**Online Supplementary Material:**

Riesch et al.: Thermal regime drives a latitudinal gradient in morphology and life history in a livebearing fish

**Section A. Collection Sites and Ecological Parameters**

**Table A1:** Summary of collection localities, water chemistry, population density estimates and sample sizes for life-history (numerator) and morphology (denominator) datasets.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Population (Code) | Latitude | Longitude | Conductivity[μS] | Chlorophyll *a*[RFU] | Density | Males | Females |
| 1 | Cape West May, NJ (NJ) | 38.93829 | -74.96416 | 2014 | 3.543 | 2 | 2/2 | 25/30 |
| 2 | Rehoboth Beach, DE (DE) | 38.71799 | -75.08268 | 249 | 6.783 | 1 | 11/8 | 30/30 |
| 3 | Suffolk, VA (VA) | 36.89134 | -76.44301 | 3894 | 0.304 | 2 | 9/9 | 19/30 |
| 4 | Hyde County, NC (NC) | 35.58629 | -76.50341 | 7541 | 6.364 | 3 | 20/20 | 29/28 |
| 5 | James Island Park, SC (SC) | 32.73412 | -79.99592 | 9818 | 6.425 | 2 | 20/20 | 26/22 |
| 6 | St. Simons, GA (GA) | 31.16918 | -81.41685 | 10000 | 10.020 | 1 | 20/27 | 17/16 |
| 7 | Daytona Beach, FL (FLDB) | 29.22824 | -81.02786 | 371 | 3.834 | 1 | 20/20 | 22/25 |
| 8 | Melbourne, FL (FLME) | 28.14445 | -80.59733 | 3075 | 25.090 | 2 | 20/19 | 28/24 |
| 9 | Port St. Lucie, FL (FLPL) | 27.29347 | -80.30026 | 338 | 5.261 | 1 | 16/16 | 22/18 |
| 10 | Zachary Taylor State Park, FL (FLZT) | 24.54694 | -81.80953 | 39839 | 7.926 | 3 | 21/21 | 5/4 |

Note: Population density was approximated as 1 = less than 100 individuals, 2 = greater than 100 but less than 500, and 3 = greater than 500.

**Section B. Principal Component Analysis**

**Table B1:** Principal components analysis of environmental variables associated with populations of *Gambusia holbrooki* sampled across a latitudinal gradient along the Atlantic Coast of the U.S.A.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Environmental Variable | PC1 | PC2 | PC3 | PC4 |
| Latitude | **0.99** | -0.14 | 0.02 | 0.00 |
| Longitude | **0.90** | -0.24 | 0.06 | 0.06 |
| Temperature seasonality | **0.98** | -0.11 | -0.05 | 0.01 |
| Annual mean temperature | **-0.99** | 0.15 | -0.05 | 0.03 |
| Annual temperature range | **0.99** | 0.09 | -0.07 | 0.05 |
| Temperature isothermality | **-0.85** | 0.45 | 0.03 | 0.04 |
| Temperature diurnal range | **0.63** | **0.72** | -0.15 | 0.19 |
| Annual precipitation | -0.08 | **0.88** | -0.18 | 0.39 |
| Conductivity | **-0.60** | **-0.69** | -0.29 | 0.02 |
| Chlorophyll *a* | -0.11 | -0.12 | **0.93** | 0.29 |
| Density | -0.13 | **-0.70** | -0.27 | **0.63** |
| % variance | 56.52 | 23.57 | 10.03 | 6.21 |
| Eigenvalue | 6.22 | 2.59 | 1.10 | 0.68 |

**Section C. Population genetic analyses**

**Supplementary Methods**

We extracted DNA from ethanol-preserved tissue samples using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s recommendations. We used primer pairs established for *G. affinis* (Spencer et al. 1999; Purcell et al. 2011) and G. *holbrooki* (Zane et al. 1999), which were arranged in three separate multiplex reactions (reaction 1: Gaaf10, Gaaf11, Gaaf13, Gafµ3; reaction 2: Gaaf7, Gaaf9, Gaaf15, Gaaf16, Gaaf22, Gafµ2, Gafµ6; reaction 3: Gafµ1, Gafµ4, Gafµ7, Mf-13) and amplified using the Type-it Mirosatellite PCR kit (Qiagen, Hilden, Germany) with thermocycling conditions as follows: initial denaturation for 5:00 min at 95°C, 30 cycles of 1:30 min at 60°C, and 0:30 min at 72°C, followed by a final extension step for 30:00 min at 60°C. Each 5 µl reaction mix included 2.5 µl Type-it master mix, 0.4 µl primer mix, 0.4 µl Q-solution, 0.9 µl RNase-free water, and 0.8 µl template DNA. Fragment sizes were scored manually after electrophoresis on a Beckman Coulter capillary sequencer CEQ 2000, using an internal size standard (Beckman Coulter).

