.42 | 1.00 | 0.77 | 0.47 | 0.71 |
| **Gaaf15** | | 13 | 72 | *HO* | 0.35 | 0.35 | 0.50 | 0.45 | 0.35 | 0.45 | 0.30 | 0.30 | 0.50 | 0.30 | 0.75 | 0.55 | 0.45 | 0.20 | 0.45 | 0.30 | 0.55 | 0.70 | 0.35 | 0.55 | 0.60 | 0.20 | 0.60 | 0.44 |
| *HE* | 0.41 | 0.50 | 0.55 | 0.51 | 0.36 | 0.45 | 0.43 | 0.27 | 0.49 | 0.51 | 0.52 | 0.50 | 0.50 | 0.19 | 0.38 | 0.34 | 0.47 | 0.57 | 0.30 | 0.51 | 0.51 | 0.33 | 0.49 | 0.44 |
| *A* | 2.00 | 2.00 | 3.00 | 2.00 | 2.00 | 2.00 | 2.00 | 3.00 | 2.00 | 2.00 | 2.95 | 2.95 | 2.00 | 2.00 | 2.95 | 2.95 | 2.95 | 3.00 | 2.00 | 3.00 | 2.00 | 2.00 | 2.00 | 2.38 |
|  | |  |  | *R* | 0.04 | 0.09 | 0.08 | 0.03 | 0.00 | 0.00 | 0.09 | 0.00 | 0.00 | 0.13 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.03 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.11 | 0.00 | 0.04 |
|  | |  |  | *F*IS | 0.15 | 0.31 | 0.09 | 0.12 | 0.02 | 0.00 | 0.31 | -0.10 | -0.02 | 0.42 | -0.45 | -0.11 | 0.10 | -0.09 | -0.23 | 0.11 | -0.19 | -0.25 | -0.19 | -0.07 | -0.19 | 0.40 | -0.23 | 0.00 |
| **Gafµ7** | | 30 | 90 | *HO* | 0.50 | 0.70 | 0.75 | 0.75 | 0.50 | 0.75 | 0.05 | 0.50 | 0.50 | 0.15 | 0.75 | 0.45 | 0.85 | 0.55 | 0.80 | 0.80 | 0.65 | 0.70 | 0.50 | 0.75 | 0.00 | 0.40 | 0.55 | 0.56 |
| *HE* | 0.63 | 0.73 | 0.81 | 0.86 | 0.57 | 0.85 | 0.52 | 0.56 | 0.71 | 0.41 | 0.71 | 0.68 | 0.73 | 0.70 | 0.73 | 0.67 | 0.78 | 0.78 | 0.62 | 0.85 | 0.10 | 0.70 | 0.75 | 0.67 |
| *A* | 4.90 | 6.90 | 7.00 | 8.00 | 4.90 | 7.95 | 3.00 | 3.95 | 5.85 | 2.00 | 4.90 | 4.00 | 5.00 | 3.95 | 5.90 | 4.95 | 5.50 | 5.95 | 4.90 | 7.95 | 2.00 | 4.95 | 6.90 | 5.29 |
|  | |  |  | *R* | 0.04 | 0.00 | 0.00 | 0.03 | 0.00 | 0.02 | 0.31 | 0.01 | 0.10 | 0.19 | 0.00 | 0.10 | 0.00 | 0.07 | 0.02 | 0.00 | 0.05 | 0.05 | 0.04 | 0.16 | 0.15 | 0.17 | 0.12 | 0.07 |
|  | |  |  | *F*IS | 0.21 | 0.05 | 0.07 | 0.13 | 0.12 | 0.12 | 0.91 | 0.11 | 0.30 | 0.64 | -0.05 | 0.35 | -0.17 | 0.21 | -0.10 | -0.20 | 0.17 | 0.10 | 0.19 | 0.12 | 1.00 | 0.44 | 0.28 | 0.22 |
| **Mf13** | | 12 | 32 | *HO* | 0.35 | 0.35 | 0.45 | 0.40 | 0.25 | 0.35 | 0.00 | 0.30 | 0.40 | 0.45 | 0.30 | 0.55 | 0.40 | 0.00 | 0.00 | 0.00 | 0.45 | 0.65 | 0.40 | 0.35 | 0.15 | 0.25 | 0.35 | 0.38 |
| *HE* | 0.67 | 0.41 | 0.51 | 0.39 | 0.38 | 0.54 | 0.00 | 0.50 | 0.53 | 0.56 | 0.30 | 0.65 | 0.47 | 0.00 | 0.00 | 0.00 | 0.48 | 0.48 | 0.50 | 0.48 | 0.14 | 0.30 | 0.36 | 0.38 |
| *A* | 4.00 | 2.00 | 3.00 | 2.00 | 3.00 | 3.00 | 1.00 | 3.00 | 3.00 | 4.90 | 2.95 | 3.95 | 2.95 | 1.00 | 1.00 | 1.00 | 2.00 | 2.00 | 3.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.47 |
|  | |  |  | *R* | 0.20 | 0.04 | 0.07 | 0.00 | 0.13 | 0.13 | - | 0.14 | 0.11 | 0.09 | 0.00 | 0.06 | 0.02 | - | - | - | 0.01 | 0.00 | 0.10 | 0.08 | 0.00 | 0.04 | 0.00 | 0.07 |
|  | |  |  | *F*IS | 0.48 | 0.15 | 0.12 | -0.04 | 0.35 | 0.36 | - | 0.41 | 0.25 | 0.20 | 0.01 | 0.15 | 0.14 | - | - | - | 0.07 | -0.36 | 0.20 | 0.28 | -0.06 | 0.16 | 0.02 | 0.15 |
| **Gafµ1** | | 9 | 70 | *HO* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *HE* | 0.10 | 0.00 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.19 | 0.00 | 0.00 | 0.49 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 |
| *A* | 2.00 | 1.00 | 1.00 | 1.00 | 2.00 | 1.00 | 1.00 | 1.00 | 1.00 | 2.00 | 1.00 | 1.00 | 2.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.17 |
|  | |  |  | *R* | 0.15 | - | - | - | 0.15 | - | - | - | - | 0.20 | - | - | 0.33 | - | - | - | - | - | - | - | - | - | - | 0.10 |
|  | |  |  | *F*IS | 1.00 | - | - | - | 1.00 | - | - | - | - | 1.00 | - | - | 1.00 | - | - | - | - | - | - | - | - | - | - | 1.00 |
| **Gafµ4** | | 61 | 170 | *HO* | 0.80 | 0.55 | 0.65 | 0.75 | 0.75 | 0.50 | 0.60 | 0.25 | 0.65 | 0.75 | 0.90 | 0.80 | 0.75 | 0.50 | 0.40 | 0.65 | 0.30 | 0.85 | 0.60 | 0.75 | 0.50 | 0.70 | 0.50 | 0.63 |
| *HE* | 0.83 | 0.83 | 0.91 | 0.90 | 0.80 | 0.80 | 0.85 | 0.75 | 0.68 | 0.80 | 0.80 | 0.87 | 0.87 | 0.73 | 0.79 | 0.80 | 0.88 | 0.88 | 0.78 | 0.82 | 0.61 | 0.90 | 0.81 | 0.81 |
| *A* | 7.90 | 10.80 | 11.90 | 12.75 | 9.75 | 8.90 | 6.00 | 7.95 | 8.85 | 9.80 | 5.95 | 10.80 | 14.50 | 4.00 | 8.95 | 6.90 | 9.90 | 10.85 | 7.90 | 9.80 | 5.85 | 13.65 | 8.80 | 9.24 |
|  | |  |  | *R* | 0.00 | 0.14 | 0.13 | 0.07 | 0.04 | 0.15 | 0.13 | 0.28 | 0.03 | 0.01 | 0.00 | 0.03 | 0.03 | 0.12 | 0.21 | 0.06 | 0.30 | 0.00 | 0.08 | 0.00 | 0.07 | 0.10 | 0.15 | 0.09 |
|  | |  |  | *F*IS | 0.04 | 0.35 | 0.29 | 0.17 | 0.06 | 0.38 | 0.30 | 0.67 | 0.05 | 0.07 | -0.13 | 0.08 | 0.15 | 0.32 | 0.50 | 0.19 | 0.67 | 0.04 | 0.24 | 0.08 | 0.18 | 0.22 | 0.39 | 0.23 |
| **Mean across loci** |  | |  | *HO* | 0.43 | 0.46 | 0.44 | 0.48 | 0.33 | 0.45 | 0.30 | 0.38 | 0.44 | 0.39 | 0.48 | 0.40 | 0.41 | 0.38 | 0.48 | 0.39 | 0.49 | 0.52 | 0.48 | 0.49 | 0.27 | 0.42 | 0.49 |  |
| *HE* | 0.50 | 0.55 | 0.58 | 0.57 | 0.41 | 0.50 | 0.42 | 0.44 | 0.52 | 0.47 | 0.52 | 0.51 | 0.55 | 0.32 | 0.45 | 0.38 | 0.56 | 0.51 | 0.434 | 0.46 | 0.23 | 0.50 | 0.48 |  |
| *A* | 3.77 | 4.36 | 4.71 | 4.70 | 3.42 | 4.25 | 2.99 | 3.39 | 4.29 | 3.62 | 3.76 | 4.04 | 4.48 | 2.38 | 3.77 | 2.85 | 4.29 | 4.49 | 3.37 | 3.83 | 2.12 | 4.29 | 3.77 |  |
|  |  | |  | *R* | 0.09 | 0.09 | 0.11 | 0.09 | 0.11 | 0.09 | 0.15 | 0.11 | 0.09 | 0.09 | 0.08 | 0.09 | 0.09 | 0.06 | 0.08 | 0.10 | 0.09 | 0.07 | 0.05 | 0.05 | 0.08 | 0.10 | 0.07 |  |
|  |  | |  | *F*IS | 0.32 | 0.30 | 0.33 | 0.29 | 0.39 | 0.32 | 0.52 | 0.30 | 0.26 | 0.28 | 0.22 | 0.30 | 0.29 | 0.18 | 0.18 | 0.28 | 0.21 | 0.12 | 0.18 | 0.12 | 0.28 | 0.29 | 0.18 |  |

**Online Supplement 3**

**Extended results—phenotypic variation in wild-caught mosquitofish**

***Phenotypic variation between populations and genetic clusters***

In the analysis of male and female body size we found significant effects of the factors ‘sex’ (MANCOVA; *F*3, 1252 = 8,842.64, *P* < 0.001, *η*p2 = 0.955), ‘year’ (*F*3, 1252 = 17.11, *P* < 0.001, *η*p2 = 0.039), ‘population(cluster)’ (*F*102, 3750 = 7.83, *P* < 0.001, *η*p2 = 0.175) and ‘cluster’ (*F*3, 1252 = 11.35, *P* <0.001, *η*p2 = 0.026), as well as the covariate ‘centroid size’ (*F*3, 1252 = 8.63, *P* < 0.001, *η*p2 = 0.020) and the interactions ‘sex × population(cluster)’ (*F*102,3750 = 4.75, *P* < 0.001, *η*p2 = 0.114), ‘year × cluster’ (*F*3, 1252 = 5.63, *P* = 0.001, *η*p2 = 0.013) and ‘year × population(cluster)’ (*F*6, 2504 = 5.41, *P* < 0.001, *η*p2 = 0.013; Table S8*a*). Sexual dimorphism was the greatest source of body shape diversity (Fig. S3), but the extent of sexual dimorphism changed between populations (‘sex × population(cluster)’-effect). Furthermore, body size varied between populations and between Spanish and French/Italian fish (‘population(cluster)’- and ‘cluster’-effects respectively), however these differences varied depending on the year in which the mosquitofish were sampled.

