**SUPPLEMENTARY MATERIAL**

**Increased polyamine levels and maintenance of γ-aminobutyric acid (Gaba) homeostasis in the gills is indicative of osmotic plasticity in killifish**

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**TABLES**

**Table S1.** Summary of generalized linear models (GLMs) and survival models performed to examine the effects of salinity, time, and the interaction between salinity and time on plasma chemistry in *F. majalis,* *F. grandis*, and *F. heteroclitus*.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Species** | **Statistical Test and Family** | **Coefficients** | **Estimate** | **SE** | ***t* or *z*** | ***P*** | ***\**** | **Figure** |
| **Plasma Osm** | *F. grandis* | GLM, Gaussian | Salinity | 5.720 | 1.692 | 3.381 | **0.002** | \*\* | Fig. 2B |
|  |  |  | Time | 0.854 | 9.782 | 0.087 | 0.931 | NS |  |
|  |  |  | Salinity\*Time | -0.375 | 1.001 | -0.375 | 0.710 | NS |  |
|  | *F. heteroclitus* | GLM, Gaussian | Salinity | 1.073 | 0.451 | 2.379 | **0.023** | \* | Fig. 2C |
|  |  |  | Time | 5.125 | 6.335 | 0.809 | 0.424 | NS |  |
|  |  |  | Salinity\*Time | -0.220 | 0.260 | -0.844 | 0.404 | NS |  |
| **Plasma [Na+]** | *F. majalis* | Survival, Weibull | Salinity | 0.007 | 0.002 | 4.490 | **<0.001** | \*\*\* | Fig. 2D |
|  |  |  | Time | -0.052 | 0.023 | -2.280 | **0.023** | \* |  |
|  |  |  | Salinity\*Time | 0.002 | 0.001 | 2.230 | **0.026** | \* |  |
|  | *F. grandis* | GLM, Gaussian | Salinity | 3.307 | 0.731 | 4.523 | **<0.001** | \*\*\* | Fig. 2E |
|  |  | Time | -6.479 | 3.742 | -1.732 | ***0.091*** | # |  |
|  |  |  | Salinity\*Time | 0.707 | 0.419 | 1.685 | ***0.099*** | # |  |
|  | *F. heteroclitus* | GLM, Gaussian | Salinity | 1.660 | 0.118 | 14.10 | **<0.001** | \*\*\* | Fig. 2F |
|  |  |  | Time | 15.31 | 1.654 | 9.257 | **<0.001** | \*\*\* |  |
|  |  |  | Salinity\*Time | -0.541 | 0.068 | -7.962 | **<0.001** | \*\*\* |  |
| **Plasma [Cl-]** | *F. majalis* | Survival, Weibull | Salinity | 0.005 | 0.002 | 3.000 | **0.003** | \*\* | Fig. 2G |
|  |  |  | Time | -0.127 | 0.024 | -5.350 | **<0.001** | \*\*\* |  |
|  |  |  | Salinity\*Time | 0.003 | 0.001 | 3.360 | **0.001** | \*\* |  |
|  | *F. grandis* | GLM, Gaussian | Salinity | 3.868 | 0.738 | 5.243 | **<0.001** | \*\*\* | Fig. 2H |
|  |  |  | Time | -8.950 | 4.173 | -2.145 | **0.038** | \* |  |
|  |  |  | Salinity\*Time | 0.517 | 0.438 | 1.179 | 0.245 | NS |  |
|  | *F. heteroclitus* | Survival, Weibull | Salinity | 0.003 | 0.002 | 1.780 | ***0.076*** | # | Fig. 2I |
|  |  |  | Time | 0.039 | 0.024 | 1.640 | 0.100 | NS |  |
|  |  |  | Salinity\*Time | -0.001 | 0.001 | -1.260 | 0.208 | NS |  |

Generalized linear models and survival models were used to assess the effects of salinity (*F. majalis* and *F. heteroclitus*: 32 ppt and 0.1 ppt, *F. grandis*: 12 ppt and 0.1 ppt), time [0 h (pre-transfer), 6 h, 1 d, and 3 d], and the interaction between salinity and time on plasma osmolality, plasma sodium concentration, and plasma chloride concentration in *F. majalis* (*n* = 5-6), *F. grandis* (*n* = 4-7), and *F. heteroclitus* (*n* = 4-6). Coefficient estimates, standard error (SE), *t*-statistics (for GLMs), *z*-statistics (for survival models), *P*-values (*P*), statistical significance (\*), and the location of figures associated with each model are shown. **Boldface** indicates *P* < 0.05, whereas **boldface** and *italics* indicate *P* < 0.10. χ2 and Nagelkerke's pseudo-*R*2 values for each model are provided in the Results section of the main text. *Abbreviations: chloride, Cl-; osmolality, Osm; sodium, Na+.* Symbols: NS (not significant, *P* > 0.10), #*P* < 0.10, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