We used ARLEQUIN v 3.5 (Excoffier and Lischer 2010) to calculate expected (*H*E) and observed heterozygosity (*H*O), and to test for deviations from Hardy-Weinberg-Equilibrium. FSTAT v 2.9.3 (Goudet 2001) was used to calculate allelic richness (*A*) and to calculate pairwise *F*ST-values between populations. We further tested for null alleles at each locus using Micro-checker v 2.2.3 (van Oosterhout et al. 2004), and then used FreeNA to calculate unbiased *F*ST-values (taking Null alleles into account; Weir 1996) between populations following the method described in Chapuis and Estoup (2007).

To explore potential footprints of latitude on the population genetic structure, we tested for a correlation between allelic richness (*A*) and latitude by means of a Pearson correlation in SPSS VS 22 (IBM Corp., Armonk, NY). Moreover, we used the software STRUCTURE 2.3.4104 to calculate individual assignment probabilities (*Q*-values) to varying numbers of genetically distinct clusters (*K*). For each value of *K* = 1–15, ten iterations were run using the admixture model with a burn-in period of 250,000 generations, followed by a sampling phase of 750,000 iterations. We detected the uppermost level of population differentiation with the method presented by Evanno et al. (2005) using the web-based tool STRUCTURE HARVESTER 0.6.93105. We detected *K* = 2 as the uppermost hierarchical level of population structure.

We furthermore calculated genetic distances (Nei’s *D*A; Nei et al. 1983) using Populations 1.2.32 and visualized a population tree using phylip 3.695.

**Supplementary Results and Discussion**

Descriptive statistics for site-specific means of different estimates of genetic variability of the *N* = 200 genotyped individuals are provided in Table B1. We found variable degrees of genetic differentiation between populations, ranging from virtual panmixis (*F*ST = 0.003) to pronounced genetic differentiation (*F*ST = 0.535; Table B2).

Historical contingency and anthropogenic impacts (translocations) may have influenced patterns of genetic diversity and population structure across the species’ range. However, the genetic data did not indicate an anthropogenic impact: the STRUCTURE analysis found a population genetic structuring with all northern populations being assigned to one cluster and all southern populations (SC to FLZT) to another; the only exception was the NC population, which was of mixed origin (Fig. B1). Nei’s genetic distances (*D*A) ranged from 0.263 (between FLMW and FLPL) to 0.831 (between DE and FLZT) and confirmed a split between a northern (NJ to NC) and a southern cluster (SC to FLZT; Table B3, Fig. B2). Average Nei’s *D*A among the northern populations was0.396 ± 0.020 (mean ± SE) and 0.420 ± 0.019 among southern populations, whereas the mean *D*A between the northern and southern clades was 0.589 ± 0.023. Our results are congruent with earlier studies based on haplotype diversity that found different haplotypes in the northern (Washington to North-Carolina), compared to the southern range (South Carolina to Florida) (Vidal et al. 2010). Our microsatellite data, as well as haplotype information from Vidal et al. (2010), however, are not fully congruent with observations by Wooten et al. (1988) and Scribner and Avise (1993) who described a more southern boundary (Savanah river drainage and Ogeechee drainage, respectively) between *G. holbrooki* ‘Types I and II’, using autosomally inherited nuclear (allozyme) loci and maternally inherited mitochondrial DNA (mtDNA) markers.

The number of alleles per locus ranged from 10 (MF-13) to 49 (Gafµ4). In general, we found a higher genetic diversity in southern populations, with a gradual decrease towards northern populations (Fig. B3). These findings might reflect the species’ historical range expansion, indicating a postglacial colonization of northern sites from southern source populations, or regular bottlenecks due to more pronounced seasonal variations (e.g., Shikano et al. 2010; Tison et al. 2014).