Similarly, male life-history traits were significantly affected by ‘SL’ (*F*3, 544 = 510.22, *P* < 0.001, *η*p2 = 0.738), but also varied between populations (‘population’-effect; *F*102, 1639 = 4.52, *P* < 0.001, *η*p2 = 0.220), genetic clusters (‘cluster’-effect; *F*3, 544 = 3.15, *P* = 0.025, *η*p2 = 0.017), and sampling years (‘year’-effect; *F*3, 544 = 12.93, *P* < 0.001, *η*p2 = 0.067). Moreover, male life histories scaled differently with body size in different populations (‘SL × population(cluster)’-effect; *F*105, 1630 = 1.35, *P* = 0.013, *η*p2 = 0.080), and the differences between clusters varied between sampling years (‘year × cluster’-effect; *F*3, 544 = 2.69, *P* = 0.046, *η*p2 = 0.015; Table S8*b*).

Lastly, in the analysis on female life-history traits we found significant effects of both the covariates ‘SL’ (*F*6, 633 = 655.89, *P* < 0.001, *η*p2 = 0.861) and ‘embryonic stage of development (*F*6, 633 = 7.08, *P* < 0.001, *η*p2 = 0.063). Female life histories also varied depending on ‘population(cluster)’ (*F*198, 3753 = 7.70, *P* < 0.001, *η*p2 = 0.265), the genetic cluster (*F*6, 633 = 14.97, *P* < 0.001, *η*p2 = 0.124) and sampling years (*F*6, 633 = 8.45, *P* < 0.001, *η*p2 = 0.074). Finally, life-history traits scaled differently with body size between populations (*F*198, 3753 = 3.11, *P* < 0.001, *η*p2 = 0.139) and genetic clusters (*F*6, 633 = 5.04, *P* < 0.001, *η*p2 = 0.046; Table S8*c*).

***Body-size variation***

In the ANCOVA on body size (SL), the factors ‘sex’ (*F*1, 1321 = 2,752.60, *P* < 0.001, *η*p2= 0.676) and ‘year’ (*F*1, 1321 = 27.55, *P* < 0.001, *η*p2= 0.020) had significant effects, as did the covariates ‘EPC1’ (*F*1, 1321 = 5.38, *P* = 0.022, *η*p2= 0.004), ‘EPC2’ (*F*1, 1321 = 5.26, *P* = 0.022, *η*p2= 0.004) and the interactions 'sex × EPC1’ (*F*1, 1321 = 23.43, *P* < 0.001, *η*p2= 0.017), ‘sex × EPC3’ (*F*1, 1321 = 3.95, *P* = 0.047, *η*p2 = 0.003), ‘year × EPC1’ (*F*1, 1321 = 8.11, *P* = 0.004, *η*p2= 0.006) and ‘year × ‘EPC2’ (*F*1, 1321 = 12.99, *P* < 0.001, *η*p2= 0.010), while ‘EPC3’ did not have a significant effect (*F*1, 1321 = 0.93, *P* = 0.334, *η*p2 = 0.001). Based on our measure of effect strength (*η*p2), ‘sex’ had the strongest overall effect, with females being larger than males. Fish sampled in 2013 were also larger than those sampled in 2017 (‘year’-effect). Among the effects of environmental variables (EPCs), fish had larger bodies in northern than in southern populations (‘EPC1’-effect), and this effect was more pronounced in males than in females (‘sex × EPC1’-effect; Fig. 3*b*). The ‘EPC2’-effect suggested that in high-oxygen, coastal environments fish tended to be smaller than in low-oxygen, inland conditions, but this response was much more pronounced in 2017 than in 2013 (‘year × EPC2’-effect).

***Body-shape variation***

In the analysis on body-shape variation, all factors and the covariates ‘EPC1’ and ‘EPC3’ had significant effects (Table 1*a*). Nonetheless, ‘sex’ had the strongest overall effect (*η*p2= 0.962), while the importance of the other significant effects was considerably lower (all *η*p2< 0.050). The main source of body-shape variation regarding sexual dimorphism concerned the enlarged abdominal region in females, while in males the anal fin (modified into the gonopodium) was shifted towards the anterior. Our analysis also highlighted the presence of allometric effects due to body size differences (‘centroid size’-effect). Additionally, body shape changed in response to environmental variation and between sampling years. However, the responses to climate variation differed between males and females (‘sex × EPC1’-effect), and between 2013 and 2017 (‘year × EPC1’-effect), as did the responses to differences in conductivity (‘year × EPC3’-effect).

*Post-hoc* ANCOVAs (Table 2; *α*’= 0.017, corrected for multiple testing) revealed that ‘sex’, ‘centroid size’ and ‘year × EPC1’ significantly affected RW1-3. ‘Year’, ‘EPC1’ and ‘year × EPC3’ had significant effects on RW1 and RW3; ‘EPC3’ on RW1 and RW2; and ‘sex × EPC1’ significantly affected RW3. The ‘sex’-effect reflected that, besides the presence of a gonopodium, males were also characterised by more streamlined bodies and relatively larger heads than females. Bigger fish had deeper and rounder bodies than smaller ones (‘centroid size’-effects), as had fish from 2013 when compared to fish from 2017 (‘year’-effect). When considering environmental variation, in colder climates (i.e., northern and Italian populations) males had rounder and deeper bodies compared to fish from warmer climates (i.e., southern and Spanish populations), whereas females had deeper and rounder bodies in southern, rather than northern populations (‘sex × EPC1’-effect). Moreover, the ‘year × EPC1’-effect suggests that these responses were more pronounced in 2013 than in 2017. Lastly, in low-conductivity environments fish had deeper bodies than in high-conductivity ones (‘EPC3’-effect), but this effect was much stronger in fish sampled in 2017 than in those sampled in 2013 (‘year × EPC3’-effect).

***Life-history variation***

In the analysis on male life-history traits we found significant effects of SL, ‘year’, ‘EPC1’, ‘EPC3’ and ‘Year × EPC1’, while EPC2 had no significant effect (Table 1*b*). ‘SL’ had the strongest effect (*η*p2= 0.838), followed by ‘year’ (*η*p2= 0.258), but ‘EPC1’, ‘EPC3’ and ‘Year × EPC1’ all had relatively weak effects (*η*p2≤ 0.100 in all cases). *Post-hoc* ANCOVAs (Table 3; *α*’= 0.017) uncovered a significant effect of body size (SL) on lean weight, fat content and GSI, with bigger males having higher lean weights and fat contents but lower GSI. ‘Year’ had a significant effect on fat content and GSI, as males were characterised by higher fat content and reproductive investment in 2017 than in 2013. Moreover, ‘EPC1’ had a significant effect on lean weight and GSI, as males from northern and colder populations were heavier and had higher GSI than those from southern/warmer populations. ‘EPC3’ had a significant effect on lean weight and fat content, showing that in high-conductivity populations males were heavier but had lower fat content than in low-conductivity ones. Lastly, ‘SL x EPC3’ significantly affected GSI, with bigger males having a higher GSI compared to smaller males in high-, but not in low-conductivity populations.

In the analysis of female life histories, the factor ‘year’, all covariates, as well as the interactions ‘year × EPC1-3’ and ‘SL × EPC1-3’ had significant effects. Based on *η*p2, ‘SL’ had by far the strongest effect (*η*p2= 0.937), followed by ‘embryonic stage of development’, ‘EPC3’, ‘year × EPC3’ and ‘year’ and (*η*p2= 0.144, 0.138, 0.131 and 0.126, respectively). ‘EPC1’, ‘EPC2’, ‘year × EPC1’, ‘year × EPC2’, and ‘SL × EPCs’, while significant, were of lower importance (*η*p2 < 0.100; Table 1*c*). This suggests that female life-history traits changed during offspring development (‘embryonic stage of development’-effect) and as a function of body size (SL-effect), differed between years, and in response to different environmental conditions. However, these responses to climate varied between 2013 and 2017 (‘year × EPC’-effects), and bigger females responded to environmental variation differently than smaller ones (‘SL × EPC’-effects). *Post-hoc* ANCOVAs (Table 4; *α*’= 0.008) revealed that female lean weight, fecundity, embryo lean weight, and RA all increased as females became bigger (SL-effects). ‘Embryonic stage of development’ affected embryo fat and lean weight, as embryos decreased in weight and lost body fat during development, and fecundity, which decreased as embryos progressed in development. ‘Year’ significantly affected lean weight, adult fat content and embryo fat content and RA, as fish were lighter and had reduced reproductive investment in 2017 than in 2013 but had increased adult and embryo fat contents. Considering the effects of environmental variation, ‘EPC1’ significantly affected RA, ‘EPC2’ offspring lean weight, and ‘EPC3’ affected adult fat content and RA, as well as embryo fat content. Females from colder populations invested more into reproduction (RA) than those from warmer populations (‘EPC1’-effect). The ‘EPC2’ effect suggested that females in high-oxygen environments tended to have bigger offspring than in low-oxygen environments, while female in low-conductivity environments had increased body and embryo fat and RA (‘EPC3’-effect).

Regarding the interaction effects, adult fat content was significantly affected by ‘SL × EPC1’, while ‘SL × EPC3’ affected adult and embryo weight, fecundity and RA. Larger females had higher fat content in warmer (southern) populations, while the difference was greatly reduced in smaller females (‘SL × EPC1’- effect). Moreover, only in high-conductivity populations did larger females have relatively higher body weight, fecundity, and offspring size compared to small-bodied females. Lastly, ‘year × EPC1’ affected RA, ‘year × EPC2’ affected fecundity, embryo weight and fat content, and ‘year × EPC3’ affected fecundity, RA, and embryo fat content. In 2017, females in colder populations were characterised by increased RA when compared with warmer populations, but this effect disappeared in 2013 (‘year × EPC1’-effect). Similarly, the ‘year × EPC2’-effect suggests that in high-oxygen environments females were characterised by reduced fecundity and increased embryo weight and fat content in 2013, while this pattern was reversed in 2017. The ‘year × EPC3’-effect suggests that in high-conductivity environments females had higher fecundity and RA, but lower embryo fat content in 2017, but these relationships were reversed in 2013.