**Table S2.** Summary of generalized linear models (GLMs) performed to examine the effects of salinity, time, and the interaction between salinity and time on gill polyamine concentrations in *F. majalis*, *F. grandis,* and *F. heteroclitus*.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Species** | **Statistical Test and Family** | **Coefficients** | **Estimate** | **SE** | ***t*** | ***P*** | ***\**** | **Figure** |
| **[Putrescine]** | *F. majalis* | GLM, Gaussian | Salinity | 1.819 | 5.275 | 0.345 | 0.733 | NS | Fig. 3A |
|  |  |  | Time | -33.34 | 80.09 | -0.416 | 0.680 | NS |  |
|  |  |  | Salinity\*Time | 3.454 | 3.233 | 1.068 | 0.294 | NS |  |
|  | *F. grandis* | GLM, Gamma | Salinity | 2.55x10-4 | 6.36x10-5 | 4.015 | **<0.001** | \*\*\* | Fig. 3B |
|  |  | Time | 3.72x10-5 | 2.20x10-4 | 0.169 | 0.866 | NS |  |
|  |  |  | Salinity\*Time | 1.29x10-4 | 4.96x10-5 | 2.598 | **0.013** | \* |  |
|  | *F. heteroclitus* | GLM, Gaussian | Salinity | -0.026 | 0.005 | -5.739 | **<0.001** | \*\*\* | Fig. 3C |
|  |  |  | Time | 0.155 | 0.057 | 2.712 | **0.011** | \* |  |
|  |  |  | Salinity\*Time | -0.008 | 0.002 | -3.196 | **0.003** | \*\* |  |
| **[Spermidine]** | *F. majalis* | GLM, Gaussian | Salinity | 1.354 | 1.748 | 0.774 | 0.445 | NS | Fig. 3D |
|  |  |  | Time | -18.39 | 26.89 | -0.684 | 0.499 | NS |  |
|  |  |  | Salinity\*Time | 0.517 | 1.081 | 0.478 | 0.636 | NS |  |
|  | *F. grandis* | GLM, Gaussian | Salinity | -21.24 | 7.357 | -2.888 | **0.006** | \*\* | Fig. 3E |
|  |  |  | Time | -116.3 | 37.68 | -3.087 | **0.004** | \*\* |  |
|  |  |  | Salinity\*Time | 3.506 | 4.128 | 0.849 | 0.400 | NS |  |
|  | *F. heteroclitus* | GLM, Gaussian | Salinity | -2.119 | 2.232 | -0.949 | 0.350 | NS | Fig. 3F |
|  |  |  | Time | 35.08 | 27.58 | 1.272 | 0.213 | NS |  |
|  |  |  | Salinity\*Time | -1.600 | 1.201 | -1.333 | 0.192 | NS |  |
| **[Spermine]** | *F. majalis* | GLM, Gaussian | Salinity | 0.010 | 0.004 | 2.698 | **0.011** | \* | Fig. 3G |
|  |  |  | Time | -0.112 | 0.054 | -2.062 | **0.047** | \* |  |
|  |  |  | Salinity\*Time | 0.001 | 0.002 | 0.543 | 0.591 | NS |  |
|  | *F. grandis* | GLM, Gaussian | Salinity | -0.037 | 0.013 | -2.798 | **0.008** | \*\* | Fig. 3H |
|  |  |  | Time | -0.294 | 0.067 | -4.366 | **<0.001** | \*\*\* |  |
|  |  |  | Salinity\*Time | 0.018 | 0.007 | 2.508 | **0.016** | \* |  |
|  | F. *heteroclitus* | GLM, Gaussian | Salinity | 0.095 | 1.004 | 0.095 | 0.925 | NS | Fig. 3I |
|  |  |  | Time | -7.931 | 12.42 | -0.639 | 0.528 | NS |  |
|  |  |  | Salinity\*Time | 0.049 | 0.540 | 0.091 | 0.928 | NS |  |