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**Table C1:** Genetic diversity in Eastern mosquitofish (*Gambusia holbrooki*). For each population and locus, observed (*H*O) and expected (*H*E) heterozygosities and allelic richness (*A*) are given. Zero values indicate that the locus is monomorphic in this population; for population information, please refer to Table 1.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Locus** | **No. of alleles** | **Range of allele size** | **Test** | **NJ** | **DE** | **VI** | **NC** | **SC** | **GE** | **FLDB** | **FLME** | **FLPL** | **FLZT** | **Mean across populations** |
|  | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 | *n* = 20 |
| **Gaaf10** | 26 | 179-327 | *HO* | 0.55 | 0.25 | 0.5 | 0.55 | 0.60 | 0.75 | 0.85 | 0.75 | 0.80 | 0.53 | 0.61 |
| *HE* | 0.65 | 0.36 | 0.7 | 0.72 | 0.73 | 0.86 | 0.79 | 0.75 | 0.79 | 0.58 | 0.69 |
| *A* | 4.95 | 2.00 | 6.00 | 8.85 | 6.95 | 9.85 | 7.90 | 6.85 | 12.79 | 6.00 | 7.21 |
| **Gaaf11** | 27 | 63-191 | *HO* | 0.55 | 0.00 | 0.20 | 0.15 | 0.55 | 0.45 | 0.60 | 0.60 | 0.75 | 0.42 | 0.43 |
| *HE* | 0.62 | 0.00 | 0.70 | 0.82 | 0.89 | 0.80 | 0.82 | 0.94 | 0.92 | 0.73 | 0.72 |
| *A* | 4.95 | 1.00 | 5.95 | 8.94 | 13.6 | 8.90 | 9.90 | 15.79 | 15.6 | 5.00 | 8.96 |
| **Gaaf13** | 33 | 127-267 | *HO* | 0.90 | 0.1 | 0.90 | 0.90 | 0.85 | 0.85 | 0.90 | 0.85 | 0.8 | 0.53 | 0.76 |
| *HE* | 0.85 | 0.1 | 0.89 | 0.93 | 0.89 | 0.89 | 0.90 | 0.96 | 0.94 | 0.92 | 0.83 |
| *A* | 11.65 | 2.00 | 11.75 | 13.75 | 11.8 | 9.90 | 12.7 | 20.45 | 17.5 | 12.00 | 12.35 |
| **Gafµ3** | 38 | 165-303 | *HO* | 0.40 | 0.45 | 0.70 | 0.65 | 0.70 | 0.85 | 0.20 | 0.65 | 0.80 | 0.84 | 0.62 |
| *HE* | 0.39 | 0.40 | 0.69 | 0.81 | 0.89 | 0.94 | 0.70 | 0.93 | 0.93 | 0.92 | 0.76 |
| *A* | 1.79 | 1.75 | 2.50 | 3.03 | 3.39 | 3.67 | 2.61 | 3.58 | 3.59 | 3.53 | 2.94 |
| **Gaaf7** | 27 | 93-213 | *HO* | 0.35 | 0 | 0.4 | 0.65 | 0.35 | 0.75 | 0.75 | 0.5 | 0.65 | 0.55 | 0.50 |
| *HE* | 0.73 | 0 | 0.71 | 0.8 | 0.63 | 0.72 | 0.7 | 0.74 | 0.77 | 0.7 | 0.65 |
| *A* | 6.9 | 1 | 5.85 | 6 | 4 | 8.8 | 5.95 | 9.8 | 15.5 | 5.9 | 6.97 |
| **Gaaf9** | 17 | 184-260 | *HO* | 0.4 | 0 | 0.65 | 0.75 | 0.9 | 0.7 | 0.7 | 0.6 | 0.65 | 0.5 | 0.59 |
| *HE* | 0.69 | 0 | 0.53 | 0.76 | 0.85 | 0.76 | 0.69 | 0.68 | 0.77 | 0.67 | 0.64 |
| *A* | 5.9 | 1 | 3.9 | 5.9 | 7 | 6.95 | 4 | 7.9 | 7.95 | 6.85 | 5.74 |
| **Gaaf15** | 14 | 70-146 | *HO* | 0.65 | 0.15 | 0 | 0.6 | 0.35 | 0.25 | 0.15 | 0.35 | 0.4 | 0.2 | 0.31 |
| *HE* | 0.65 | 0.15 | 0.1 | 0.57 | 0.35 | 0.54 | 0.15 | 0.67 | 0.5 | 0.28 | 0.40 |
| *A* | 5.9 | 2.95 | 2 | 3.95 | 0.95 | 7.9 | 2.95 | 5 | 5.9 | 4.9 | 4.54 |
| **Gaaf16** | 23 | 179-315 | *HO* | 0.55 | 0 | 0.35 | 0.35 | 0.35 | 0.65 | 0.85 | 0.6 | 0.85 | 0.6 | 0.52 |
| *HE* | 0.59 | 0 | 0.46 | 0.32 | 0.5 | 0.7 | 0.79 | 0.83 | 0.83 | 0.56 | 0.56 |
| *A* | 5.9 | 1 | 4 | 4.95 | 7.75 | 9.7 | 6.85 | 11.65 | 11.65 | 6.9 | 7.04 |
| **Gaaf22** | 48 | 235-367 | *HO* | 0.55 | 0.9 | 0.9 | 0.8 | 0.95 | 0.85 | 0.95 | 0.75 | 1 | 0.6 | 0.83 |
| *HE* | 0.69 | 0.68 | 0.79 | 0.89 | 0.93 | 0.93 | 0.87 | 0.92 | 0.96 | 0.84 | 0.85 |
| *A* | 11.6 | 4.9 | 8.8 | 13.74 | 16.64 | 16.5 | 9.9 | 16.6 | 19.5 | 13.6 | 13.18 |
| **Gafµ2** | 31 | 104-182 | *HO* | 0.55 | 0.05 | 0 | 0.55 | 0.75 | 0.9 | 0.95 | 0.7 | 0.9 | 0.75 | 0.61 |
| *HE* | 0.53 | 0.05 | 0.1 | 0.72 | 0.72 | 0.91 | 0.85 | 0.81 | 0.93 | 0.69 | 0.63 |
| *A* | 5.9 | 1.95 | 2 | 5.95 | 10.65 | 13.75 | 8.9 | 9.85 | 16.64 | 6.85 | 8.24 |
| **Gafµ6** | 20 | 89-209 | *HO* | 0.55 | 0 | 0.2 | 0.2 | 0.85 | 0.65 | 0.7 | 0.55 | 0.85 | 0.6 | 0.52 |
| *HE* | 0.6 | 0.1 | 0.53 | 0.7 | 0.86 | 0.82 | 0.76 | 0.57 | 0.9 | 0.77 | 0.66 |
| *A* | 3.95 | 2 | 4 | 5 | 8.9 | 7.9 | 7.9 | 8.75 | 12.75 | 7.85 | 6.90 |
| **Gafµ1** | 9 | 70-140 | *HO* | 0.05 | 0 | 0.05 | 0 | 0.05 | 0.25 | 0.85 | 0.85 | 0.7 | 0.1 | 0.29 |
| *HE* | 0.05 | 0 | 0.05 | 0 | 0.15 | 0.39 | 0.71 | 0.71 | 0.7 | 0.1 | 0.29 |
| *A* | 1.95 | 1 | 1.95 | 1 | 2.95 | 4 | 5 | 4 | 4 | 2.9 | 2.88 |
| **Gafµ4** | 49 | 173-297 | *HO* | 0.7 | 0.45 | 0.75 | 0.79 | 0.75 | 0.85 | 0.5 | 0.75 | 0.7 | 0.6 | 0.68 |
| *HE* | 0.81 | 0.55 | 0.88 | 0.97 | 0.91 | 0.96 | 0.89 | 0.96 | 0.96 | 0.9 | 0.88 |
| *A* | 9.8 | 4.9 | 10.85 | 22 | 17.4 | 19.5 | 10.9 | 22.34 | 20.49 | 12.8 | 15.10 |
| **Gafµ7** | 28 | 155-245 | *HO* | 0.65 | 0.3 | 0.45 | 0.95 | 1 | 0.75 | 0.85 | 0.7 | 0.55 | 0.45 | 0.67 |
| *HE* | 0.75 | 0.58 | 0.77 | 0.92 | 0.94 | 0.83 | 0.88 | 0.91 | 0.77 | 0.62 | 0.80 |
| *A* | 7.85 | 3.95 | 6.95 | 15 | 16.64 | 10.75 | 8.95 | 12.75 | 8.75 | 5.85 | 9.74 |
| **Mf-13** | 10 | 153-181 | *HO* | 0.1 | 0 | 0 | 0.53 | 0.2 | 0.2 | 0.55 | 0.75 | 0.85 | 0.65 | 0.38 |
| *HE* | 0.1 | 0 | 0 | 0.53 | 0.46 | 0.49 | 0.69 | 0.77 | 0.8 | 0.71 | 0.46 |
| *A* | 2.9 | 1 | 1 | 3 | 5.85 | 4.95 | 5 | 7.8 | 7.85 | 4.95 | 4.43 |
| **Mean across loci** |   |   | *HO* | 0.5 | 0.18 | 0.4 | 0.56 | 0.61 | 0.65 | 0.69 | 0.66 | 0.75 | 0.53 |   |
| *HE* | 0.58 | 0.2 | 0.53 | 0.7 | 0.71 | 0.77 | 0.74 | 0.81 | 0.83 | 0.67 |
| *A* | 6.13 | 2.16 | 5.17 | 8.07 | 9.16 | 9.53 | 7.29 | 10.87 | 12.03 | 7.06 |