**Table S5**. Overview of relative warps (RWs) used for the body-shape analysis of wild-caught mosquitofish. For each RW, eigenvalues and the % of variance explained are presented.

|  |  |  |  |
| --- | --- | --- | --- |
| RW | Eigenvalue | % variance explained | Cumulative % var. explained |
| RW1 | 2.6306 | 87.30 | 87.30 |
| RW2 | 0.4061 | 2.08 | 89.30 |
| RW3 | 0.3813 | 1.83 | 91.21 |

**Table S6**.Descriptive statistics (mean ± s.e.m.) of life-history traits of wild-caught male *G. holbrooki*.

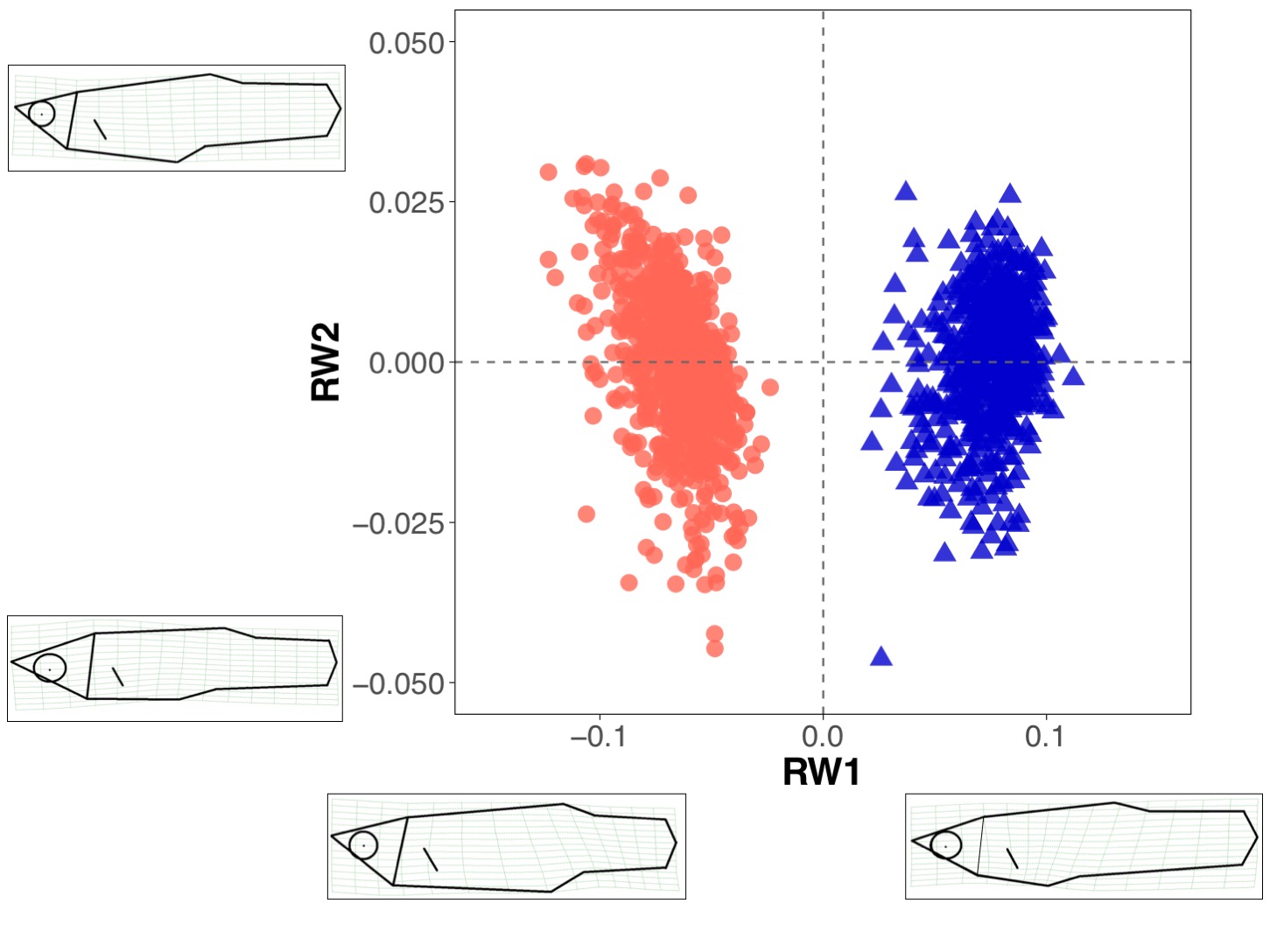
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site number |  | Population name and code | Year | *N* | SL [mm] | Lean weight [g] | Fat content [%] | GSI [%] |
| 1 | Italy | Lago di Garda – LdG | 2013 | 5 | 21.2 ± 0.6 | 0.038 ± 0.004 | 0.28 ± 0.14 | 2.57 ± 0.19 |
| 2 | Italy | Comacchio – Com | 2013 | 22 | 23.3 ± 0.3 | 0.051 ± 0.003 | 0.75 ± 0.06 | 3.16 ± 0.10 |
| 3 | Italy | Lago di Bolsena – LdB | 2013 | 15 | 22.0 ± 0.6 | 0.038 ± 0.003 | 1.52 ± 0.17 | 2.21 ± 0.07 |
| 4 | Italy | Barletta – Blt | 2013 | 20 | 21.3 ± 0.3 | 0.033 ± 0.002 | 1.29 ± 0.13 | 1.69 ± 0.07 |
| 5 | Italy | Torre Castiglione – TCa | 2013 | 24 | 21.5 ± 0.2 | 0.037 ± 0.001 | 0.70 ± 0.08 | 1.91 ± 0.08 |
| 6 | Italy | Porto Cesareo - PCe | 2013 | 16 | 20.3 ± 0.3 | 0.031 ± 0.001 | 0.30 ± 0.09 | 2.32 ± 0.12 |
| 7 | France | Briere – Bri | 2013 | 12 | 19.4 ± 0.4 | 0.027 ± 0.002 | 0.16 ± 0.09 | 2.08 ± 0.15 |
| 8 | France | La Ligneron – LLi | 2013 | 22 | 18.9 ± 0.4 | 0.026 ± 0.002 | 0.51 ± 0.17 | 2.25 ± 0.07 |
| 9 | France | La Charente – LCh | 2013 | 16 | 21.1 ± 0.4 | 0.030 ± 0.002 | 4.17 ± 0.41 | 2.06 ± 0.12 |
| 10 | France | Garonne – Gar | 2013 | 21 | 19.4 ± 0.4 | 0.025 ± 0.002 | 3.51 ± 0.45 | 2.58 ± 0.08 |
| 11 | France | Avignon – Avi | 2013 | 13 | 18.8 ± 0.5 | 0.027 ± 0.003 | 0.67 ± 0.15 | 2.78 ± 0.17 |
| 12 | France | Arles – Arl | 2013 | 24 | 19.4 ± 0.5 | 0.026 ± 0.002 | 1.73 ± 0.26 | 1.73 ± 0.09 |
| 13 | France | Montpellier – Mon | 2013 | 19 | 18.6 ± 0.4 | 0.022 ± 0.002 | 0.28 ± 0.10 | 2.60 ± 0.11 |
| 14 | Spain | Zadorra – Zad | 2013 | 25 | 18.6 ± 0.3 | 0.025 ± 0.002 | 0.32 ± 0.09 | 2.14 ± 0.08 |
| 15 | Spain | Rio Ter – Rte | 2013 | 12 | 20.3 ± 0.6 | 0.027 ± 0.003 | 1.37 ± 0.33 | 1.64 ± 0.13 |
| 16 | Spain | Barcelona – Bcn | 2013 | 19 | 20.2 ± 0.3 | 0.030 ± 0.002 | 0.66 ± 0.12 | 2.17 ± 0.12 |
| 17 | Spain | Ebro Delta – EDe | 2013 | 17 | 19.1 ± 0.5 | 0.024 ± 0.002 | 1.82 ± 0.37 | 2.27 ± 0.08 |
| 18 | Spain | Sagunt – Sag | 2013 | 19 | 19.2 ± 0.6 | 0.023 ± 0.003 | 0.77 ± 0.27 | 2.00 ± 0.11 |
| 19 | Spain | Rio Júcar S – RJS | 2013 | 9 | 19.9 ± 0.5 | 0.022 ± 0.003 | 0.95 ± 0.19 | 2.07 ± 0.10 |
| 20 | Spain | Rio Segura – RSe | 2013 | 23 | 19.0 ± 0.3 | 0.023 ± 0.001 | 0.73 ± 0.11 | 2.28 ± 0.11 |
| 21 | Spain | Almanzora – Alm | 2013 | 9 | 18.3 ± 0.2 | 0.020 ± 0.001 | 0.59 ± 0.16 | 1.84 ± 0.09 |
| 22 | Spain | Guadalquivir – Gdq | 2013 | 23 | 18.4 ± 0.2 | 0.021 ± 0.001 | 0.74 ± 0.17 | 1.83 ± 0.08 |
| 23 | Spain | Guadiaro – Gdr | 2013 | 19 | 18.9 ± 0.6 | 0.026 ± 0.003 | 0.56 ± 0.12 | 2.04 ± 0.08 |
| 24 | Italy | Lago di Fimon W – LFW | 2017 | 18 | 19.0 ± 0.4 | 0.023 ± 0.002 | 4.33 ± 0.92 | 2.73 ± 0.13 |
| 25 | Italy | Lago di Fimon S – LFS | 2017 | 11 | 18.1 ± 0.4 | 0.022 ± 0.002 | 4.10 ± 1.16 | 3.29 ± 0.11 |
| 26 | Italy | Marina Grosseto – MGr | 2017 | 22 | 18.0 ± 0.2 | 0.021 ± 0.001 | 3.05 ± 0.68 | 3.56 ± 0.15 |
| 27 | Italy | Grosseto – Gro | 2017 | 6 | 17.5 ± 0.6 | 0.019 ± 0.002 | 1.56 ± 1.03 | 4.51 ± 0.40 |
| 5 | Italy | Torre Castiglione – TCa | 2017 | 21 | 20.9 ± 0.3 | 0.033 ± 0.002 | 1.96 ± 0.52 | 2.52 ± 0.16 |
| 6 | Italy | Porto Cesareo – PCe | 2017 | 5 | 15.7 ± 0.6 | 0.012 ± 0.001 | 1.25 ± 1.25 | 2.99 ± 0.35 |
| 28 | Spain | Gualta – Glt | 2017 | 22 | 17.9 ± 0.2 | 0.019 ± 0.001 | 2.38 ± 0.79 | 2.94 ± 0.14 |
| 29 | Spain | Tortosa – Tor | 2017 | 10 | 19.4 ± 0.4 | 0.027 ± 0.002 | 2.82 ± 0.82 | 3.25 ± 0.23 |
| 30 | Spain | L’Estanyol – Est | 2017 | 8 | 17.5 ± 0.2 | 0.018 ± 0.001 | 5.48 ± 1.82 | 3.05 ± 0.50 |
| 18 | Spain | Sagunt – Sag | 2017 | 3 | 18.2 ± 0.5 | 0.019 ± 0.002 | 3.33 ± 3.33 | 2.87 ± 0.22 |
| 31 | Spain | El Palmar - EPa | 2017 | 18 | 19.2 ± 0.7 | 0.028 ± 0.003 | 3.70 ± 0.95 | 2.98 ± 0.21 |
| 32 | Spain | Rio Júcar N – RJN | 2017 | 11 | 17.4 ± 0.4 | 0.020 ± 0.002 | 3.58 ± 1.16 | 3.01 ± 0.09 |
| 33 | Spain | Rio Vaca - RVa | 2017 | 9 | 19.1 ± 0.6 | 0.026 ± 0.003 | 2.48 ± 1.38 | 2.17 ± 0.12 |
| 34 | Spain | Lebrija – Leb | 2017 | 11 | 19.8 ± 0.3 | 0.024 ± 0.001 | 3.55 ± 1.12 | 2.95 ± 0.16 |
| 22 | Spain | Guadalquivir – Gdq | 2017 | 11 | 19.7 ± 0.5 | 0.026 ± 0.002 | 4.31 ± 1.18 | 2.52 ± 0.17 |
| 35 | Spain | Doñana N – DoN | 2017 | 9 | 17.8 ± 0.3 | 0.022 ± 0.002 | 2.51 ± 1.14 | 4.64 ± 0.21 |
| 36 | Spain | Doñana W – DoW | 2017 | 21 | 18.4 ± 0.3 | 0.020 ± 0.002 | 3.06 ± 0.90 | 1.98 ± 0.14 |

