**Chapter 1 Figures and Tables**

**Chart

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**Figure 1.1.** Temperature- and *P*CO2-dependent routine metabolic rates (RMR) of *M. menidia* embryos and larvae.(A) Whole-body RMR (μmol O2 individual-1 h-1) of embryos and (B) mass-specific RMR (μmol O2 mg-1 h-1) of newly hatched larvae from experiments 1-4. Regression lines are fitted to the metabolic rates as a function of temperature to illustrate the significant effect of temperature (MLR, embryos: *F*3,247 = 10.96 , *p* < 0.001; larvae: *F*3,364 = 69.87, *p* < 0.001). Sample sizes for each temperature and *P*CO2 treatment combination are *n*=10-46 for embryos and *n*=9-68 for larvae.

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**Figure 1.2.** The effect of *P*CO2 on *Q*10 of routine metabolic rates in *M. menidia* embryos and larvae. Bootstrapped mean *Q*10 values of *M. menidia* embryos and larvae calculated from routine metabolic rates at 17°C and 28°C, from experiments 1-4. Error bars indicate bootstrapped 95% confidence intervals, and sample sizes for each *P*CO2 level are *n*=10-27 for embryos and *n*=9-41 for larvae.

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**Figure 1.3.** *P*O2- and *P*CO2-dependent routine metabolic rates of *M. menidia* embryos and larvae.(A) Whole-body RMR (μmol O2 individual-1 h-1) of embryos and (B) mass-specific RMR (μmol O2 mg-1 h-1) of newly hatched larvae from experiments 5-6. Regression lines are fitted to embryonic metabolic rates (A) as a function of *P*O2 within each *P*CO2 treatment to illustrate the significant *P*CO2 and *P*O2 interaction (MLR, *F*3,258 = 7.96, *p* = 0.005). No regression lines are shown for larvae (B) because there were no significant effects (MLR, *F*3,142 = 0.325, *p* > 0.05). Sample sizes in each *P*O2 and *P*CO2 combination are *n*=8-31 for embryos and *n*=10-22 for larvae.

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**Figure 1.4.** Conceptual diagram of the relationship between *P*O2 and routine metabolic rate of *M. menidia* embryos in ambient and elevated *P*CO2.Hypothesized shifts in the relationship between embryonic RMR and *P*O2 are shown for elevated (orange) versus ambient (blue) *P*CO2. Our results (measured at the *P*O2 levels marked by black dots) suggest that *P*CO2 can influence both the critical oxygen partial pressure (*P*crit, gray lines) and the oxygen-independent RMR. At higher *P*O2 levels, RMR increases with *P*CO2, potentially due to increased metabolic demand. As *P*O2 decreases, embryonic RMR reaches *P*crit and becomes oxygen-dependent at a higher *P*O2 level in acidified than in ambient *P*CO2 conditions. Low intracellular red blood cell pH caused by high *P*CO2 can be expected to reduce hemoglobin-O2 affinity (Bohr effect) and make embryonic RMR less hypoxia-resistant, which could manifest as an increase in *P*crit for embryos in elevated *P*CO2. See text for more information.

**Table 1.1**. Overview of target levels for *P*CO2, temperature, and oxygen partial pressure (*P*O2) for six experiments for which respirometry was conducted on embryos (E) and newly hatched larvae (L) of *Menidia menidia*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Experiment** | **Fertilization Date** | | ***P*CO2 (μatm)** | **Temperature (°C)** | ***P*O2**  **(kPa)** | **Stage** |
| 1 | | 4/22/2016 | 400, 2200 | 17, 24 | 21-23 | E, L |
| 2 | | 5/3/2016 | 400, 2200, 4200 | 17, 20, 24 | 21-23 | L |
| 3 | | 5/19/2016 | 400, 2200, 4200 | 17, 20, 24 | 21-23 | E, L |
| 4 | | 5/26/2017 | 400, 2200, 4200 | 24, 28 | 21-23 | E, L |
| 5 | | 5/9/2017 | 400, 2200, 4200 | 24 | 7.5, 12.0, 23.0 | E, L\* |
| 6 | | 6/9/2017 | 400, 2200, 4200 | 24 | 9.0, 12.0, 23.0 | E, L |

\*In Experiment 5, respirometry was only done on larvae from the 23.0 and 12.0 kPa *P*O2 treatments due to low hypoxic hatch survival.

**Table 1.2.** Age at sampling (equivalent to exposure time) in days-post-fertilization (dpf) and mean routine metabolic rates (±s.e.m.) of *M. menidia* embryos and larvae across *P*CO2, temperature, and *P*O2 treatments.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Temp (°C)** | ***P*O2**  **(kPa)** | **Age at Embryonic Sampling (dpf)** | **Age at Larval Sampling (dpf)** | ***P*CO2 (μatm)** | **Embryonic Sample Size** | **Mean Embryonic RMR (±s.e.m.)**  **(μmol O2 h-1)** | **Larval Sample Size** | **Mean Larval RMR (±s.e.m.) (μmol O2 mg-1 h-1)** |
| 17 | >20 | 11-12 | 14-15 | 400 | 26 | 0.016(±0.002) | 41 | 0.078(±0.004) |
| 2200 | 27 | 0.020(±0.001) | 35 | 0.071(±0.005) |
| 4200 | 12 | 0.026(±0.002) | 24 | 0.066(±0.005) |
| 20 | >20 | 8 | 10-11 | 400 | 12 | 0.027(±0.002) | 23 | 0.071(±0.007) |
| 2200 | 12 | 0.020(±0.002) | 26 | 0.088(±0.006) |
| 4200 | 13 | 0.024(±0.002) | 24 | 0.087(±0.009) |
| 24 | >20 | 5 | 6-7 | 400 | 55 | 0.024(±0.001) | 65 | 0.126(±0.006) |
| 2200 | 46 | 0.021(±0.002) | 68 | 0.118(±0.006) |
| 4200 | 16 | 0.017(±0.002) | 33 | 0.118(±0.009) |
| 28 | >20 | 4 | 5 | 400 | 11 | 0.039(±0.004) | 9 | 0.196(±0.018) |
| 2200 | 11 | 0.035(±0.004) | 10 | 0.191(±0.018) |
| 4200 | 11 | 0.034(±0.002) | 10 | 0.207(±0.022) |
| 24 | 23.0 | 5 | 6-7 | 400 | 26 | 0.022(±0.002) | 21 | 0.165(±0.013) |
| 2200 | 28 | 0.028(±0.003) | 19 | 0.148(±0.018) |
| 4200 | 29 | 0.034(±0.003) | 19 | 0.174(±0.017) |
| 24 | 12.0 | 5-6 | 7-8 | 400 | 26 | 0.018(±0.002) | 15 | 0.182(±0.019) |
| 2200 | 31 | 0.026(±0.002) | 22 | 0.179(±0.013) |
| 4200 | 30 | 0.025(±0.002) | 18 | 0.173(±0.019) |
| 24 | 9.0 | 7 | 9 | 400 | 13 | 0.025(±0.004) | 11 | 0.126(±0.030) |
| 2200 | 14 | 0.022(±0.003) | 11 | 0.142(±0.020) |
| 4200 | 8 | 0.027(±0.004) | 10 | 0.130(±0.024) |
| 24 | 7.5 | 7 | - | 400 | 18 | 0.025(±0.003) | - | - |
| 2200 | 17 | 0.018(±0.003) | - | - |
| 4200 | 19 | 0.017(±0.002) | - | - |

Dashes (-) indicates treatments for which too few embryos survived to hatching for larval respirometry to be done.