**Table C2:** Pairwise genetic divergence among ten populations of *Gambusia holbrooki*. Above the diagonal are *F*ST distances calculates using FSTAT (Goudet 2001), while below the diagonal are *F*ST distances calculated in FreeNA (Chapuis and Estoup 2007) using the ENA method, which considers potential null alleles. For population information, please refer to Table 1.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **NJ** | **DE** | **VA** | **NC** | **SC** | **GA** | **FLDB** | **FLME** | **FLPL** | **FLZT** |
| **NJ** |  | 0.32883 | 0.17223 | 0.12239 | 0.21580 | 0.20996 | 0.24160 | 0.16772 | 0.19117 | 0.32506 |
| **DE** | 0.31537 |  | 0.36047 | 0.34793 | 0.46099 | 0.44366 | 0.44438 | 0.39667 | 0.40868 | 0.54666 |
| **VA** | 0.15047 | 0.35291 |  | 0.12510 | 0.25184 | 0.25008 | 0.26274 | 0.22378 | 0.22979 | 0.35543 |
| **NC** | 0.11994 | 0.34290 | 0.10402 |  | 0.14889 | 0.14236 | 0.16866 | 0.12846 | 0.12371 | 0.24983 |
| **SC** | 0.20750 | 0.45049 | 0.22221 | 0.13675 |  | 0.06493 | 0.12591 | 0.12000 | 0.12000 | 0.20151 |
| **GA** | 0.20230 | 0.43408 | 0.21975 | 0.13252 | 0.05670 |  | 0.11737 | 0.09554 | 0.06034 | 0.17958 |
| **FLDB** | 0.23489 | 0.43763 | 0.24239 | 0.16386 | 0.10828 | 0.10809 |  | 0.08047 | 0.05588 | 0.16168 |
| **FLME** | 0.16642 | 0.39400 | 0.20098 | 0.12569 | 0.10678 | 0.08560 | 0.07619 |  | 0.03534 | 0.14949 |
| **FLPL** | 0.18332 | 0.40025 | 0.20272 | 0.11650 | 0.05512 | 0.05055 | 0.04856 | 0.03218 |  | 0.16271 |
| **FLZT** | 0.31248 | 0.53479 | 0.32662 | 0.24102 | 0.18120 | 0.16644 | 0.14994 | 0.13899 | 0.15097 |  |