**Table S7**.Descriptive statistics (mean ± s.e.m.) of life-history traits of wild-caught female *G. holbrooki*.

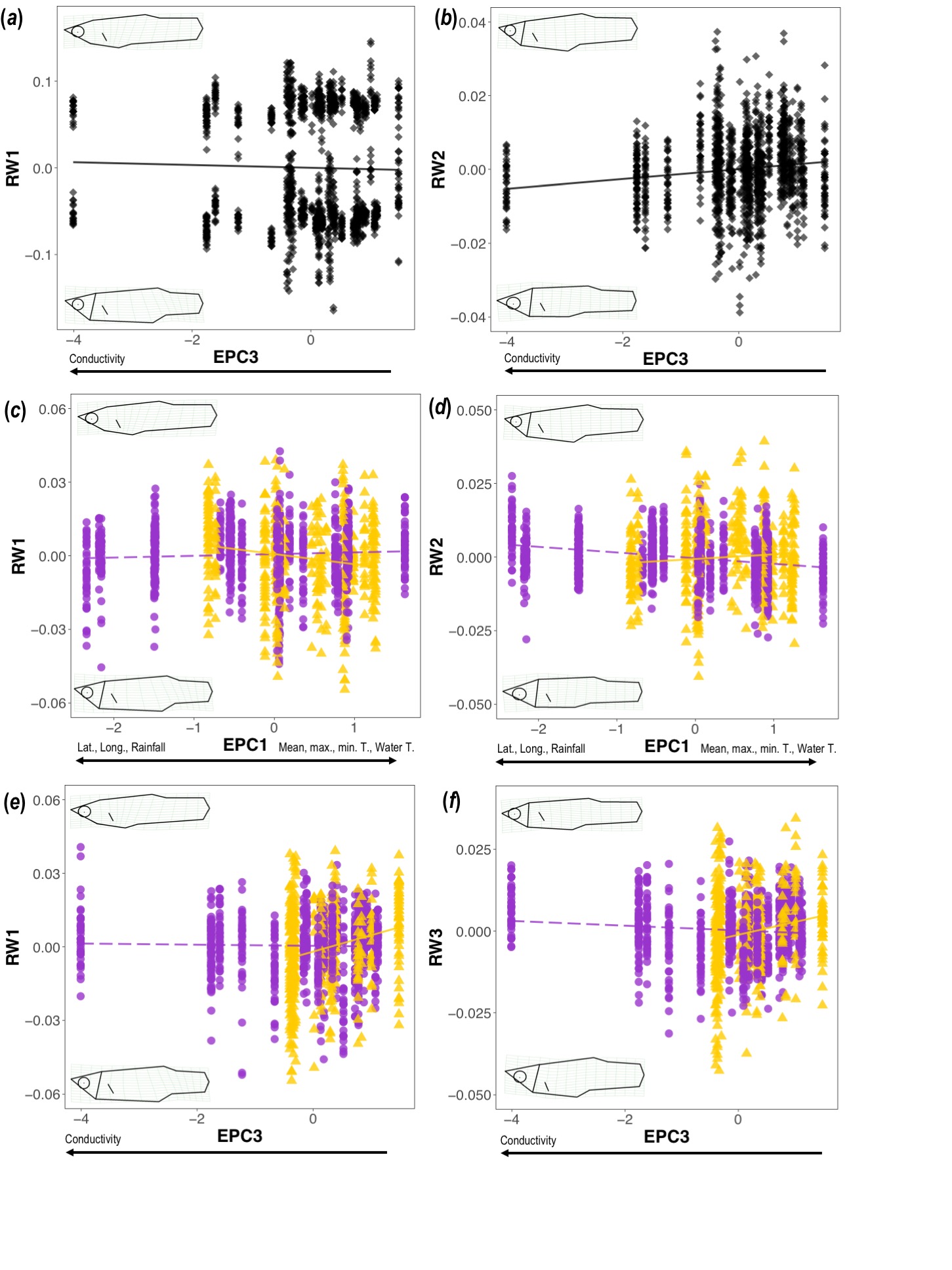
|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site number |  | Population name and code | Year | *N* | SL [mm] | Lean weight [g] | Fat content [%] | Fecundity | Embryo fat content [%] | Embryo lean weight [mg] | RA [%] |
| 1 | Italy | Lago di Garda – LdG | 2013 | 23 | 28.2 ± 0.4 | 0.099 ± 0.004 | 0.59 ± 0.05 | 35.74 ± 3.20 | 0.95 ± 0.21 | 0.66 ± 0.03 | 18.78 ± 1.28 |
| 2 | Italy | Comacchio – Com | 2013 | 17 | 35.5 ± 0.7 | 0.214 ± 0.015 | 0.68 ± 0.10 | 57.41 ± 3.49 | 0.68 ± 0.18 | 0.82 ± 0.03 | 18.99 ± 0.98 |
| 3 | Italy | Lago di Bolsena – LdB | 2013 | 21 | 34.0 ± 1.0 | 0.167 ± 0.017 | 0.42 ± 0.10 | 39.86 ± 2.29 | 1.63 ± 0.32 | 0.87 ± 0.04 | 18.48 ± 1.28 |
| 4 | Italy | Barletta – Blt | 2013 | 18 | 26.0 ± 0.6 | 0.069 ± 0.005 | 2.47 ± 0.33 | 14.22 ± 1.22 | 1.88 ± 0.28 | 0.84 ± 0.07 | 14.57 ± 1.37 |
| 5 | Italy | Torre Castiglione – TCa | 2013 | 20 | 30.2 ± 0.5 | 0.122 ± 0.008 | 0.36 ± 0.07 | 12.55 ± 1.30 | 1.72 ± 0.64 | 1.20 ± 0.05 | 11.92 ± 1.62 |
| 6 | Italy | Porto Cesareo – PCe | 2013 | 18 | 27.4 ± 0.4 | 0.081 ± 0.004 | 0.61 ± 0.09 | 15.33 ± 1.53 | 0.78 ± 0.34 | 1.12 ± 0.03 | 17.12 ± 0.89 |
| 7 | France | Briere – Bri | 2013 | 14 | 23.9 ± 0.5 | 0.053 ± 0.004 | 0.13 ± 0.06 | 11.50 ± 1.17 | 0.45 ± 0.21 | 0.80 ± 0.04 | 14.97 ± 1.24 |
| 8 | France | La Ligneron – LLi | 2013 | 21 | 27.4 ± 0.6 | 0.088 ± 0.006 | 0.42 ± 0.09 | 24.90 ± 2.67 | 1.18 ± 0.32 | 0.79 ± 0.02 | 17.71 ± 0.77 |
| 9 | France | La Charente – LCh | 2013 | 25 | 27.9 ± 0.9 | 0.082 ± 0.010 | 2.75 ± 0.16 | 23.84 ± 4.46 | 0.86 ± 0.15 | 0.64 ± 0.02 | 14.33 ± 0.82 |
| 10 | France | Garonne – Gar | 2013 | 25 | 26.2 ± 0.4 | 0.066 ± 0.003 | 2.38 ± 0.14 | 28.00 ± 4.71 | 0.93 ± 0.10 | 0.64 ± 0.02 | 19.74 ± 0.93 |
| 11 | France | Avignon – Avi | 2013 | 23 | 32.7 ± 0.4 | 0.152 ± 0.007 | 1.21 ± 0.09 | 89.04 ± 5.62 | 0.78 ± 0.16 | 0.49 ± 0.02 | 21.90 ± 0.82 |
| 12 | France | Arles – Arl | 2013 | 25 | 35.4 ± 0.7 | 0.164 ± 0.009 | 2.13 ± 0.19 | 39.44 ± 3.29 | 2.08 ± 0.48 | 1.00 ± 0.05 | 18.46 ± 0.70 |
| 13 | France | Montpellier – Mon | 2013 | 18 | 26.5 ± 1.1 | 0.063 ± 0.005 | 0.31 ± 0.10 | 20.83 ± 1.70 | 0.58 ± 0.23 | 0.70 ± 0.04 | 18.87 ± 1.26 |
| 14 | Spain | Zadorra – Zad | 2013 | 14 | 26.3 ± 0.4 | 0.076 ± 0.002 | 0.44 ± 0.09 | 33.86 ± 2.57 | 0.78 ± 0.27 | 0.52 ± 0.02 | 18.76 ± 0.96 |
| 15 | Spain | Rio Ter – RTe | 2013 | 15 | 27.9 ± 0.6 | 0.079 ± 0.005 | 1.44 ± 0.17 | 20.13 ± 2.77 | 0.45 ± 0.10 | 0.90 ± 0.04 | 17.59 ± 1.15 |
| 16 | Spain | Barcelona – Bcn | 2013 | 20 | 26.8 ± 0.6 | 0.076 ± 0.005 | 0.49 ± 0.10 | 14.70 ± 1.94 | 0.43 ± 0.10 | 1.02 ± 0.03 | 15.95 ± 1.41 |
| 17 | Spain | Ebro Delta – EDe | 2013 | 21 | 31.9 ± 0.7 | 0.133 ± 0.011 | 3.86 ± 0.75 | 54.95 ± 4.36 | 7.41 ± 0.69 | 0.68 ± 0.03 | 22.57 ± 0.79 |
| 18 | Spain | Sagunt – Sag | 2013 | 22 | 29.5 ± 0.8 | 0.104 ± 0.010 | 0.72 ± 0.11 | 17.82 ± 1.45 | 0.57 ± 0.10 | 0.92 ± 0.06 | 13.80 ± 0.87 |
| 19 | Spain | Rio Júcar S – RJS | 2013 | 24 | 33.5 ± 0.5 | 0.140 ± 0.008 | 1.90 ± 0.36 | 36.92 ± 2.73 | 1.33 ± 0.29 | 0.99 ± 0.02 | 20.30 ± 0.68 |
| 20 | Spain | Rio Segura – RSe | 2013 | 25 | 23.2 ± 0.4 | 0.047 ± 0.003 | 0.69 ± 0.09 | 11.04 ± 1.27 | 0.59 ± 0.11 | 0.86 ± 0.04 | 15.60 ± 1.14 |
| 21 | Spain | Almanzora – Alm | 2013 | 18 | 26.2 ± 0.7 | 0.074 ± 0.007 | 0.70 ± 0.11 | 9.28 ± 1.05 | 1.31 ± 0.27 | 0.98 ± 0.04 | 11.66 ± 1.28 |
| 22 | Spain | Guadalquivir – Gdq | 2013 | 22 | 29.9 ± 0.9 | 0.110 ± 0.010 | 0.28 ± 0.07 | 18.68 ± 1.46 | 0.29 ± 0.09 | 0.97 ± 0.03 | 14.59 ± 0.88 |
| 23 | Spain | Guadiaro – Gdr | 2013 | 23 | 31.9 ± 1.0 | 0.127 ± 0.010 | 0.46 ± 0.08 | 34.13 ± 3.87 | 1.67 ± 0.41 | 0.83 ± 0.05 | 17.73 ± 1.12 |
| 24 | Italy | Lago di Fimon W – LFW | 2017 | 29 | 27.5 ± 1.0 | 0.092 ± 0.011 | 2.35 ± 0.33 | 16.45 ± 2.35 | 5.78 ± 0.92 | 0.84 ± 0.03 | 13.41 ± 0.83 |
| 25 | Italy | Lago di Fimon S – LFS | 2017 | 10 | 30.0 ± 1.8 | 0.120 ± 0.022 | 2.29 ± 0.45 | 24.20 ± 2.28 | 3.24 ± 1.08 | 0.76 ± 0.03 | 14.73 ± 0.87 |
| 26 | Italy | Marina Grosseto – MGr | 2017 | 20 | 24.5 ± 1.1 | 0.063 ± 0.009 | 3.43 ± 0.69 | 23.35 ± 3.29 | 3.63 ± 1.01 | 0.83 ± 0.03 | 23.52 ± 1.28 |
| 27 | Italy | Grosseto – Gro | 2017 | 10 | 25.6 ± 1.1 | 0.070 ± 0.008 | 2.49 ± 0.54 | 27.90 ± 5.10 | 0.72 ± 0.41 | 0.55 ± 0.01 | 16.67 ± 1.28 |
| 5 | Italy | Torre Castiglione – TCa | 2017 | 27 | 30.9 ± 0.5 | 0.132 ± 0.008 | 1.46 ± 0.28 | 33.37 ± 2.00 | 2.13 ± 0.55 | 1.11 ± 0.04 | 22.43 ± 1.11 |
| 6 | Italy | Porto Cesareo – PCe | 2017 | 7 | 20.6 ± 0.3 | 0.037 ± 0.002 | 2.07 ± 1.07 | 5.86 ± 0.40 | 0.63 ± 0.43 | 0.83 ± 0.08 | 11.81 ± 1.45 |
| 28 | Spain | Gualta – Glt | 2017 | 24 | 24.4 ± 0.6 | 0.059 ± 0.005 | 2.63 ± 0.46 | 14.54 ± 1.40 | 4.97 ± 0.86 | 0.82 ± 0.03 | 17.25 ± 0.94 |
| 29 | Spain | Tortosa – Tor | 2017 | 12 | 26.9 ± 0.8 | 0.080 ± 0.008 | 2.88 ± 0.41 | 26.5 ± 3.97 | 1.57 ± 0.47 | 0.75 ± 0.03 | 19.05 ± 1.89 |
| 30 | Spain | L'Estanyol – Est | 2017 | 0 | - | - | - | - | - | - | - |
| 18 | Spain | Sagunt – Sag | 2017 | 6 | 26.7 ± 2.1 | 0.081 ± 0.022 | 1.53 ± 0.57 | 14.17 ± 4.33 | 2.26 ± 1.46 | 1.02 ± 0.06 | 14.77 ± 1.20 |
| 31 | Spain | El Palmar – EPa | 2017 | 28 | 29.6 ± 0.6 | 0.113 ± 0.007 | 1.64 ± 0.24 | 43.07 ± 5.78 | 1.27 ± 0.22 | 0.70 ± 0.03 | 18.63 ± 1.74 |
| 32 | Spain | Rio Júcar N – RJN | 2017 | 8 | 35.8 ± 1.0 | 0.196 ± 0.019 | 1.43 ± 0.23 | 60.00 ± 8.09 | 0.30 ± 0.16 | 0.75 ± 0.04 | 17.88 ± 1.84 |
| 33 | Spain | Rio Vaca – RVa | 2017 | 9 | 24.2 ± 1.9 | 0.060 ± 0.014 | 2.12 ± 0.73 | 8.89 ± 2.53 | 2.88 ± 1.42 | 0.72 ± 0.04 | 9.49 ± 1.07 |
| 34 | Spain | Lebrija – Leb | 2017 | 10 | 28.1 ± 0.9 | 0.085 ± 0.008 | 1.78 ± 0.41 | 14.90 ± 1.93 | 0.94 ± 0.23 | 1.16 ± 0.03 | 16.67 ± 1.02 |
| 22 | Spain | Guadalquivir – Gdq | 2017 | 10 | 29.4 ± 0.6 | 0.105 ± 0.006 | 0.53 ± 0.24 | 19.60 ± 1.63 | 1.67 ± 0.49 | 1.15 ± 0.04 | 17.68 ± 0.96 |
| 35 | Spain | Doñana N – DoN | 2017 | 10 | 30.8 ± 1.1 | 0.127 ± 0.015 | 0.50 ± 0.22 | 58.10 ± 11.26 | 2.74 ± 1.53 | 0.56 ± 0.05 | 19.31 ± 2.17 |
| 36 | Spain | Doñana W – DoW | 2017 | 19 | 28.9 ± 0.8 | 0.103 ± 0.009 | 5.90 ± 1.04 | 12.58 ± 1.88 | 7.58 ± 1.43 | 1.10 ± 0.07 | 11.58 ± 1.24 |