# 

# Appendix 1: Chapter 1 Supplemental Tables

**Table S1.1.** Treatment conditions and carbon chemistry for CO2 × temperature experiments shown as mean (±standard deviation) temperature (°C), pHNIST, salinity, *P*O2 (kPa), *P*CO2 and *f*CO2, (µatm), and *A*T, DIC, and CO32- (μmol kg1-) measured over the course of each experiment for each corresponding target treatment. Measurements are described in the methods section.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Exp. | Target Temp | Measured Temp | Target *P*CO2 | Measured *P*CO2 | Measured pH | Sal. | *P*O2 | *A*T | DIC | *f*CO2 | CO32- |
| 1 | 17 | 16.9 ± 0.3 | 400 | 368 ± 18 | 8.17 ± 0.12 | 30 | 21.2 | 2038 ± 17 | 1851 ± 8 | 367 ± 18 | 135 ± 6 |
| 16.9 ± 0.3 | 2200 | 2037 ± 188 | 7.49 ± 0.13 | 30 | 21.2 | 2031 ± 12 | 2058 ± 21 | 2030 ± 188 | 32 ± 2 |
| 24 | 23.5 ± 0.3 | 400 | 427 ± 29 | 8.13 ± 0.09 | 30 | 21.2 | 2042 ± 11 | 1838 ± 16 | 426 ± 29 | 150 ± 7 |
| 23.6 ± 0.3 | 2200 | 2190 ± 277 | 7.49 ± 0.12 | 30 | 21.2 | 2041 ± 11 | 2048 ± 7 | 2183 ± 276 | 5 ± 5 |
| 2 | 17 | 16.9 ± 0.3 | 400 | 341 | 8.17 ± 0.04 | 31 | 22.0 ± 0.4 | 2059 | 1852 | 340.0 | 147.3 |
| 17.1 ± 0.2 | 2200 | 1869 | 7.47 ± 0.09 | 31 | 23.0 ± 0.3 | 2023 | 2037 | 1862.6 | 35.3 |
| 17.0 ± 0.2 | 4200 | 3936 | 7.22 ± 0.07 | 31 | 23.2 ± 0.3 | 2044 | 2159 | 3921.6 | 17.8 |
| 20 | 20.2 ± 0.3 | 400 | 373 | 8.13 ± 0.06 | 31 | 24.5 ± 0.3 | 2038 | 1827 | 371.5 | 150.3 |
| 19.9 ± 0.2 | 2200 | 2184 | 7.51 ± 0.05 | 31 | 23.8 ± 0.4 | 2039 | 2060 | 2176.7 | 34.8 |
| 19.9 ± 0.2 | 4200 | 3996 | 7.20 ± 0.09 | 31 | 23.4 ± 0.3 | 2059 | 2161 | 3982.0 | 20.2 |
| 24 | 24.1 ± 0.2 | 400 | 426 | 8.20 ± 0.05 | 31 | 23.6 ± 0.5 | 2044 | 1828 | 424.2 | 154.9 |
| 23.9 ± 0.2 | 2200 | 2043 | 7.52 ± 0.04 | 31 | 23.8 ± 0.5 | 2022 | 2020 | 2036.7 | 42.6 |
| 24.0 ± 0.3 | 4200 | 4310 | 7.21 ± 0.07 | 31 | 24.5 ± 0.4 | 2031 | 2127 | 4295.7 | 21.6 |
| 3 | 17 | 17.4 ± 0.2 | 400 | 322 ± 12 | 8.22 ± 0.01 | 31 | 21.5 ± 0.3 | 2054 ± 8 | 1838 ± 26 | 321 ± 12 | 153 ± 2 |
| 17.6 ± 0.3 | 2200 | 1952 ± 39 | 7.51 ± 0.01 | 31 | 22.4 ± 0.4 | 2047 ± 20 | 2066 ± 21 | 1945 ± 39 | 35 ± 1 |
| 17.4 ± 0.2 | 4200 | 4056 ± 204 | 7.20 ± 0.02 | 31 | 22.9 ± 0.4 | 2053 ± 24 | 2174 ± 16 | 4042 ± 203 | 18 ± 1 |
| 20 | 19.7 ± 0.2 | 400 | 345 ± 15 | 8.20 ± 0.02 | 31 | 23.6 ± 0.4 | 2048 ± 29 | 1833 ± 3 | 345 ± 15 | 160 ± 6 |
| 19.6 ± 0.3 | 2200 | 1964 ± 109 | 7.51 ± 0.03 | 31 | 22.6 ± 0.4 | 2031 ± 14 | 2039 ± 10 | 1957 ± 108 | 38 ± 2 |
| 19.7 ± 0.2 | 4200 | 4066 ± 227 | 7.21 ± 0.02 | 31 | 22.1 ± 0.5 | 2058 ± 6 | 2153 ± 37 | 4063 ± 226 | 20 ± 1 |
| 24 | 23.7 ± 0.2 | 400 | 331 ± 14 | 8.22 ± 0.02 | 31 | 22.0 ± 0.5 | 2044 ± 9 | 1798 ± 8 | 330 ± 14 | 185 ± 5 |
| 23.7 ± 0.3 | 2200 | 2157 ± 92 | 7.49 ± 0.02 | 31 | 22.4 ± 0.4 | 2048 ± 22 | 2050 ± 25 | 2151 ± 92 | 42 ± 1 |
| 23.6 ± 0.2 | 4200 | 4339 ± 169 | 7.20 ± 0.02 | 31 | 23.2 ± 0.4 | 2059 ± 51 | 2140 ± 8 | 4325 ± 169 | 22 ± 1 |
| 4 | 24 | 24.3 ± 0.4 | 400 | 389 ± 23 | 8.19 ± 0.02 | 32 | 21.2 | 2137 ± 3 | 1897 ± 13 | 388 ± 23 | 175 ± 8 |
| 24.1 ± 0.2 | 2200 | 2265 ± 228 | 7.50 ± 0.04 | 32 | 21.2 | 2151 ± 14 | 2156 ± 27 | 2258 ± 227 | 43 ± 4 |
| 24.2 ± 0.3 | 4200 | 4432 ± 180 | 7.21 ± 0.02 | 32 | 21.2 | 2130 ± 27 | 2230 ± 25 | 4418 ± 179 | 23 ± 1 |
| 28 | 28.2 ± 0.2 | 400 | 350 ± 19 | 8.23 ± 0.02 | 32 | 21.2 | 2157 ± 24 | 1857 ± 29 | 348 ± 19 | 215 ± 4 |
| 28.1 ± 0.2 | 2200 | 2439 ± 84 | 7.48 ± 0.02 | 32 | 21.2 | 2176 ± 50 | 2172 ± 48 | 2431 ± 83 | 49 ± 2 |
| 28.2 ± 0.3 | 4200 | 4720 ± 217 | 7.20 ± 0.03 | 32 | 21.2 | 2155 ± 20 | 2244 ± 18 | 4714 ± 204 | 26 ± 1 |