**Table C3:** Pairwise Nei's *DA* distances among ten populations of *Gambusia holbrooki* (Nei et al. 1983). For population information, please refer to Table 1.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **NJ** | **DE** | **VA** | **NC** | **SC** | **GA** | **FLDB** | **FLME** | **FLPL** | **FLZT** |
| **NJ** |  |  |  |  |  |  |  |  |  |  |
| **DE** | 0.395 |  |  |  |  |  |  |  |  |  |
| **VA** | 0.412 | 0.429 |  |  |  |  |  |  |  |  |
| **NC** | 0.337 | 0.460 | 0.344 |  |  |  |  |  |  |  |
| **SC** | 0.526 | 0.701 | 0.565 | 0.438 |  |  |  |  |  |  |
| **GA** | 0.558 | 0.738 | 0.658 | 0.530 | 0.380 |  |  |  |  |  |
| **FLDB** | 0.554 | 0.701 | 0.615 | 0.500 | 0.456 | 0.447 |  |  |  |  |
| **FLME** | 0.420 | 0.655 | 0.563 | 0.439 | 0.425 | 0.404 | 0.372 |  |  |  |
| **FLPL** | 0.483 | 0.653 | 0.577 | 0.419 | 0.361 | 0.371 | 0.333 | 0.263 |  |  |
| **FLZT** | 0.678 | 0.831 | 0.725 | 0.620 | 0.516 | 0.493 | 0.502 | 0.462 | 0.511 |  |

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**Figure C1:** Population assignment using STRUCTURE version 2.3.4104.*K* = 2 was recovered as the most likely number of genetic clusters;for population information, please refer to Table 1.



**Figure C2:** Neighbor-joining tree based on Nei's *DA* distances among ten populations of Gambusia holbrooki (Nei et al. 1983). For population information, please refer to Table 1.