Generalized linear models were used to assess the effects of salinity (*F. majalis* and *F. heteroclitus*: 32 ppt and 0.1 ppt, *F. grandis*: 12 ppt and 0.1 ppt), time [0 h (pre-transfer), 6 h, 1 d, and 3 d], and the interaction between salinity and time on putrescine, spermidine, and spermine levels in the gills of *F. majalis* (*n* = 4-6), *F. grandis* (*n* = 6-7), and *F. heteroclitus* (*n* = 5-6). Coefficient estimates, standard error (SE), *t*-statistics, *P*-values (*P*), statistical significance (\*), and the location of figures associated with each model are shown. **Boldface** indicates *P* < 0.05. χ2 and Nagelkerke's pseudo-*R*2 values for each model are provided in the Results section of the main text. Symbols: NS (not significant, *P* > 0.10), \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

**Table S3.** Summary of generalized linear models (GLMs) performed to examine the effects of salinity, time, and the interaction between salinity and time on gill glutamate and γ-aminobutyric acid (Gaba) concentrations in *F. majalis*, *F. grandis*, and *F. heteroclitus*.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Species** | **Statistical Test and Family** | **Coefficients** | **Estimate** | **SE** | ***t*** | ***P*** | ***\**** | **Figure** |
| **[Glutamate]** | *F. majalis* | GLM, Gaussian | Salinity | 0.033 | 0.009 | 3.820 | **<0.001** | \*\*\* | Fig. 4A |
|  |  |  | Time | 0.107 | 0.132 | 0.810 | 0.424 | NS |  |
|  |  |  | Salinity\*Time | -0.005 | 0.005 | -1.021 | 0.315 | NS |  |
|  | *F. grandis* | GLM, Gaussian | Salinity | -0.005 | 0.011 | -0.436 | 0.665 | NS | Fig. 4B |
|  |  | Time | -0.219 | 0.056 | -3.944 | **<0.001** | \*\*\* |  |
|  |  |  | Salinity\*Time | 0.002 | 0.006 | 0.408 | 0.686 | NS |  |
|  | *F. heteroclitus* | GLM, Gaussian | Salinity | 3.798 | 4.632 | 0.820 | 0.420 | NS | Fig. 4C |
|  |  |  | Time | 150.3 | 55.46 | 2.709 | **0.012** | \* |  |
|  |  |  | Salinity\*Time | -5.040 | 2.720 | -1.853 | ***0.075*** | # |  |
| **[Gaba]** | *F. majalis* | GLM, Gaussian | Salinity | 0.049 | 0.015 | 3.258 | **0.003** | \*\* | Fig. 4D |
|  |  |  | Time | -0.684 | 0.229 | -2.982 | **0.006** | \*\* |  |
|  |  |  | Salinity\*Time | 0.002 | 0.009 | 0.168 | 0.868 | NS |  |
|  | *F. grandis* | GLM, Gaussian | Salinity | 0.069 | 0.030 | 2.346 | **0.024** | \* | Fig. 4E |
|  |  |  | Time | 0.102 | 0.151 | 0.674 | 0.504 | NS |  |
|  |  |  | Salinity\*Time | -0.011 | 0.017 | -0.650 | 0.520 | NS |  |
|  | *F. heteroclitus* | GLM, Gaussian | Salinity | -0.008 | 0.012 | -0.700 | 0.490 | NS | Fig. 4F |
|  |  |  | Time | 0.028 | 0.147 | 0.187 | 0.853 | NS |  |
|  |  |  | Salinity\*Time | -0.001 | 0.007 | -0.139 | 0.890 | NS |  |

Generalized linear models were used to assess the effects of salinity (*F. majalis* and *F. heteroclitus*: 32 ppt and 0.1 ppt, *F. grandis*: 12 ppt and 0.1 ppt), time [0 h (pre-transfer), 6 h, 1 d, and 3 d], and the interaction between salinity and time on glutamate and Gaba levels in the gills of *F. majalis* (*n* = 4-6), *F. grandis* (*n* = 6-7), and *F. heteroclitus* (*n* = 4-6). Coefficient estimates, standard error (SE), *t*-statistics, *P*-values (*P*), statistical significance (\*), and the location of figures associated with each model are shown. **Boldface** indicates *P* < 0.05, whereas **boldface** and *italics* indicate *P* < 0.10. χ2 and Nagelkerke's pseudo-*R*2 values for each model are provided in the Results section of the main text. Symbols: NS (not significant, *P* > 0.10), #*P* < 0.10, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.