**Table S8.** MANCOVA on phenotypic variation in invasive mosquitofish between different populations and population genetic clusters. We inferred population genetic cluster information for our Italian and Spanish populations sampled in 2017 from the Structure analysis of our 2013 samples (Fig. 2*b*) for *K* = 4. (*a*) Male and female body shape, (*b*) male and (*c*) female life-history traits. Significant effects are highlighted in bold.

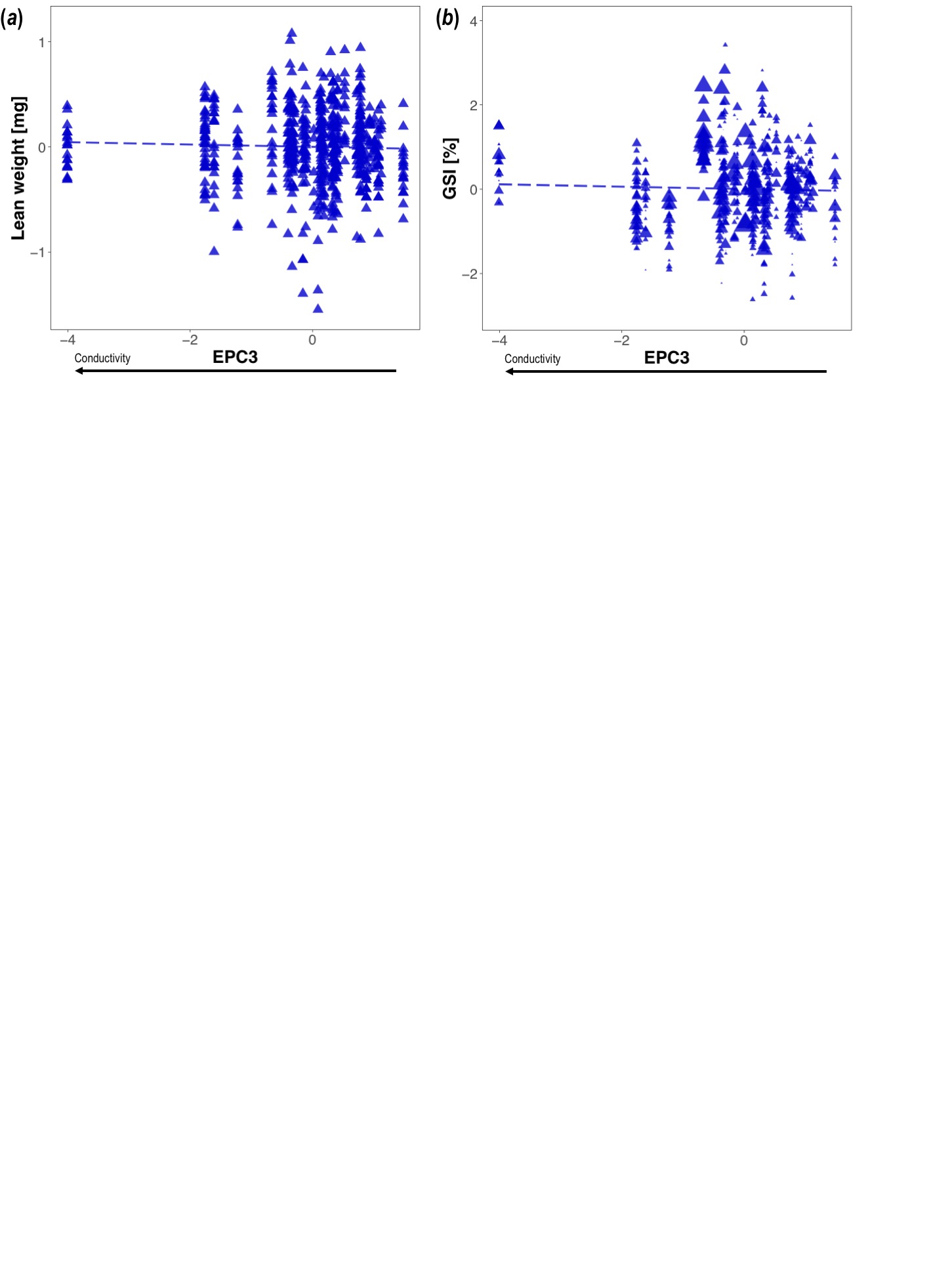
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| --- | --- | --- | --- | --- | --- | --- |
|  | Factor | *F* | Degrees of freedom | *P* | | Partial *η*2 |
| (*a*) male and female body shape | **Sex** | 8,842.644 | 3, 1252 | **< 0.001** | 0.955 | |
| **Year** | 17.112 | 3, 1252 | **< 0.001** | 0.039 | |
| **Centroid size** | 8.630 | 3, 1252 | **< 0.001** | 0.020 | |
| **Cluster** | 11.347 | 3, 1252 | **< 0.001** | 0.026 | |
| **Population(cluster)** | 7.829 | 102, 3750 | **< 0.001** | 0.175 | |
| Sex × year | 2.169 | 3, 1252 | 0.090 | 0.005 | |
| **Sex × population(cluster)** | 4.754 | 102, 3750 | **< 0.001** | 0.114 | |
| **Year × cluster** | 5.626 | 3, 1252 | **0.001** | 0.013 | |
| **Year × population(cluster)** | 5.414 | 6, 2504 | **< 0.001** | 0.013 | |
| (*b*) male life-history traits | **SL** | 510.221 | 3, 544 | **< 0.001** | 0.738 | |
| **Year** | 12.931 | 3, 544 | **< 0.001** | 0.067 | |
| **Cluster** | 3.152 | 3, 544 | **0.025** | 0.017 | |
| **Population(cluster)** | 4.522 | 102, 1639 | **< 0.001** | 0.220 | |
| **SL × population(cluster)** | 1.347 | 105, 1630 | **0.013** | 0.080 | |
| **Year × cluster** | 2.688 | 3, 544 | **0.046** | 0.015 | |
| (*c*) female life-history traits | **Embryo stage** | 7.081 | 6, 633 | **< 0.001** | 0.063 | |
| **SL** | 655.886 | 6, 633 | **< 0.001** | 0.861 | |
| **Year** | 8.453 | 6, 633 | **< 0.001** | 0.074 | |
| **Cluster** | 14.974 | 6, 633 | **< 0.001** | 0.124 | |
| **Population(cluster)** | 7.698 | 198, 3753 | **< 0.001** | 0.265 | |
| **SL × cluster** | 5.040 | 6, 633 | **< 0.001** | 0.046 | |
| **SL × population(cluster)** | 3.108 | 198, 3753 | **< 0.001** | 0.139 | |
| **Year × cluster** | 7.763 | 6, 633 | **< 0.001** | 0.069 | |