**Figure C3:** Relationship between allelic richness (*A*) and latitude (Pearson’s *r*= -0.66, *P* = 0.038).

**Section D. Descriptive Life Histories**

**Table D1:** Descriptive life-history traits (mean±S.E.M.) for 159 male eastern mosquitofish (*Gambusia holbrooki*) from 10 populations along the U.S. Atlantic Coast, spanning >14 degrees of latitude.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Population | Code | *N* | SL [mm] | Wet Weight [mg] | Lean Weight [mg] | Fat Content [%] | GSI [%] |
| 1 | NJ | 2 | 21.15±1.55 | 133.00±17.00 | 24.12±2.14 | 0.60±2.28 | 2.39±0.34 |
| 2 | DE | 11 | 21.23±0.66 | 152.64±16.99 | 25.79±0.95 | 6.36±1.01 | 2.61±0.15 |
| 3 | VA | 9 | 20.66±0.36 | 113.11±7.24 | 19.81±1.02 | 0.00±1.09 | 2.20±0.16 |
| 4 | NC | 20 | 18.75±0.33 | 88.85±4.46 | 20.29±0.67 | 1.40±0.72 | 2.00±0.11 |
| 5 | SC | 20 | 18.45±0.43 | 85.45±6.24 | 22.24±0.67 | 2.10±0.72 | 1.30±0.11 |
| 6 | GA | 20 | 19.07±0.47 | 103.00±8.02 | 25.91±0.67 | 2.81±0.72 | 1.54±0.11 |
| 7 | FLDB | 20 | 18.44±0.43 | 86.90±6.91 | 23.01±0.67 | 1.98±0.72 | 1.63±0.11 |
| 8 | FLME | 20 | 20.16±0.47 | 121.20±9.98 | 23.80±0.69 | 5.76±0.74 | 1.64±0.11 |
| 9 | FLPL | 16 | 16.89±0.18 | 68.50±2.37 | 23.89±0.78 | 4.48±0.84 | 1.70±0.12 |
| 10 | FLZT | 21 | 17.62±0.67 | 79.29±11.84 | 25.89±0.67 | 2.57±0.72 | 1.19±0.11 |

Note: Lean weight, fat content, and GSI (gonadosomatic index) are given as estimated marginal means corrected for differences in SL (evaluated at SL = 18.86 mm). For population information, please refer to Table 1.

**Table D2:** Descriptive life-history traits (mean±S.E.M.) for 223 pregnant female eastern mosquitofish (*Gambusia holbrooki*) from 10 populations along the U.S. Atlantic Coast, spanning >14 degrees of latitude.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Population | Code | *N* | SL [mm] | Wet Weight [mg] | Lean Weight [mg] | Fat Content [%] | Fecundity | Embryo Fat Content [%] | Estimated Offspring Dry Weight at Birth [mg] | RA [%] |
| 1 | NJ | 25 | 26.17±0.76 | 419.08±38.93 | 63.44±1.48 | 2.88±0.47 | 16.41±1.37 | 9.76±0.65 | 0.67 | 18.40±0.90 |
| 2 | DE | 30 | 27.86±0.45 | 548.47±36.46 | 62.33±1.43 | 7.66±0.45 | 46.19±1.32 | 9.95±0.60 | 0.62 | 29.39±0.82 |
| 3 | VA | 19 | 28.29±0.48 | 443.74±23.71 | 55.73±1.78 | 4.62±0.57 | 9.59±1.65 | 13.05±0.75 | 0.99 | 18.02±1.03 |
| 4 | NC | 29 | 21.63±0.37 | 185.72±10.50 | 64.95±1.52 | 2.61±0.49 | 11.30±1.41 | 13.49±0.61 | 0.76 | 11.66±0.85 |
| 5 | SC | 26 | 22.19±0.57 | 206.85±21.45 | 66.21±1.55 | 3.75±0.49 | 12.90±1.43 | 9.57±0.65 | 1.10 | 15.68±0.90 |
| 6 | GA | 17 | 22.71±0.54 | 256.12±23.10 | 73.53±1.84 | 2.73±0.59 | 13.44±1.71 | 10.79±0.79 | 0.96 | 18.00±1.09 |
| 7 | FLDB | 22 | 27.56±0.51 | 401.55±24.32 | 60.43±1.63 | 2.38±0.52 | 11.33±1.51 | 9.95±0.69 | 0.86 | 16.48±0.96 |
| 8 | FLME | 28 | 27.58±0.72 | 444.46±43.83 | 65.38±1.46 | 10.48±0.47 | 4.42±1.35 | 13.46±0.62 | 1.63 | 15.18±0.85 |
| 9 | FLPL | 22 | 23.23±0.30 | 259.14±11.11 | 58.02±1.61 | 6.82±0.52 | 21.31±1.49 | 7.00±0.69 | 0.64 | 22.73±0.96 |
| 10 | FLZT | 5 | 23.70±1.40 | 264.40±50.10 | 69.80±3.30 | 4.87±1.06 | 6.71±3.06 | 14.19±1.46 | 1.43 | 10.59±2.01 |

Note: Lean weight, fat content, and fecundity are given as estimated marginal means corrected for differences in SL (evaluated at SL = 25.23 mm); embryo fat content and RA (reproductive allocation) are given as estimated marginal means corrected for differences in embryonic stage of development (evaluated at stage = 30.46). For population information, please refer to Table 1.