**Table S1.2.** Treatment conditions and carbon chemistry for CO2 × oxygen experiments shown as mean (±standard deviation) temperature (°C), pHNIST, *P*O2 (kPa), salinity, *P*CO2 and *f*CO2, (µatm), and *A*T, DIC, and CO32- (μmol kg1-) measured over the course of each experiment for each corresponding target treatment. Measurements are described in the methods section. The target temperature for all treatments was 24°C.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Exp.** | **Measured Temp** | **Target** ***P*O2** | **Measured  *P*O2** | **Target  *P*CO2** | ***P*CO2** | **Measured pH** | **Sal.** | ***A*T** | **DIC** | ***f*CO2** | **CO32-** |
| 5 | 24.7 ± 0.4 | 24.0 | 23.7 ± 0.3 | 400 | 370 ± 1 | 8.18 ± 0.02 | 30 | 2001 ± 6 | 1769 ± 5 | 369 ± 1 | 165.2 ± 0.5 |
| 24.5 ± 0.3 | 23.3 ± 0.3 | 2200 | 1826 ± 7 | 7.56 ± 0.1 | 30 | 2005 ± 8 | 1990 ± 8 | 1821 ± 7 | 46.7 ± 0.2 |
| 24 ± 0.6 | 23.1 ± 0.3 | 4200 | 4368 ± 19 | 7.19 ± 0.07 | 30 | 1998 ± 9 | 2099 ± 9 | 4354 ± 19 | 20.3 ± 0.1 |
| 24.6 ± 0.4 | 12.0 | 12.4 ± 1.2 | 400 | 439 ± 2 | 8.12 ± 0.04 | 30 | 1996 ± 7 | 1793 ± 6 | 437 ± 2 | 145.8 ± 0.5 |
| 24.7 ± 0.4 | 12.1 ± 1.2 | 2200 | 2338 ± 5 | 7.46 ± 0.06 | 30 | 2002 ± 4 | 2015 ± 4 | 2330 ± 5 | 37.5 ± 0.1 |
| 24.2 ± 0.5 | 12.3 ± 0.9 | 4200 | 4119 ± 17 | 7.22 ± 0.03 | 30 | 2003 ± 8 | 2093 ± 8 | 4105 ± 16 | 21.7 ± 0.1 |
| 24.9 ± 0.3 | 7.5 | 8.2 ± 1.2 | 400 | 400 ± 1 | 8.15 ± 0.05 | 30 | 2004 ± 3 | 1783 ± 3 | 399 ± 1 | 157.8 ± 0.2 |
| 24.2 ± 0.5 | 8.1 ± 1.2 | 2200 | 2189 ± 4 | 7.48 ± 0.08 | 30 | 2010 ± 3 | 2017 ± 3 | 2182 ± 4 | 39.3 ± 0.1 |
| 24.3 ± 0.4 | 7.5 ± 0.9 | 4200 | 4337 ± 36 | 7.2 ± 0.05 | 30 | 2014 ± 17 | 2112 ± 18 | 4323 ± 36 | 21 ± 0.2 |
| 6 | 24.4 ± 0.3 | 24.0 | 23.5 ± 0.3 | 400 | 385 ± 2 | 8.17 ± 0.08 | 30 | 2062 ± 11 | 1829 ± 10 | 384 ± 2 | 167.5 ± 0.9 |
| 24.7 ± 0.3 | 23.7 ± 0.3 | 2200 | 2173 ± 22 | 7.5 ± 0.07 | 30 | 2060 ± 21 | 2062 ± 21 | 2166 ± 22 | 42.2 ± 0.4 |
| 24.4 ± 0.4 | 23.2 ± 0.3 | 4200 | 4539 ± 55 | 7.19 ± 0.11 | 30 | 2064 ± 25 | 2167 ± 26 | 4524 ± 55 | 21.1 ± 0.3 |
| 24.4 ± 0.4 | 12.0 | 12.7 ± 0.9 | 400 | 505 ± 1 | 8.07 ± 0.09 | 30 | 2046 ± 4 | 1861 ± 4 | 503 ± 1 | 137.2 ± 0.3 |
| 24.4 ± 0.3 | 12.4 ± 1.2 | 2200 | 2157 ± 12 | 7.5 ± 0.05 | 30 | 2055 ± 12 | 2057 ± 12 | 2151 ± 12 | 42 ± 0.2 |
| 24.4 ± 0.4 | 12.4 ± 1.2 | 4200 | 4512 ± 20 | 7.19 ± 0.07 | 30 | 2060 ± 9 | 2162 ± 10 | 4498 ± 20 | 21.2 ± 0.1 |
| 24 ± 0.4 | 9.0 | 9.3 ± 1.5 | 400 | 520 ± 5 | 8.06 ± 0.09 | 30 | 2050 ± 19 | 1871 ± 18 | 518 ± 5 | 133.3 ± 1.3 |
| 24.1 ± 0.4 | 9.0 ± 0.9 | 2200 | 2151 ± 19 | 7.5 ± 0.05 | 30 | 2039 ± 17 | 2043 ± 18 | 2144 ± 18 | 41 ± 0.4 |
| 24.2 ± 0.5 | 9.0 ± 0.9 | 4200 | 4473 ± 64 | 7.19 ± 0.06 | 30 | 2053 ± 29 | 2155 ± 31 | 4459 ± 64 | 21 ± 0.3 |

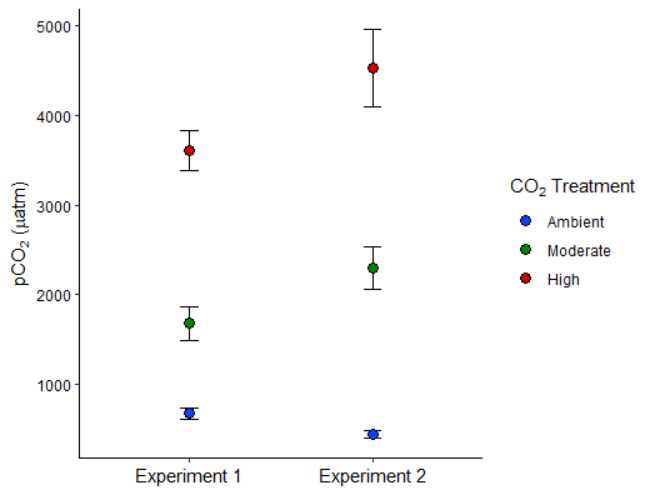
**Table S1.3.** Coefficient estimates, t-values, and p-values from multiple linear regression models of square-root transformed metabolic rates of *M. menidia* embryos and larvae.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Stage** | **Predictor Variable** | **Coefficient Estimate (±s.e.m.)** | **t** | **p** |
| Embryos | *P*CO2 | 1.5e-5 (±8.2e-6) | 1.82 | 0.070 |
| Temp | 4.5e-3 (±9.0e-4) | 4.99 | **<0.001** |
| *P*CO2 × Temp | -6.8e-7 (±3.6e-7) | -1.90 | 0.059 |
| Larvae | *P*CO2 | -1.8e-5 (±1.4e-5) | -1.32 | 0.189 |
| Temp | 1.3e-2 (±1.6e-3) | 7.86 | **<0.001** |
| *P*CO2 × Temp | 7.6e-7 (±6.2e-7) | 1.22 | 0.224 |
| Embryos | *P*CO2 | -7.2e-6 (±4.0e-6) | -1.81 | 0.071 |
| *P*O2 | -2.1e-4 (±6.9e-4) | -0.301 | 0.763 |
| *P*CO2× *P*O2 | 7.1e-7 (±2.4e-7) | 2.94 | **0.004** |
| Larvae | *P*CO2 | -1.4e-6 (±1.4e-5) | -0.245 | 0.807 |
| *P*O2 | 5.9e-4 (±2.3e-3) | 0.264 | 0.792 |
| *P*CO2 × *P*O2 | 2.7e-7 (±8.1e-7) | 0.336 | 0.737 |