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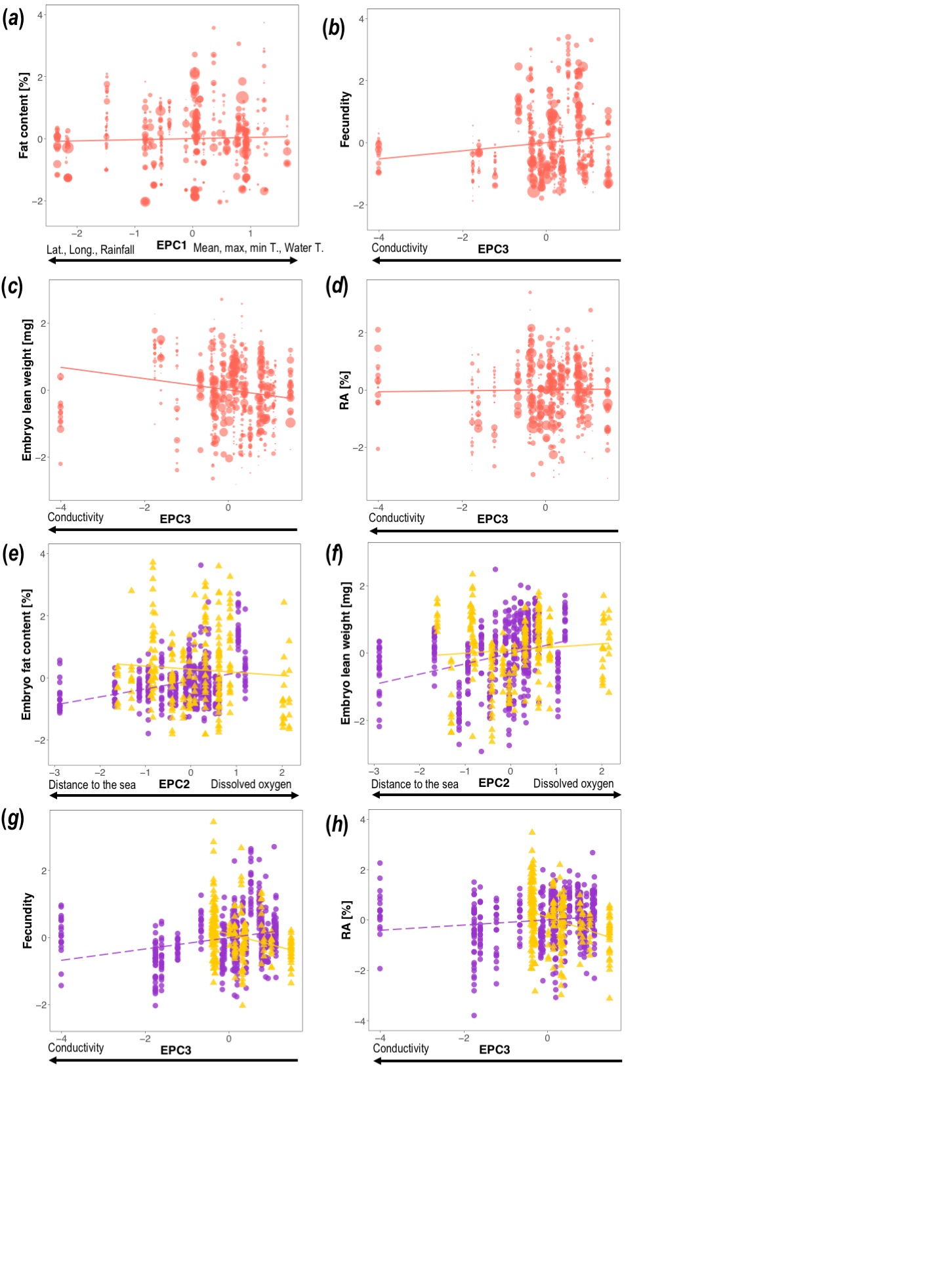
**Figure S3.** Sexual dimorphism in body shape in wild-caught *G. holbrooki*. Males: blue triangles, females: red circles. Morphological variation is visualised using thin-plate spline transformation. RW1 mainly separates males (anal fin positioned towards the anterior, creating a small body cavity) from females (anal fin positioned towards the posterior, creating a large body cavity), whereas RW2 describes differences in body depth and head size.



**Figure S4**. Partial regression plots (residuals corrected for all other terms in the model) of climate-dependent (EPC1) and -independent (EPC3) variation of body shape in wild-caught *G. holbrooki*. (*a*), (b) variation along EPC3; (*c*) RW1 and (d) RW2 variation along EPC1; (*e*) RW1 and (*f*) RW2 variation across EPC3 in fish sampled in 2013 (purple circles) and 2017 (yellow triangles).



**Figure S5**. Partial regression plots (residuals corrected for all other terms in the model) of EPC3-effects on (*a*) lean weight, and (*b*) GSI in wild-caught male *Gambusia holbrooki*. In (*b*), the size of the data points is proportional to SL.



**Figure S6**. Partial regression plots (residuals corrected for all other terms in the model) of climate-dependent (EPC1) and -independent (EPC2, EPC3) variation of female life histories in wild-caught *G. holbrooki*. (*a*) Fat content variation along EPC1; (*b*) fecundity, (*c*) embryo lean weight, and (*d*) RA variation along EPC3. The size of the data points is proportional to SL. (*e*) Embryo fat content, and (*f*) embryo lean weight variation along EPC2, and (*g*) fecundity, and (*h*) RA variation across EPC3 in fish sampled in 2013 (purple circles) and 2017 (yellow triangles).

**Online Supplement 4**

**Extended results and discussion—phenotypic differences between wild-caught and laboratory-raised mosquitofish**

The ANCOVA on male and female SL yielded significant effects of ‘sex’ (*F*1 ,224 = 469.54, *P* < 0.001, *η*p2= 0.689), ‘population’ (*F*3, 224 = 8.51, *P* < 0.001; *η*p2= 0.105), ‘generation’ (*F*1, 224 = 138.85, *P* < 0.001, *η*p2= 0.396), but also of the interactions ‘population × generation’ (*F*3, 224 = 32.24, *P* < 0.001, *η*p2= 0.349), ‘sex × population’ (*F*3, 224 = 9.26, *P* < 0.001, *η*p2= 0.129), and ‘sex × population × generation’ (*F*4, 224 = 11.08, *P* < 0.001, *η*p2= 0.249). Males (estimated marginal mean ± s.e.m. = 23.16 ± 0.29 mm) were smaller than females (32.31 ± 0.29 mm), and overall, the largest fish came from Lago di Garda (29.32 ± 0.48 mm), with intermediate-sized fish at Comacchio (28.20 ± 0.40 mm) and Torre Castiglione (27.14 ± 0.32 mm), while the smallest fish originated from Zadorra (26.29 ± 0.43 mm). However, the smallest males came from Zadorra (21.26 ± 0.59 mm) and the largest ones from Torre Castiglione (24.10 ± 0.45 mm) and Comacchio (24.09 ± 0.55 mm), whereas the smallest females came from Torre Castiglione (30.18 ± 0.45 mm) and by far the largest females from Lago di Garda (35.44 ± 0.66 mm). In addition, second-generation laboratory-reared fish were larger (30.23 ± 0.32 mm) than wild-caught individuals (25.25 ± 0.26 mm), but this size difference was population-specific, with the largest increase in fish from Lago di Garda (ΔSL = 9.37 mm), followed by fish from Zadorra (ΔSL = 8.14 mm) and Torre Castiglione (ΔSL = 4.33 mm), while fish from Comacchio actually became smaller (ΔSL = 2.28 mm). For males, the relative size differences between wild-caught males from different populations were largely replicated for F2 laboratory-reared males, but population-specific patterns of female size differed between wild-caught and laboratory-reared females (Fig. 4*a*).

The MANCOVA on body shape revealed significant effects of the covariate ‘centroid size’ (*F*3, 242 = 10.28, *P* < 0.001) but also of all factors and interactions (sex: *F*3, 242 = 1,890.81, *P* < 0.001; population: *F*9, 589 = 3.02, *P* = 0.002; generation: *F*3, 242 = 13.74, *P* < 0.001; sex × generation: *F*3, 242 = 5.09, *P* = 0.002; sex × population: *F*9, 589 = 2.80, *P* = 0.003; population × generation: *F*9, 589 = 6.85, *P* < 0.001; sex × population × generation: *F*9, 589 = 3.14, *P* = 0.001). ‘Sex’ had by far the strongest effect (*η*p2= 0.959), followed by ‘generation’ (*η*p2= 0.146) and ‘centroid size’ (*η*p2= 0.113), while ‘population’ and all interactions were of relatively minor importance (*η*p2< 0.079 in all cases). *Post-hoc* ANCOVAs (*α’* = 0.017, corrected for multiple testing) revealed that sexes differed along RW1 (*F*1, 244 = 5,070.23, *P* < 0.001, *η*p2= 0.954) with males being characterized by larger eyes, smaller heads, deeper caudal peduncles, and an anal fin that was positioned more anteriorly compared to females. Populations differed significantly in RW3 (*F*3, 244 = 1.07, *P* = 0.001, *η*p2= 0.065), with fish from Zadorra having slightly deeper bodies, smaller eyes, a slightly more superiorly-positioned mouth, slightly more ventrally-positioned pectoral fins, and the dorsal part of their heads extending further into the body compared to fish from Torre Castiglione, while fish from Comacchio and Lago di Garda showed intermediate character state combinations. Lastly, wild-caught and laboratory-reared fish differed significantly in RW1 (*F*1, 244 = 21.67, *P* < 0.001, *η*p2= 0.082) and RW2 (*F*1, 244 = 20.24, *P* < 0.001, *η*p2= 0.077). Wild-caught fish had slightly deeper bodies, shorter caudal peduncles, smaller eyes, and their anal fin was positioned more posteriorly compared to laboratory-reared individuals (RW1), and showed slightly deeper bodies, larger heads coupled with larger eyes, and more posteriorly-positioned pectoral fins (RW2). However, the interaction effects indicated that the differences between populations were both sex-specific and moderated by differences between wild-caught and laboratory reared fish.

The MANCOVA on male life histories revealed significant effects of the covariate SL (*F*3, 111 = 207.68, *P* < 0.001, *η*p2= 0.849), the factors ‘population’ (*F*9, 270 = 6.02, *P* < 0.001, *η*p2= 0.138) and ‘generation’ (*F*3, 111 = 87.80, *P* < 0.001, *η*p2= 0.704), as well as the interaction ‘population × generation’ (*F*9, 270 = 11.44, *P* < 0.001, *η*p2= 0.230). *Post-hoc* ANCOVAs (*α’* = 0.017) of the same model structure indicated that SL had significant effects on all three traits (*P* < 0.001 in all cases), and both lean weight (*η*p2= 0.848) and body fat (*η*p2= 0.327) increased with increasing SL, while GSI (*η*p2= 0.142) decreased. Populations differed significantly in lean weight (*P* = 0.007, *η*p2= 0.102) and fat content (*P* < 0.001, *η*p2= 0.193), with males from Torre Castiglione having the smallest and males from Zadorra the greatest lean weight (Fig 4*b*; Table S9). Wild-caught fish differed from F2 laboratory-reared fish in all three traits (*P* < 0.001 in all cases), with lean weight (*η*p2= 0.245) and GSI (*η*p2= 0.225) being lower in laboratory-reared fish and fat content (*η*p2= 0.575) being higher (Table S10). Nonetheless, there were also population-specific responses to the laboratory environment in fat content (*η*p2= 0.314) and GSI (*η*p2= 0.436; *P* < 0.001 in both cases), while this was not evident for lean weight (*P* = 0.889). Fat content increased in all populations, but showed by far the strongest increase in Comacchio (Δfat content = 18.60%) and the weakest increase in Zadorra (Δfat content = 3.10%; Table S7). GSI, on the other hand, increased from wild-caught to laboratory-raised males only in Zadorra, while it decreased in all other populations (Table S11).