**Section E. Model Selection Results**

**Table E1:** Summary of best-fitting models and model-averaged coefficients (β) and standard errors (SE) for our tests of environmental drivers (PC1-4) of life-history trait variation in *Gambusia holbrooki*.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Trait | Model # | ∆ AIC*c* | Weight | PC1 | PC2 | PC3 | PC4 | Sex | Sex × PC4 |
| Body Size  | 1 | 0.00 | 0.25 | 0.0278 |  | 0.0240 |  | <0.0001 |  |
| (length) | 2 | 1.12 | 0.14 | 0.0232 |  | 0.0199 | 0.1366 | <0.0001 |  |
|  | β |  |  | 0.0057 | -0.0004 | 0.0144 | -0.0042 | -0.0570 |  |
|  | SE |  |  | 0.0026 | 0.0017 | 0.0064 | 0.0051 | 0.0074 |  |
| Body Size  | 1 | 0.00 | 0.31 | 0.0052 |  | 0.0019 | 0.0363 | <0.0001 |  |
| (weight) | 2 | 0.35 | 0.26 | 0.0033 |  | 0.0012 | 0.0261 | <0.0001 | 0.0805 |
|  | 3 | 1.86 | 0.12 | 0.0104 |  | 0.0042 |  | <0.0001 |  |
|  | β |  |  | 0.0262 | -0.0008 | 0.0731 | -0.0409 | -0.2517 | 0.0173 |
|  | SE |  |  | 0.0080 | 0.0038 | 0.0194 | 0.0200 | 0.0194 | 0.0148 |
| Reproductive  | 1 | 0.00 | 0.27 | 0.0215 |  |  | 0.0147 | <0.0001 | 0.0212 |
| Effort | 2 | 1.74 | 0.11 | 0.0179 |  | 0.1457 | 0.0121 | <0.0001 | 0.0176 |
|  | 3 | 1.85 | 0.11 | 0.0182 | 0.1532 |  | 0.0123 | <0.0001 | 0.0179 |
|  | β |  |  | 0.0074 | 0.0019 | 0.0030 | -0.0259 | -0.1486 | 0.0225 |
|  | SE |  |  | 0.0032 | 0.0027 | 0.0042 | 0.0102 | 0.0089 | 0.0097 |
| Fecundity | 1 | 0.00 | 0.32 | 0.1131 |  |  |  |  |  |
|  | 2 | 0.21 | 0.29 |  |  |  | 0.1250 |  |  |
|  | 3 | 1.30 | 0.17 | 0.0734 |  |  | 0.0797 |  |  |
|  | β |  |  | 0.1417 | 0.0231 | 0.0255 | -0.3720 |  |  |
|  | SE |  |  | 0.0982 | 0.0856 | 0.1186 | 0.2802 |  |  |
| Offspring  | 1 | 0.00 | 0.65 | 0.0392 |  |  |  |  |  |
| Size | β |  |  | -0.0329 | -0.0054 | 0.0015 | 0.0028 |  |  |
|  | SE |  |  | 0.0137 | 0.0105 | 0.0121 | 0.0160 |  |  |
| Offspring Fat | 1 |  |  |  | 0.0496 |  |  |  |  |
|  | β |  |  | -0.0001 | -0.0124 | 0.0000 | 0.0029 |  |  |
|  | SE |  |  | 0.0015 | 0.0056 | 0.0029 | 0.0058 |  |  |
| Relative Lean  | 1 | 0.00 | 0.10 |  |  | 0.0915 |  | 0.0609 |  |
| Weight | 2 | 0.22 | 0.09 |  |  | 0.0772 | 0.1046 | 0.0500 |  |
|  | 3 | 0.28 | 0.08 |  |  |  |  | 0.0743 |  |
|  | 4 | 0.63 | 0.07 |  |  |  | 0.1272 | 0.0647 |  |
|  | 5 | 1.09 | 0.06 |  |  | 0.1153 |  |  |  |
|  | 6 | 1.55 | 0.04 |  |  | 0.1035 | 0.1356 |  |  |
|  | 7 | 1.60 | 0.04 |  |  |  | 0.1541 |  |  |
|  | β |  |  | -0.0005 | -0.0011 | 0.0064 | -0.0063 | -0.0088 |  |
|  | SE |  |  | 0.0014 | 0.0022 | 0.0050 | 0.0059 | 0.0055 |  |
| Fat Content | 1 | 0.00 | 0.37 |  |  | 0.0018 |  | 0.0065 |  |
|  | 2 | 1.41 | 0.18 | 0.1905 |  | 0.0016 |  | 0.0059 |  |
|  | β |  |  | -0.0019 | 0.0003 | 0.0426 | -0.0003 | -0.0344 |  |
|  | SE |   |   | 0.0025 | 0.0025 | 0.0115 | 0.0048 | 0.0111 |   |

Note:For models with ∆ AIC*c* ≤ 2.0, we provide Akaike model weights and the *P* values for each term included in the model.