**Table S1.4.** Bootstrapped means and 95% confidence interval lower (LL) and upper (UL) limits obtained by sampling from Q10 values calculated using metabolic rates for every possible pairing of individuals reared in 17°C and 28°C treatments, for *M. menidia* embryos and larvae across three *P*CO2 treatments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Stage** | ***P*CO2 (μatm)** | **LL** | **Mean** | **UL** |
| Embryo | 400 | 2.33 | 2.47 | 2.61 |
| 2200 | 1.83 | 1.95 | 2.07 |
| 4200 | 1.28 | 1.36 | 1.44 |
| Larvae | 400 | 2.51 | 2.65 | 2.79 |
| 2200 | 3.00 | 3.32 | 3.67 |
| 4200 | 3.04 | 3.26 | 3.49 |

**Chapter 2 Figures and Tables**



**Figure 2.1.** Experiment 1 and 2 mean pCO2 levels for each treatment with error bars showing standard error. In Experiment 1, each rearing tank was sampled twice. In Experiment 2, each equilibration tank was sampled three times.

Diagram

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**Figure 2.2.** Routine metabolic rates of whole *M. menidia* embryos (A, µmol O2 individual-1 h-1) and mass-specific routine metabolic rates (µmol O2 mg-1 h-1) of 2-dph larvae (B) and 5-dph larvae (C) as a function of pCO2 (µatm). Error bars display standard error about the mean. Closed circles represent means from Experiment 1 (24°C) and open circles represent means from Experiment 2 (22°C). Letters denote significant differences between pCO2 levels within an experiment (temperature level), as detected with a Tukey test, with points that do not share a letter being significantly different. Points that lack significance letters had no significant pCO2 effect according to ANOVA.

Diagram, engineering drawing

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**Figure 2.3.** Examples of a typical MO2-O2 curve with a fitted breakpoint regression (A), an MO2-O2 curve with a sudden transient increase in MO2 at low oxygen with a fitted breakpoint regression (B), and an oxyconforming MO2-O2 curve that frequently occurred in embryos with a fitted linear regression (C). In (A) and (B) the dashed line shows Pcrit, the breakpoint identified by the regression and the point at which relatively oxygen-independent metabolism shifts to highly oxygen-dependent. In (C) no such breakpoint exists because metabolism is consistently oxygen-dependent throughout the trial. The proportions of individuals exhibiting the low-oxygen increase in metabolism in (B) and the oxyconformity shown in (C) for each treatment group are listed in Table 3.

Diagram

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**Figure 2.4.** Critical oxygen levels of *M. menidia* offspring in response to pCO2 treatments at the embryo (A), 2dph larval (B), and 5dph larval (C) stages. Error bars display standard error about the mean. Closed circles represent means from Experiment 1 (24°C) and open circles represent means from Experiment 2 (22°C). Letters denote significant differences between pCO2 levels within an experiment (temperature level), as detected with a Tukey test, with points that do not share a letter being significantly different. Points that lack significance letters had no significant pCO2 effect according to ANOVA.

**Table 2.1.** Target and measured mean pH, pCO2, and temperature levels in Experiments 1 and 2.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **pH** | | | **pCO2 (µatm)** | | | **Temperature (°C)** |
|  | **Amb** | **Mod** | **High** | **Amb** | **Mod** | **High** |
| **Target levels** | 8.1 | 7.5 | 7.2 | 450 | 2000 | 4000 | 24 (Exp. 1),  22 (Exp. 2) |
| **Exp. 1** | 7.94 | 7.41 | 7.13 | 680.0 | 1683.0 | 3609.1 | 23.8 |
| **Exp. 2** | 8.08 | 7.39 | 7.09 | 441.7 | 2299.2 | 4530.9 | 22.3 |

**Table 2.2.** Mean routine MO2 and Pcrit (±standard error) for *M. menidia* embryos and larvae reared in three pCO2 treatments in two experiments (Experiment 1 at 24°C and Experiment 2 at 22°C). Routine MO2 for embryos is µmol O2 individual-1 h-1 and for larvae is µmol O2 mg dw-1 h-1. Pcrit is in mg l-1.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Routine MO2** | | | **Pcrit** | | |
| **Ambient pCO2** | **Moderate pCO2** | **High pCO2** | **Ambient pCO2** | **Moderate pCO2** | **High pCO2** |
| **Embryos** | **Exp. 1** | 0.0044(±0.00066) | 0.0036(±0.00075) | 0.0030(±0.00043) | 2.44(±0.54) | 3.01(±0.44) | 2.80(±0.32) |
| **Exp. 2** | 0.0016(±0.00030) | 0.0023(±0.00020) | 0.0047(±0.00054) | 1.90(±0.21) | 1.90(±0.32) | 2.51(±0.23) |
| **2dph Larvae** | **Exp. 1** | 0.29(±0.041) | 0.28(±0.033) | 0.25(±0.047) | 2.04(±0.25) | 1.56(±0.21) | 1.21(±0.26) |
| **Exp. 2** | 0.19(±0.017) | 0.15(±0.0071) | 0.21(±0.024) | 1.23(±0.29) | 1.42(±0.23) | 1.34(±0.31) |
| **5dph Larvae** | **Exp. 1** | 0.23(±0.016) | 0.17(±0.014) | 0.23(±0.022) | 1.23(±0.18) | 0.94(±0.11) | 0.72(±0.17) |
| **Exp. 2** | 0.18(±0.024) | 0.20(±0.011) | 0.12(±0.0092) | 1.99(±0.29) | 1.65(±0.16) | 1.17(±0.16) |