The MANCOVA on female life histories demonstrated significant effects of the covariates ‘embryonic stage of development’ (*F*6, 103 = 2.26, *P* = 0.043, *η*p2= 0.116) and SL (*F*6, 103 = 40.03, *P* < 0.001, *η*p2= 0.700), the factors ‘population’ (*F*18, 292 = 12.06, *P* < 0.001, *ηp*2= 0.408) and ‘generation’ (*F*6, 103 = 127.39, *P* < 0.001, *ηp*2= 0.881), as well as the interaction ‘population × generation’ (*F*18, 292 = 6.97, *P* < 0.001, *η*p2= 0.286). *Post-hoc* ANCOVAs (*α*’ = 0.008) of the same model structure revealed that ‘embryonic stage of development’ did not significantly affect any individual life-history trait, while SL had significant effects on lean weight (*η*p2= 0.652), fat content (*η*p2= 0.140), fecundity (*η*p2= 0.234) and embryo fat (*η*p2= 0.108; *P* < 0.001 in all cases), all of which increased with increasing female size. Populations differed significantly in fat content (*η*p2= 0.404), fecundity (*η*p2= 0.377), embryo lean weight (*η*p2= 0.314; *P* < 0.001 in all cases), and embryo fat content (*P* = 0.001, *η*p2= 0.144; Fig. 4*c*). Females from Zadorra had the greatest somatic fat content and also had embryos with the greatest fat content, and together with females from Comacchio, had the highest fecundity (Table S12). Females from Torre Castiglione had the lowest fat content and fecundity, coupled with the heaviest embryos, and females from Comacchio had embryos with the lowest fat content (Table S12). Wild-caught females differed from F2 laboratory-reared females in somatic fat content (*η*p2= 0.861), embryo lean weight (*η*p2= 0.538), embryo fat content (*η*p2= 0.626; all *P* < 0.001), and fecundity (*P* = 0.001, *η*p2= 0.092), with somatic fat content, embryo lean weight and embryo fat content being higher in laboratory-reared fish and fecundity being higher in wild-caught fish (Table S13). Furthermore, there were again population-specific responses to the laboratory environment. These were uncovered for fat content (*η*p2= 0.428), fecundity (*η*p2= 0.268), embryo lean weight (*η*p2= 0.334), and RA (*η*p2= 0.179; *P* < 0.001 in all cases). Fat content increased in all populations, but showed the strongest increase in Comacchio (Δfat content = 29.4%) and the weakest increase in Torre Castiglione (Δfat content = 10.9%; Table S14). Fecundity, on the other hand, decreased from wild-caught to laboratory-raised fish in Zadorra, Comacchio and Lago di Garda, while it increased in Torre Castiglione (Table S14). Embryo lean weight increased in all populations from wild-caught to lab-raised females, with the strongest increase in Lago di Garda females (Δembryo lean weight = 0.880 mg) and the weakest increase in females from Torre Castiglione (Δembryo lean weight = 0.256 mg). Lastly, RA strongly increased from wild-caught to lab-raised females in Torre Castiglione (ΔRA = 9.3%), remained rather unchanged in both Zadorra (ΔRA = 0.2%) and Lago di Garda (ΔRA = 0.5%), but strongly decreased in Comacchio (ΔRA = 4.6%; Table S14).

In general, phenotypic responses to the laboratory environment—rounder, deeper bodies, increased body size and fat content, coupled with reduced GSI in males and increased offspring size and lower fecundity in females—appear to be plastic and likely reflect increased food availability (i.e. *ad libitum* feeding), as they follow predictions from life-history theory (Stearns, 1989; Reznick et al., 2002; Spoljaric and Reimchen, 2007).

**Table S9.** Estimated marginal means and standard errors (s.e.m.) for significant population-level differences in male lean weight and somatic fat content (derived from MANCOVA; evaluated at SL = 23.17 mm).

|  |  |  |  |
| --- | --- | --- | --- |
| Trait | Population | Mean | s.e.m. |
| Lean weight [mg] | Zadorra (ES) | 52.26 | 1.84 |
|  | Torre Castiglione (IT) | 43.93 | 1.34 |
|  | Comacchio (IT) | 45.69 | 1.62 |
|  | Lago di Garda (IT) | 49.42 | 2.07 |
| Fat content [%] | Zadorra (ES) | 8.55 | 0.90 |
|  | Torre Castiglione (IT) | 4.72 | 0.66 |
|  | Comacchio (IT) | 10.03 | 0.80 |
|  | Lago di Garda (IT) | 8.96 | 1.01 |

**Table S10.** Estimated marginal means and standard errors (s.e.m.) for significant differences in lean weight, fat content and GSI between wild-caught and second-generation laboratory-reared male *G. holbrooki* (derived from MANCOVA; evaluated at SL = 23.17 mm).

|  |  |  |  |
| --- | --- | --- | --- |
| Trait | Generation | Mean | s.e.m. |
| Lean weight [mg] | Wild-caught | 55.51 | 1.40 |
|  | Lab-reared | 41.14 | 1.37 |
| Fat content [%] | Wild-caught | 3.48 | 0.69 |
|  | Lab-reared | 12.64 | 0.67 |
| GSI [%] | Wild-caught | 2.29 | 0.09 |
|  | Lab-reared | 1.27 | 0.09 |

**Table S11.** Estimated marginal means and standard errors (s.e.m.) for significant population-by-generation differences in male fat content and GSI (derived from MANCOVA; evaluated at SL = 23.17 mm).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Trait | Population | Generation | Mean | s.e.m. |
| Fat content [%] | Zadorra (ES) | Wild-caught | 6.99 | 1.18 |
|  |  | Lab-reared | 10.10 | 1.43 |
|  | Torre Castiglione (IT) | Wild-caught | 3.04 | 0.94 |
|  |  | Lab-reared | 6.39 | 1.08 |
|  | Comacchio (IT) | Wild-caught | 0.73 | 0.90 |
|  |  | Lab-reared | 19.32 | 1.31 |
|  | Lago di Garda (IT) | Wild-caught | 3.16 | 1.84 |
|  |  | Lab-reared | 14.76 | 0.99 |
| GSI [%] | Zadorra (ES) | Wild-caught | 1.77 | 0.16 |
|  |  | Lab-reared | 2.39 | 0.20 |
|  | Torre Castiglione (IT) | Wild-caught | 1.78 | 0.13 |
|  |  | Lab-reared | 1.28 | 0.15 |
|  | Comacchio (IT) | Wild-caught | 3.18 | 0.12 |
|  |  | Lab-reared | 0.69 | 0.18 |
|  | Lago di Garda (IT) | Wild-caught | 2.41 | 0.25 |
|  |  | Lab-reared | 0.74 | 0.13 |

**Table S12.** Estimated marginal means and standard errors (s.e.m.) for significant population-level differences in female fat content, fecundity, embryo lean weight, and embryo fat content (derived from MANCOVA; evaluated at SL = 30.78 mm and an embryonic stage of development = 15.38).

|  |  |  |  |
| --- | --- | --- | --- |
| Trait | Population | Mean | s.e.m. |
| Fat content [%] | Zadorra (ES) | 16.07 | 0.73 |
|  | Torre Castiglione (IT) | 7.35 | 0.50 |
|  | Comacchio (IT) | 13.83 | 0.66 |
|  | Lago di Garda (IT) | 13.21 | 0.95 |
| Fecundity | Zadorra (ES) | 34.31 | 2.56 |
|  | Torre Castiglione (IT) | 17.07 | 1.77 |
|  | Comacchio (IT) | 34.97 | 2.32 |
|  | Lago di Garda (IT) | 32.30 | 3.35 |
| Embryo lean weight [mg] | Zadorra (ES) | 0.99 | 0.05 |
|  | Torre Castiglione (IT) | 1.31 | 0.03 |
|  | Comacchio (IT) | 1.15 | 0.04 |
|  | Lago di Garda (IT) | 1.12 | 0.06 |
| Embryo fat content [%] | Zadorra (ES) | 10.59 | 0.68 |
|  | Torre Castiglione (IT) | 7.67 | 0.47 |
|  | Comacchio (IT) | 5.72 | 0.61 |
|  | Lago di Garda (IT) | 6.85 | 0.88 |

**Table S13.** Estimated marginal means and standard errors (s.e.m.) for significant differences in female fat content, fecundity, embryo lean weight and embryo fat content between wild-caught and second-generation laboratory-reared *G. holbrooki* (derived from MANCOVA; evaluated at SL = 30.78 mm and an embryonic stage of development = 15.38).

|  |  |  |  |
| --- | --- | --- | --- |
| Trait | Generation | Mean | s.e.m. |
| Fat content [%] | Wild-caught | 1.68 | 0.42 |
|  | Lab-reared | 23.54 | 0.74 |
| Fecundity | Wild-caught | 35.18 | 1.47 |
|  | Lab-reared | 24.15 | 2.58 |
| Embryo lean weight [mg] | Wild-caught | 0.82 | 0.03 |
|  | Lab-reared | 1.47 | 0.05 |
| Embryo fat content [%] | Wild-caught | 1.74 | 0.39 |
|  | Lab-reared | 13.68 | 0.68 |

**Table S14.** Estimated marginal means and standard errors (s.e.m.) for significant population-by-generation differences in female fat content, fecundity, embryo lean weight and RA (derived from MANCOVA; evaluated at SL = 30.78 mm and embryonic stage of development = 15.38).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Trait | Population | Generation | Mean | s.e.m. |
| Fat content [%] | Zadorra (ES) | Wild-caught | 4.08 | 0.96 |
|  |  | Lab-reared | 27.99 | 1.28 |
|  | Torre Castiglione (IT) | Wild-caught | 1.91 | 0.72 |
|  |  | Lab-reared | 12.77 | 0.74 |
|  | Comacchio (IT) | Wild-caught | < 0.01 | 0.92 |
|  |  | Lab-reared | 28.54 | 1.07 |
|  | Lago di Garda (IT) | Wild-caught | 1.55 | 0.79 |
|  |  | Lab-reared | 24.85 | 1.80 |
| Fecundity | Zadorra (ES) | Wild-caught | 38.43 | 3.39 |
|  |  | Lab-reared | 29.75 | 4.52 |
|  | Torre Castiglione (IT) | Wild-caught | 14.22 | 2.54 |
|  |  | Lab-reared | 19.95 | 2.61 |
|  | Comacchio (IT) | Wild-caught | 49.44 | 3.22 |
|  |  | Lab-reared | 20.20 | 3.78 |
|  | Lago di Garda (IT) | Wild-caught | 37.85 | 2.79 |
|  |  | Lab-reared | 26.92 | 6.35 |
| Embryo lean weight [mg] | Zadorra (ES) | Wild-caught | 0.63 | 0.06 |
|  |  | Lab-reared | 1.34 | 0.08 |
|  | Torre Castiglione (IT) | Wild-caught | 1.19 | 0.05 |
|  |  | Lab-reared | 1.44 | 0.05 |
|  | Comacchio (IT) | Wild-caught | 0.76 | 0.06 |
|  |  | Lab-reared | 1.51 | 0.07 |
|  | Lago di Garda (IT) | Wild-caught | 0.68 | 0.05 |
|  |  | Lab-reared | 1.56 | 0.12 |
| RA [%] | Zadorra (ES) | Wild-caught | 18.78 | 1.81 |
|  |  | Lab-reared | 18.85 | 2.42 |
|  | Torre Castiglione (IT) | Wild-caught | 10.77 | 1.36 |
|  |  | Lab-reared | 20.06 | 1.40 |
|  | Comacchio (IT) | Wild-caught | 20.58 | 1.72 |
|  |  | Lab-reared | 15.87 | 2.02 |
|  | Lago di Garda (IT) | Wild-caught | 16.89 | 1.50 |
|  |  | Lab-reared | 17.38 | 3.40 |



**Figure S4.** Thin-plate transformation grids visualising variation in body shape between wild-caught and laboratory-reared mosquitofish. RW1 mainly described differences between males and females, which were mostly due to the anteriorly-shifted anal fin—modified into the gonopodium—in males. RW2 described differences in body depth and head size, while RW3 described differences in both body depth and caudal peduncle depth.