**Table E2:** Summary of best-fitting models and model-averaged coefficients (β) and standard errors (SE) for our tests of environmental drivers (PC1-4) of body shape variation in *Gambusia holbrooki*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Trait | Model # | ∆ AIC*c* | Weight | PC1 | PC2 | PC3 | PC4 |
| Male d1 | 1 | 0.00 | 0.43 |  | 0.1239 |  |  |
|  | 2 | 1.30 | 0.23 | 0.2389 |  |  |  |
|  | β |  |  | 0.0014 | 0.0050 | 0.0004 | -0.0006 |
|  | SE |  |  | 0.0018 | 0.0036 | 0.0029 | 0.0037 |
| Male d2 | 1 | 0.00 | 0.63 | 0.0449 |  |  |  |
|  | β |  |  | 0.0024 | 0.0000 | -0.0009 | 0.0001 |
|  | SE |  |  | 0.0011 | 0.0005 | 0.0014 | 0.0012 |
| Male d3 | 1 | 0.00 | 0.42 | 0.1546 |  |  |  |
|  | 2 | 1.28 | 0.22 |  |  | 0.3026 |  |
|  | β |  |  | -0.0009 | 0.0001 | -0.0008 | -0.0001 |
|  | SE |  |  | 0.0007 | 0.0007 | 0.0013 | 0.0013 |
| Male d4 | 1 | 0.00 | 0.43 |  |  | 0.1384 |  |
|  | 2 | 1.86 | 0.17 |  |  |  | 0.5169 |
|  | β |  |  | -0.0001 | 0.0003 | 0.0020 | -0.0004 |
|  | SE |  |  | 0.0004 | 0.0007 | 0.0015 | 0.0012 |
| Female d1 | 1 | 0.00 | 0.40 | 0.0249 | 0.0353 |  |  |
|  | 2 | 0.77 | 0.27 | 0.0620 |  |  |  |
|  | 3 | 1.68 | 0.17 |  | 0.0947 |  |  |
|  | β |  |  | 0.0030 | 0.0035 | 0.0004 | -0.0001 |
|  | SE |  |  | 0.0013 | 0.0018 | 0.0013 | 0.0012 |
| Female d2 | 1 | 0.00 | 0.31 | 0.0449 |  |  |  |
|  | 2 | 0.75 | 0.21 |  |  |  | 0.9431 |
|  | 3 | 0.76 | 0.21 |  |  | 0.9624 |  |
|  | 4 | 0.76 | 0.21 |  | 0.9857 |  |  |
|  | β |  |  | 0.0005 | 0.0000 | 0.0000 | -0.0001 |
|  | SE |  |  | 0.0009 | 0.0012 | 0.0019 | 0.0024 |
| Female d3 | 1 | 0.00 | 0.81 |  |  | 0.0027 | 0.0103 |
|  | β |  |  | 0.0000 | 0.0000 | -0.0056 | 0.0049 |
|  | SE |  |  | 0.0001 | 0.0001 | 0.0012 | 0.0014 |
| Female d4 | 1 | 0.00 | 0.63 | 0.0346 |  |  |  |
|  | β |  |  | 0.0018 | -0.0004 | -0.0001 | 0.0000 |
|  | SE |  |  | 0.0007 | 0.0006 | 0.0006 | 0.0005 |
| Female d5 | 1 | 0.00 | 0.61 |  | 0.0752 |  |  |
|  | β |  |  | 0.0001 | -0.0014 | 0.0001 | 0.0000 |
|  | SE |  |  | 0.0003 | 0.0007 | 0.0005 | 0.0006 |

Note: For models with ∆ AIC*c* ≤ 2.0, we provide Akaike model weights and the *P* values for each term included in the model.

**Section F: Visualization of Body Shape Divergence**



**Figure F1:** Visualization of body shape variation (based on thin-plate spline transformations for each sex) along divergence vectors (**d**) that exhibited associations with latitude that were explained by covariation with life-history traits (see main results).