**Table 2.3.** The percentages of *M. menidia* offspring in which full oxyconformity or a low-oxygen increase in MO2 following a decline in MO2 below Pcrit, for each Experiment, pCO2 treatment, and life stage.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Ambient pCO2** | **Moderate pCO2** | **High pCO2** | ***p*-value** |
| **Oxyconformity** | **Embryos** | Exp. 1 (24°C) | 40.0% | 22.2% | 33.3% | 0.7065 |
| Exp. 2 (22°C) | 0.00% | 8.33% | 71.4% | **7.7e-5** |
| **Low-DO increase** | **Embryos** | Exp. 1 (24°C) | 10.0% | 22.2% | 11.1% | 0.71 |
| Exp. 2 (22°C) | 18.2% | 16.7% | 35.7% | 0.45 |
| **2dph Larvae** | Exp. 1 (24°C) | 100% | 90.0% | 77.8% | 0.28 |
| Exp. 2 (22°C) | 66.7% | 83.3% | 53.8% | 0.29 |
| **5dph Larvae** | Exp. 1 (24°C) | 55.6% | 37.5% | 33.3% | 0.60 |
| Exp. 2 (22°C) | 85.7% | 75.0% | 40.0% | 0.10 |

# Appendix 2: Chapter 2 Supplemental Table

**Table S2.1.** Mean and standard error of measured salinity, temperature (°C), pH, total alkalinity (TA, µmol kg-1), and dissolved inorganic carbon (DIC, µmol kg-1) , and calculated partial pressure of CO2 (pCO2, µatm), fugacity of CO2 (fCO2, µatm), concentration of bicarbonate ([HCO3-], µmol kg-1), concentration of carbonate ([CO3-], µmol kg-1), aragonite saturation state (Ωarag), and calcite saturation state (Ωcalc) for each treatment within Experiments 1 and 2. Means and standard errors were calculated across repeat samples and replicate tanks. One measured DIC sample within Experiment 2, High CO2 treatment was sealed improperly and could not be used, so measured pH was instead used to calculate DIC and the other calculated carbonate chemistry parameters.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Exp.** | **Treatment** | **Salinity** | **Temp.** | **pCO2** | **pH** | **TA** | **DIC** | **fCO2** | **[HCO3-]** | **[CO32-]** |
| 1 | Ambient | 27.6 (±1.0) | 23.8 (±0.1) | 680.0 (±62.3) | 7.94 (±0.07) | 2135.3 (±59.0) | 1989.0 (±36.8) | 677.8 (±62.1) | 1850.5 (±24.8) | 117.9 (±15.1) |
| Moderate | 28.0 (±1.0) | 23.8 (±0.1) | 1683.0 (±186.4) | 7.41 (±0.06) | 2177.6 (±67.7) | 2145.7 (±60.4) | 1677.5 (±185.8) | 2035.9 (±56.9) | 58.8 (±7.6) |
| High | 27.7 (±1.0) | 23.8 (±0.1) | 3609.1 (±219.4) | 7.13 (±0.08) | 2161.9 (±63.5) | 2232.2 (±59.1) | 3597.4 (±218.7) | 2095.0 (±57.9) | 27.7 (±2.9) |
| 2 | Ambient | 27.9 (±0.8) | 22.4 (±0.1) | 441.7 (±40.9) | 8.08 (±0.01) | 2097.0 (±32.0) | 1920.8 (±27.4) | 440.2 (±40.8) | 1742.0 (±15.6) | 146.0 (±14.3) |
| Moderate | 28.0 (±0.8) | 22.3 (±0.1) | 2299.2 (±243.0) | 7.39 (±0.06) | 2129.6 (±40.2) | 2137.0 (±43.1) | 2291.6 (±242.2) | 2037.1 (±44.7) | 38.1 (±2.0) |
| High | 28.1 (±0.8) | 22.2 (±0.3) | 4530.9 (±431.0) | 7.09 (±0.04) | 2139.8 (±46.7) | 2217.1 (±44.5) | 4515.8 (±429.5) | 2090.7 (±48.3) | 20.2 (±0.7) |

**Chapter 3 Figures and Tables**

Graphical user interface

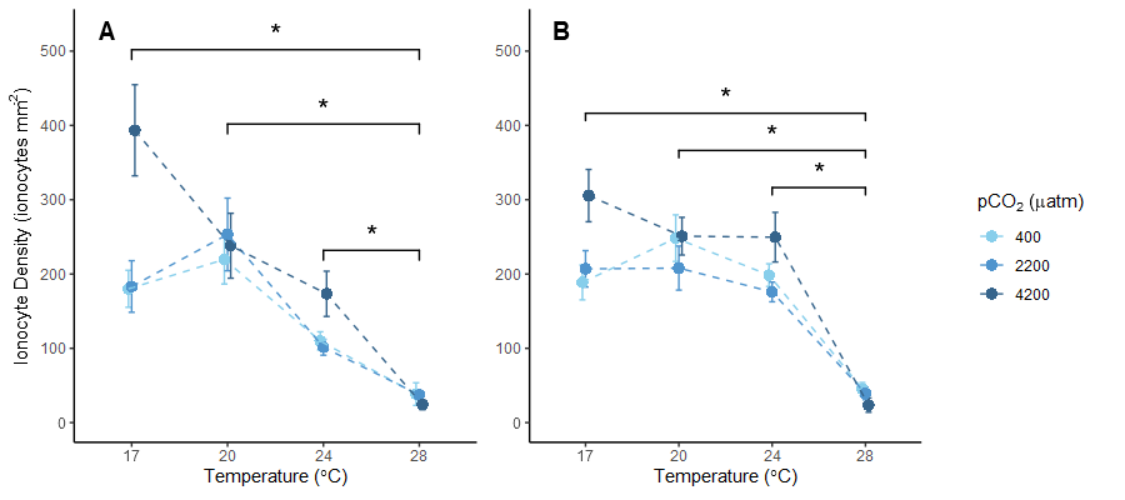
Description automatically generated

**Figure 3.1.** Microscope images of an *M. menidia* embryo, hatchling, and 10-mm larva (from top to bottom) with ionocytes stained dark purple.

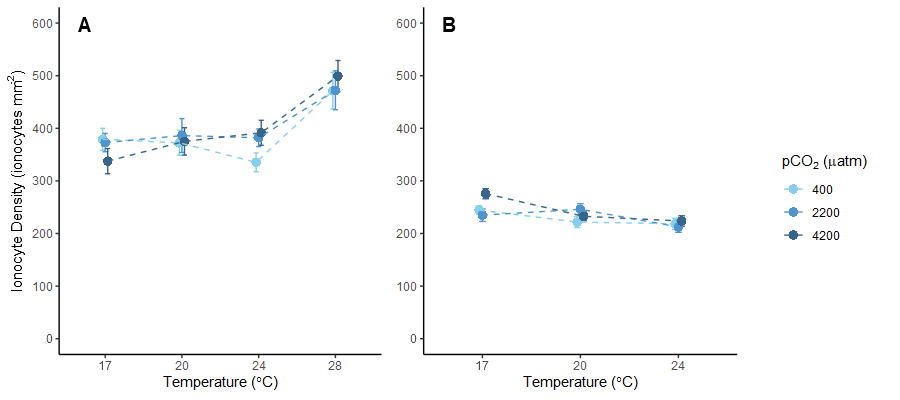
Chart, histogram

Description automatically generated

**Figure 3.2.** Frequency distribution of ionocyte densities at the embryo stage (yolk and skin) and hatchlings. In embryos the data are positively skewed, particularly for the yolk, but immediately after hatching the ionocyte densities are more normally distributed. The 10-mm larvae also have normally distributed ionocyte densities, although around a lower mean, but we do not display them here so the difference in distribution before and after hatching can be emphasized.