**Online supplement 5**

**Additional discussion—yearly variation in life-histories and body shape**

While some responses to climate variation (and dissolved oxygen) were consistent across both sampling years, several traits showed pronounced inter-annual differences, and their responses to climate gradients were sometimes opposite between 2013 and 2017. These results might be due to the fact that we sampled (mostly) different populations between the two sampling years, or they might point towards the presence of plastic responses to selective pressures that we did not quantify with our sampling protocol, but may have differed between sampling years. These could include habitat productivity and food availability (Vondraceck et al., 1988), population density (Bisazza and Marin, 1995; Smith and Sargent, 2006; Reznick et al., 2019), or parasite load (Benejam et al., 2009).

In particular, both males and females were bigger in 2013 than in 2017. In 2013, males also had reduced fat content and lower GSI, possibly reflecting a trade-off between somatic growth and sperm production (but see Locatello et al., 2008; O’Dea et al., 2014). Additionally, in females, some life-history responses to the latitudinal gradient were reversed between 2013 and 2017. In 2017, they were characterised by increased investment into reproduction in colder environments, congruent with what has been reported for native *G. holbrooki* (Riesch et al., 2018), and with predictions from life-history theory regarding the effects of a high extrinsic mortality in harsh environments (Stearns, 1989; Reznick et al., 2002). In 2013, however, these patterns were reversed, as females from southern populations now showed an increased RA. This aligns with results of laboratory studies on the effects of constant temperature differences on *G. affinis* life histories (Vondraceck et al., 1988): higher temperatures caused mosquitofish to mature earlier at bigger body sizes, and to invest more into reproduction. At present, we do not have a convincing explanation for the underlying mechanisms driving these disparate patterns. Nevertheless, our results point towards phenotypic plasticity as a strategy to cope with changing environmental conditions, as demonstrated in other fishes (e.g., *Pomatoschistus microps*,Pampoulie et al., 2000; *Lepomis gibbosus*, Belk, 1995; *Gasterosteus aculeatus*, Baker et al., 2015).

Since we sampled our fish at different times throughout the reproductive season in 2013 and 2017, seasonal changes in life-history traits (Hughes, 1985) can also play a role in creating the observed phenotypic differences between sampling trips. During the reproductive season, which lasts from early spring to mid-autumn (Fraile et al., 1994; Reznick et al., 2006), mosquitofish increase population sizes (Pyke, 2005), which in turn drives body-size and life-history shifts (Hughes, 1985; Pyke, 2005; Reznick et al., 2006). For example, males born early in the reproductive season reach sexual maturity at smaller body sizes than those born towards the end of the reproductive season (Zulian et al., 1993; Reznick et al., 2006). Small males have higher reproductive success at low population densities, as they are more manoeuvrable and less easily spotted by females during sneaky copulation attempts (Pilastro et al., 1997), while big males have an advantage towards the end of the reproductive season, as they are able to monopolise access to the females (Bisazza and Marin, 1995). We accounted for seasonal differences in temperature in our climate data, but we could not properly control for other seasonal differences (e.g., population density). Indeed, inter-annual life-history differences were reduced when Spanish populations (sampled at more or less the same time in 2013 and 2017) were analysed separately, while they were maintained in Italian populations (sampled at the beginning and towards the end of the reproductive season in 2013 and 2017, respectively; results not shown). Future studies that quantify additional environmental selection factors and their seasonal fluctuation are needed in order to properly decipher these mechanisms.

**Online supplement 6**

**Trait heritability: cross-validation discriminant function analyses**

***Supplementary Methods***

To provide an intuitive measure of how similar laboratory-reared F2 fish were phenotypically compared to their respective wild-caught ancestors, we employed discriminant function analyses (DFA) to test for classification success based on population-level differentiation. To facilitate the DFAs, the effects of “centroid size” (for relative warps), “SL” (for the other three male life histories), or “SL” and “Embryonic stage of development” (for the other six female life histories) were removed first by using the residuals of preparatory MANCOVAs. We then ran four different cross-validation DFAs, one each for male and female body shapes, as well as for male and female life histories. For each model, all data from laboratory-reared F2 were initially withheld, and a DFA model was built solely on phenotypic data derived from wild-caught specimens (i.e., the training data set). Phenotypic data from laboratory-reared F2 (i.e., the testing data set) were then inserted into the discriminant functions and assigned to the most parsimonious population [i.e., Zadorra (ES), Torre Castiglione (IT), Comacchio (IT) and Lago di Garda (IT)].

***Supplementary Results***

For male and female body shape, cross-validation DFA success was very poor (11.9% for males and 10.5% for females; compared to a random classification success of 23.0% and 25.0%, respectively). For males, cross-validation success was highest for fish from Zadorra (75%) and lowest for fish from Comacchio and Lago di Garda (both 0%); in fact, almost all laboratory raised F2 (80%) were classified (correctly or incorrectly) as originating from Zadorra. Cross-validation success based on body shapes of females was again highest for fish from Zadorra (90%) and lowest for fish from Comacchio and Lago di Garda (both 0%); similar to males, almost all laboratory raised F2 (93%) were (mis-)classified as originating from Zadorra.

For male and female life histories, cross-validation DFA success was also quite poor but slightly better compared to models based on body shape (36.8% for males and 16.7% for females; compared to a random classification success of 23.1% and 25.3%, respectively). For males, cross-validation success was highest for fish from Torre Castiglione (100%) and lowest for fish from Zadorra and Lago di Garda (both 0%); for male life histories, almost all laboratory raised F2 (94.8%) were (mis-)classified as originating from Torre Castiglione. Cross-validation success based on female life histories, however, was highest for fish from Zadorra (57.1%) and lowest for fish from Lago di Garda (0%). For females, almost all laboratory raised F2 (95.2%) were (mis-)classified as originating from either Zadorra (59.5%) or Comacchio (35.7%).

**Supplementary references**

Baker, J.A., Wund, M.A., Heins, D.C., King, R.W., Reyes, M.L., Foster, S.A., 2015. Life-history plasticity in female threespine stickleback. Heredity 115, 322–334.

Belk, M.C., 1995. Variation in growth and age at maturity in bluegill sunfish: genetic or environmental effects? J. Fish Biol. 47, 237–247.

Benejam, L., Alcaraz, C., Sasal, P., Simon-Levert, G., García-Berthou, E., 2009. Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. Biol. Invasions 11, 2256–2277.

Bisazza, A., Marin, G., 1995. Sexual selection and sexual size dimorphism in the eastern mosquitofish *Gambusia holbrooki* (Pisces: Poeciliidae). Ethol. Ecol. Evol. 7, 169–183.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol. Ecol. 14, 2611–2620.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and Windows. Mol. Ecol. Res. 10, 564–567.

Fraile, B., Sáez, F.J., Vicentini, C.A., González, A., de Miguel, M.P., Paniagua, R., 1994. Effects of temperature and photoperiod on the *Gambusia affinis holbrooki* testis during the spermatogenesis period. Copeia 1, 216–221.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. http://www2.unil.ch/popgen/softwares/fstat.htm.

Hughes, A.L., 1985. Seasonal changes in fecundity and size at first reproduction in an Indiana population of the mosquitofish *Gambusia affinis*. Am. Midl. Nat. 1, 30–36.

Locatello, L., Rasotto, M.B., Adriaenssens, B., Pilastro, A., 2008. Ejaculate traits in relation to male body size in the eastern mosquitofish *Gambusia holbrooki*. J. Fish Biol. 73, 1600–1611.

O’Dea, R.E., Jennions, M.D., Head, M.L., 2014. Male body size and condition affects sperm number and production rates in mosquitofish, *Gambusia holbrooki*. J. Evol. Biol. 27, 2739–2744.

Pampoulie, C., Bouchereau, J.L., Rosecchie, E., Poizat, G., Crivelli, A.J., 2000. Annual variations in reproductive traits of *Pomatoschistus microps* in a Mediterranean lagoon undergoing environmental changes: evidence of phenotypic plasticity. J. Fish Biol. 57, 1441–1452.

Pilastro, A., Giacomello, E., Bisazza, A., 1997. Sexual selection for small size in male mosquitofish (*Gambusia holbrooki*). Proc. R. Soc. Lond. B 264, 1125–1129.

Pyke, G.H., 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. Rev. Fish Biol. Fisher. 5, 339–365.

Reznick, D.N., Bryant, M.J., Bashley, F., 2002. *r*- and *K*-selection revisited: the role of population regulation in life-history evolution. Ecology 83, 1509.

Reznick, D.N., Bassar, R.D., Handelsma, C.A., Ghalambor, C.K., Arendt, J., Coulson, T., Potter, T., Ruell, E.W., Torres-Dowdall, J., Bentzen, P., Travis, J., 2019. Eco-evolutionary feedbacks predict the time course of rapid life history evolution. Am. Nat. doi:10.1086/705380.

Reznick, D.N., Schultz, E., Morey, S., Roff, D., 2006. On the virtue of being the first born: the influence of date of birth on fitness in the mosquitofish, *Gambusia affinis*. Oikos 114, 135–147.

Riesch, R., Martin, R.A., Diamond, S.E., Jourdan, J., Plath, M., Langerhans, R.B., 2018. Thermal regime drives a latitudinal gradient in morphology and life history in a livebearing fish. Biol. J. Linn. Soc. 125, 126–141.

Rousset, F., 2019. Genepop version 4.7.3. This documentation: 06 December 2019.

Smith, C.C., Sargent, R.C., 2006. Female fitness declines with increasing female density but not male harassment in the western mosquitofish, *Gambusia affinis*. Anim. Behav. 71, 401–407.

Spoljaric, M.A., Reimchen, T.E., 2007. 10 000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. J. Fish Biol. 70, 1484–1503.

Stearns, S.C., 1989. Trade-offs in life-history evolution. Funct. Ecol. 3, 259.

Vondraceck, B., Wurtsbaughm, W.A., Cech, J.J., 1988. Growth and reproduction of the mosquitofish, *Gambusia affinis*, on relation to temperature and ration level: consequences for life history. Environ. Biol. Fish. 21, 45–57.

Zulian, E., Bisazza, A., Marin, G., 1993. Determinants of size in male eastern mosquitofish (*Gambusia holbrooki*): inheritance and plasticity of a sexual selected character. Ita. J. Zool. 60, 317–322.