**Figure 3.3.** Embryo yolk sac (A) and skin (B) ionocyte density means plotted with respect to temperature and pCO2. Error bars show standard error and brackets with asterisks represent significant differences between temperature treatments (EMM, p<0.05).



**Figure 3.4.** Hatchling (A) and 10-mm larvae (B) ionocyte density means plotted with respect to temperature and pCO2. Error bars show standard error.

Chart, scatter chart

Description automatically generated

**Figure 3.5.** Metabolic rates of embryos (A) and mass-specific metabolic rates of hatchlings (B) plotted with respect to ionocyte density, with shades of blue indicating the treatment temperature ranging from 17°C (lightest shade) to 28°C (darkest shade). Each point represents an individual fish for which both RMR and ionocyte density were quantified. The black line and gray shading are a linear regression of RMR ~ Ionocyte Density with 95% confidence intervals.

**Table 3.1.** Spawning dates, target temperature, target pCO2 levels, and stages sampled in each experiment. E stands for embryos, H stands for hatchlings, and L stands for mature (10-mm) larvae.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **400 µatm** | **2200 µatm** | **4200 µatm** |
| **Experiment 1 – April 22, 2016** | | | |
| **17** | E, H | E, H | - |
| **20** | E, H | E, H | - |
| **24** | E, H | E, H | - |
| **Experiment 2 – May 3, 2016** | | | |
| **17** | E, H, L | E, H, L | E, H, L |
| **20** | E, H, L | E, H, L | E, H, L |
| **24** | E, H, L | E, H, L | E, H, L |
| **Experiment 3 – May 19, 2016** | | | |
| **17** | E, H, L | E, H, L | E, H, L |
| **20** | E, H, L | E, H, L | E, H, L |
| **24** | E, H, L | E, H, L | E, H, L |
| **Experiment 4 – May 26, 2017** | | | |
| **24** | E, H | E, H | E, H |
| **28** | E, H | E, H | E, H |

**Table 3.2.** Mean, standard error, sample size, and age at sampling (range in days post fertilization) for ionocyte density of embryo yolk sac and body skin surface in each treatment.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Temp (°C)** | **pCO2 (µatm)** | **N** | **Age (dpf)** | **Mean** | **SE** |
| Embryo (yolk sac) | 17 | 400 | 29 | 14-15 | 180.1 | 24.9 |
| 2200 | 30 | 14-15 | 183.2 | 34.7 |
| 4200 | 10 | 14-15 | 393.4 | 61.3 |
| 20 | 400 | 13 | 10-11 | 219.6 | 33.0 |
| 2200 | 13 | 10-11 | 253.3 | 48.9 |
| 4200 | 13 | 10-11 | 238.1 | 43.7 |
| 24 | 400 | 60 | 6-7 | 110.0 | 12.2 |
| 2200 | 64 | 6-7 | 101.4 | 10.7 |
| 4200 | 24 | 6-7 | 173.3 | 30.5 |
| 28 | 400 | 9 | 5 | 38.4 | 15.2 |
| 2200 | 9 | 5 | 37.4 | 5.9 |
| 4200 | 10 | 5 | 24.2 | 6.8 |
| Embryo (skin) | 17 | 400 | 29 | 14-15 | 188.7 | 23.4 |
| 2200 | 30 | 14-15 | 207.0 | 24.4 |
| 4200 | 10 | 14-15 | 305.5 | 35.2 |
| 20 | 400 | 13 | 10-11 | 248.3 | 31.3 |
| 2200 | 13 | 10-11 | 208.0 | 29.6 |
| 4200 | 13 | 10-11 | 250.9 | 25.3 |
| 24 | 400 | 60 | 6-7 | 198.5 | 15.3 |
| 2200 | 65 | 6-7 | 184.3 | 15.6 |
| 4200 | 24 | 6-7 | 249.6 | 33.4 |
| 28 | 400 | 9 | 5 | 45.2 | 8.7 |
| 2200 | 9 | 5 | 39.2 | 8.4 |
| 4200 | 10 | 5 | 23.6 | 9.6 |

**Table 3.3.** Mean, standard error, sample size, and age at sampling (range in days post hatching) for ionocyte density of newly hatched and 10-mm larvae in each treatment.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Temp (°C)** | **pCO2 (µatm)** | **N** | **Age (dph)** | **Mean** | **SE** |
| Hatchlings | 17 | 400 | 42 | 1 | 379.2 | 14.5 |
| 2200 | 42 | 1 | 372.8 | 11.8 |
| 4200 | 27 | 1 | 337.5 | 16.8 |
| 20 | 400 | 27 | 1 | 372.3 | 11.8 |
| 2200 | 27 | 1 | 386.1 | 17.4 |
| 4200 | 25 | 1 | 375.1 | 16.9 |
| 24 | 400 | 66 | 1 | 335.4 | 15.1 |
| 2200 | 69 | 1 | 382.0 | 12.2 |
| 4200 | 37 | 1 | 391.4 | 18.1 |
| 28 | 400 | 12 | 1 | 471.5 | 22.4 |
| 2200 | 12 | 1 | 472.3 | 23.6 |
| 4200 | 11 | 1 | 499.3 | 14.7 |
| 10-mm larvae | 17 | 400 | 40 | 15-23 | 244.6 | 8.5 |
| 2200 | 37 | 15-23 | 234.8 | 9.0 |
| 4200 | 33 | 15-23 | 275.7 | 8.9 |
| 20 | 400 | 40 | 12-14 | 221.7 | 9.2 |
| 2200 | 39 | 12-14 | 246.0 | 10.0 |
| 4200 | 38 | 12-14 | 232.7 | 7.7 |
| 24 | 400 | 38 | 10 | 218.5 | 10.2 |
| 2200 | 37 | 10 | 212.4 | 8.4 |
| 4200 | 39 | 10 | 223.7 | 8.4 |

**Table 3.4.** Linear model coefficients and p-values for ionocyte density

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Coefficient** | **St. Err.** | **t-value** | **p-value** |
| Embryo (yolk sac)1 | pCO2 | 7.8e-4 | 2.5e-4 | 3.1 | **0.0020** |
| Temp | -7.2e-2 | 2.7e-2 | -2.7 | **0.0082** |
| pCO2 x Temp | -3.2e-5 | 1.1e-5 | -2.9 | **0.0039** |
| Embryo (skin)2 | pCO2 | 4.6e-3 | 1.2e-3 | 3.8 | **0.0002** |
| Temp | -0.11 | 0.13 | -0.82 | 0.415 |
| pCO2 x Temp | -1.9e-4 | 5.3e-5 | -3.7 | **0.0003** |
| Hatchlings | pCO2 | -5.9e-2 | 2.0e-2 | -3.0 | **0.0031** |
| Temp | -0.88 | 2.3 | -0.38 | 0.706 |
| pCO2 x Temp | 3.0e-3 | 9.0e-4 | 3.3 | **0.0010** |
| 10-mm larvae | pCO2 | 2.5e-2 | 1.4e-2 | 1.7 | 0.0818 |
| Temp | -2.6 | 1.9 | -1.4 | 0.169 |
| pCO2 x Temp | -1.0e-3 | 7.0e-4 | -1.5 | 0.145 |

1Square-root transformed

2Natural log-transformed

**Table 3.5.** Comparison of ionocyte density results with previously reported effects of pCO2 and temperature on growth and survival of fish from the same experiments. Experiment 2 is omitted because growth and survival were not quantified. Green symbol is sole pCO2 effect and orange symbol is sole temperature effect, ‘↑’=positive effect, ‘↓’=negative effect, ‘↑↓’=both positive and negative, ‘×’=interaction, and ‘–’=no effect. The reference numbering is the experiment number used in the publication where the growth and survival data are reported, Murray and Baumann (2018), because the numbering for the same experiments differs in this study.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Embryo ionocytes | Hatchling ionocytes | Hatch survival | Hatch length | Larval survival | Larval growth | Reference  numbering |
| Exp. 1 | **–** | **×** | **–** | **–** | **↑** | **↑** | ‘Exp. 2’ |
| Exp. 3 | **↓** | **↓** | **–** | **×** | **↑↓** | **↑** | ‘Exp. 3’ |
| Exp. 4 | **↓** | **–** | **–** | **↑, ↓, ×** | **↓** | **↑** | ‘Exp. 5’ |

**Chapter 4 Figures and Tables**

Diagram

Description automatically generated

**Figure 4.1.** The DEBkiss model (diagram adapted from Jager et al., 2013) with stage-specific survival parameters. The candidate parameters for hypoxia stress mechanisms are highlighted in red boxes. The left panel shows the energy budget for the full life cycle and the right panel shows the stage-specific survival modification.

Diagram

Description automatically generated

**Figure 4.2.** Predicted (lines) and observed data (dots) for the DEBkiss model of *M. menidia*. The state variables are (A) total length (mm) over time (days), (B) cumulative reproduction (eggs) over time (days), (C) egg buffer mass (mg) over time (days), and (D) survival over time (days). Predicted data lines are calculated with the parameter values listed in Table 1.

Diagram

Description automatically generated

**Figure 4.3.** The effect of DO on correction factor *c* (A) at three different values of the exponential coefficient *Z*, and correction factor *c1* (B) as a function of *c*. Actual estimated *Z* values are listed in Table 4, and the three *Z* values used in (A) are sample values to show how *Z* affects the relationship between DO and *c*. Correction factor *c1* is secondarily impacted by DO through *c*, so that *c1* increases as DO and *c* decrease.

Diagram

Description automatically generated

**Figure 4.4.** Simulated effect of hypoxia on the state variables total length (A), egg buffer mass (B), and survival (C), brought about by reducing *JaAm*. These sample plots use *JaAm* as an example to show how we simulated the hypothesized effect of hypoxia on each parameter to test whether a given parameter met Criterion 1. The solid line is the predicted data for the control value of *JaAm* while the dotted lines show how the predicted data changes as *JaAm* is reduced (to arbitrary example values), as it would be by correction factor *c*. Table 3 shows the full results of running these simulations on all parameters.

Diagram

Description automatically generated

**Figure 4.5.** Simulated effect of hypoxia on the state variables total length (A), egg buffer mass (B), and survival (C), brought about by increasing *JvM*. These sample plots use *JvM* as an example to show how we simulated the hypothesized effect of hypoxia on each parameter to test whether a given parameter met Criterion 1. The solid line is the predicted data for the control value of *JvM* while the dotted lines show how the predicted data changes as *JvM* is increased (to arbitrary example values), as it would be by correction factor *c1*. Table 3 shows the full results of running these simulations on all parameters.

Diagram

Description automatically generated

**Figure 4.6.** Simulated effect of hypoxia on the state variables total length (A), egg buffer mass (B), and survival (C), brought about by increasing *μemb* and *μlar* simultaneously. These sample plots use *μemb* and *μlar* as an example to show how we simulated the hypothesized effect of hypoxia on each parameter to test whether a given parameter met Criterion 1. The solid line is the predicted data for the control values of *μemb* and *μlar* while the dotted lines show how the predicted data changes as mortality is increased (to arbitrary example values), as it would be by correction factor *c1*. Table 3 shows the full results of running these simulations on all parameters.

Chart, diagram

Description automatically generated

**Figure 4.7.** Best fit of DEBkiss model to experimental data from four DO levels, showing fit to early life data only. The best fitting model was selected based on a combination of initial criteria that all three response variables’ predicted values are affected by the hypoxia correction factor and ΔAICc. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days), with means rather than all data plotted for survival for ease of viewing patterns.

**Table 4.1.** DEBkiss parameters, their abbreviations, and their fixed or estimated values from fitting to full life data. Units are given with the value unless the parameter is a unitless ratio.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Symbol** | **Fixed or estimated** | **Value** |
| Max. area-specific assimilation rate | *JaAm* | Estimated | 0.333 mg mm-2 d-1 |
| Max. volume-specific maintenance rate | *JvM* | Fixed | 0.0214 mg mm-3 d-1 |
| Initial egg buffer mass | *WB0* | Fixed | 0.15 mg |
| Total length at puberty | *LVp* | Fixed | 102 mm |
| Yield of assimilates on volume | *yAV* | Fixed | 0.8 |
| Yield of egg buffer on assimilates | *yBA* | Fixed | 0.95 |
| Yield of structure on assimilates | *yVA* | Estimated | 0.365 |
| Fraction of assimilates allocated to soma | *κ* | Fixed | 0.8 |
| Scaled food level | *f* | Fixed | 1 |
| Scaled food level for embryo | *fB* | Fixed | 1 |
| Half-saturation total length | *LVf* | Fixed | 0 |
| Mortality rate for embryos | *μemb* | Estimated | 0.0639 |
| Mortality rate for larvae | *μlar* | Estimated | 0.0294 |

**Table 4.2.** Fluxes, state variables, and differential equations in the DEBkiss model.

|  |  |  |  |
| --- | --- | --- | --- |
| **Flux** | **Symbol** | **Equation** | **Units** |
| Assimilation flux | *JA* |  | mg day-1 |
| Maintenance flux | *JM* |  | mg day-1 |
| Flux to structural growth | *JV* |  | mg day-1 |
| Flux to reproduction buffer | *JR* |  | mg day-1 |
| Flux to maturity | *JJ* |  | mg day-1 |
|  | | | |
| **State Variable** | **Symbol** | **Equation** | **Units** |
| Structural dry mass over time | *WV* |  | mg day-1 |
| Continuous reproduction rate | *R* |  | eggs day-1 |
| Egg buffer (yolk) mass | *WB* |  | mg day-1 |
| Survival | *S* |  | unitless  (range 0-1) |
|  | | | |
| **Other variables and conversions** | **Symbol** | **Equation** | **Units** |
| Total physical length | *LM* |  | mm |
| Volumetric length | *L* |  | mm (cubic root of volume) |
| Shape coefficient | *δM* |  | unitless |
| Dry weight density of structure | *dV* |  | mg mm-3 |
| Dry mass at puberty | *WVp* |  | mg |
| Volume-specific maturity maintenance costs | *JvJ* | *-* | mg mm-3 day-1 |
| Structural volume at puberty | *LVp3* | - | mm-3 |
| Scaled measure of resource availability | *f* | - | unitless  (range 0-1) |

**Table 4.3.** The mean survival to hatching, hatch time (at which egg buffer is zero), length at hatching, length at 15 dph, and survival to 15 dph from the different oxygen treatments in Cross et al. (2019). The control DO level means (7.7 mg l-1) also include data from Murray and Baumann (2018).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **7.7 mg L-1** | **4.2 mg L-1** | **3.1 mg L-1** | **2.7 mg L-1** |
| Survival to hatching | 74.3% | 70.6% | 85.8% | 30.2% |
| Hatch time (egg buffer mass = 0) | 6 days | 7 days | 8 days | 9 days |
| Length at hatching | 5.3 mm | 4.6 mm | 4.4 mm | 4.1 mm |
| Larval length at 15 dph | 15.8 mm | 12.2 mm | 9.2 mm | - |
| Larval survival to 15 dph | 44.0% | 22.2% | 20.9% | 0% |

**Table 4.4.** Summary of impacts of altering each DEBkiss parameter on predicted data for total length, time to hatching (egg buffer mass = 0), and survival over time. We used this information to choose which parameters to which to apply hypoxia-based correction factors by identifying those that best meet our requirement of allowing hypoxia effects on at least one of three state variables, within the range of our data. The last column indicates whether the effect of changing the parameter matches the overall patterns observed in the data (i.e. an increase or decrease in at least one state variable).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Impact on predicted values of:** | | |  |
| **Parameter** | **Correction factor** | **Total length (mm)** | **Time to hatching (d)** | **Survival proportion** | **Criterion 1 met?** |
| *JaAm* | *c* | ✓ | ✓ | ✓ | yes |
| *JvM* | *c1* | ✓ | ✓ (weak) | ✓ (weak) | yes |
| *WB0* | *c* |  |  |  | no |
| *LVp* | *c, c1* |  |  |  | no |
| *yAV* | *c* |  |  |  | no |
| *yBA* | *c* |  |  |  | no |
| *yVA* | *c* | ✓ | ✓ | ✓ | yes |
| *κ* | *c* | ✓ | ✓ | ✓ | yes |
| *f* | *c* | ✓ | ✓ | ✓ | yes |
| *fB* | *c* | ✓ (prehatch only) | ✓ | ✓ | yes |
| *LVf* | *c1* |  |  |  | no |
| *μemb* | *c1* |  |  | ✓ | yes |
| *μlar* | *c1* |  |  | ✓ | yes |

**Table 4.5.** The estimated *Z* value and AICc when the correction factors were applied to each parameter or combination of parameters. ΔAICc and Akaike weights are listed only for models that satisfied Criterion 2 as the ones that do not fit the criteria are not eligible to be selected as the best model, and was calculated with AICcmin = 584.65 for the *yVA* + *JvM* + *μemb* + *μlar* model.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter(s) affected by hypoxia** | **Correction factor(s)** | **Estimated *Z* [95% CI]** | **AICc** | **ΔAICc** | **Akaike weight** |
| *JaAm* | *c* | 1.70 [1.69-2.70] | 600.73 | 16.08 | 1.16e-4 |
| *yVA* | *c* | 1.48 [1.20-3.21] | 602.38 | 17.73 | 5.07e-5 |
| *JvM* | *c1* | 0.365 [0.302-0.5178] | 599.42 | - | - |
| *μemb* | *c1* | 0.626 [0.435-0.992] | 585.76 | - | - |
| *μlar* | *c1* | 0.303 [0.201-0.492] | 575.06 | - | - |
| *JaAm* + *JvM* | *c* + *c1* | 1.72 [1.72-2.69] | 600.65 | 16.00 | 1.20e-4 |
| *yVA* + *JvM* | *c* + *c1* | 1.47 [1.22-3.08] | 602.23 | 17.58 | 5.46e-5 |
| *JvM* + *μemb* | *c1* + *c1* | 0.520 [0.374-0.851] | 582.83 | - | - |
| *JaAm* + *μemb* | *c* + *c1* | 1.70 [1.69-2.04] | 590.30 | 5.95 | 0.0213 |
| *yVA* + *μemb* | *c* + *c1* | 1.31 [1.20-1.78] | 589.29 | 4.64 | 0.0353 |
| *JvM* + *μlar* | *c1* + *c1* | 0.354 [0.299-0.448] | 568.13 | - | - |
| *JaAm* + *μlar* | *c* + *c1* | 1.70 [1.69-2.25] | 595.45 | 10.80 | 0.00162 |
| *yVA* + *μlar* | *c* + *c1* | 1.34 [1.20-1.98] | 594.67 | 10.02 | 0.00239 |
| *μemb* + *μlar* | *c1* + *c1* | 0.766 [0.543-1.15] | 580.11 | - | - |
| *JaAm* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.70 [1.69-2.02] | 586.75 | 2.10 | 0.126 |
| *yVA* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.32 [1.20-1.76] | 584.78 | 0.13 | 0.336 |
| *JvM* + *μemb* + *μlar* | *c1* + *c1* + *c1* | 0.712 [0.482-1.09] | 578.82 | - | - |
| *JaAm* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.72 [1.72-2.04] | 586.86 | 2.21 | 0.119 |
| *yVA* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.31 [1.22-1.75] | 584.65 | 0 | 0.359 |

**Table 4.6.** The value of the DEBkiss parameters that best reproduce the hypoxia effects observed experimentally, calculated (along with 95% confidence intervals in brackets) for each DO treatment level using the correction factors *c* and *c1* and the estimated value of *Z* = 1.315.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Product of correction factor and initial parameter value** | | | |
| **7.7 mg L-1** | **4.2 mg L-1** | **3.1 mg L-1** | **2.7 mg L-1** |
| ***yVA*** | 0.364  [0.364, 0.365] | 0.343  [0.337, 0.356] | 0.274  [0.261, 0.308] | 0.211  [0.198, 0.249] |
| ***μemb*** | 0.175  [0.175, 0.175] | 0.186  [0.179, 0.190] | 0.234  [0.207, 0.244] | 0.303  [0.256, 0.322] |
| ***μlar*** | 0.0807  [0.0806, 0.0807] | 0.0856  [0.0825, 0.0872] | 0.107  [0.0956, 0.112] | 0.139  [0.118, 0.148] |

# Appendix 3: Chapter 4 Supplemental Figure

Chart, diagram

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**Figure S4.1.** Best fit of DEBkiss model to all experimental data from four DO levels. The best fitting model was selected based on a combination of initial criteria that all three response variables’ predicted values are affected by the hypoxia correction factor and ΔAICc. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days).