**The combined effects of acidification and acute warming on the embryos of Pacific herring (*Clupea pallasii*)**

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**1. Supplementary Figures and Tables**

Diagram

Description automatically generated

**Figure S1.** Schematic of the control system for the winter experiment, performed at Shannon Point Marine Center (SPMC). 3 header tanks designated as “ambient ” received -free air to reduce inlet seawater. 3 header tanks designated as “high ” received additional gas from a peristaltic pump, which was broken up into small bubbles by powerhead pumps. Water from header tanks was adjusted to the main tank temperature with 8 feet of plastic coils used for heat exchange. After passing through the coil, water was distributed into an individual cup that housed embryos. 6 replicate cups per treatment were placed in each of the 4 temperature tank for a total of 48 cups (2 treatments 6 cups 4 temperature tanks). Water in the main tanks flowed from ambient (10°C) to extreme (16°C) in increments of 2°C (spring experiment: omission of 12° and 14°C; 8 replicate cups per treatment).

Chart, bubble chart

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**Figure S2.** Superior view of temperature tank for the winter experiment, performed at Shannon Point Marine Center (SPMC). Temperature tanks were controlled with 4 temperature controllers (Elitech® STC-1000) connected to three heaters per tank. Circulation pumps increased heat exchange throughout the temperature tanks. 6 replicate cups per treatment were placed in each temperature tank. Water from header tanks were fed through 8-foot-long plastic tubes, coiled, and submerged in the temperature tank to enhance heat exchange. Tubing was connected to stopcocks and dripped into replicate cups where embryos were housed. (Spring: 8 replicate cups per treatment).

A close up of a fish

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**Figure S3. A.** Example of a healthy Pacific herring hatchling. **B.** Developmental malformation: notochord curvature. **C.** Development malformation: underdeveloped jaw. **D.** Developmental malformation: spinal deviation. **E.** Developmental malformation: yolk sac edema and notochord shortening.

**Table S1.** Average *in-situ* parameters and calculated carbonate chemistry values at incubation conditions for the winter experiment, performed in March 2021. Treatment combinations of + temperature are represented at ambient and high (average 677.91 ± 152 ; 1365 ± 407 ) and 4 discrete temperatures (10, 12, 14, 16) with mean ± 1 SD. Typical nutrient concentrations of seawater source include total P: 2.2 µmol kg−1 and total Si: 2 µmol kg−1. Equilibrium constants for the dissociation of carbonic acid in seawater (K1 and K2) followed Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and the constant for KHSO4 was supplied by Dickson (1990). Sample size (N) varies from 14 to 21 for treatment combination.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | *In-Situ* Measurements | | Carbonate Chemistry Values | | |
| **TestTemp.**  () | **Test** **p** | **pH**  (NIST) | **Temperature**  () | **p**  () | **pH**  (Total) | **DIC** |
| 10 | Ambient | 7.90 ± 0.14 | 10.20 ± 0.17 | 613.49 ± 120.84 | 7.86 ± 0.06 | 1986.08 ± 23.95 |
| 10 | High | 7.49 ± 0.10 | 10.35 ± 0.12 | 1222.15 ± 330.88 | 7.59 ± 0.14 | 2063.56 ± 18.67 |
| 12 | Ambient | 7.88 ± 0.05 | 12.07 ± 0.78 | 630.37 ± 92.43 | 7.85 ± 0.06 | 1983.77 ± 23.22 |
| 12 | High | 7.41 ± 0.04 | 12.27 ± 0.31 | 1363.59 ± 443.03 | 7.56 ± 0.17 | 2068.34 ± 18.88 |
| 14 | Ambient | 7.86 ± 0.05 | 13.48 ± 0.26 | 670.66 ± 94.77 | 7.83 ± 0.06 | 1985.30 ± 13.84 |
| 14 | High | 7.42 ± 0.11 | 13.53 ± 0.15 | 1401.15 ± 469.50 | 7.55 ± 0.16 | 2062.04 ± 30.27 |
| 16 | Ambient | 7.81 ± 0.06 | 15.56 ± 0.38 | 797.12 ± 300.12 | 7.77 ± 0.12 | 1991.85 ± 29.91 |
| 16 | High | 7.41 ± 0.07 | 15.57 ± 0.25 | 1473.59 ± 385.12 | 7.52 ± 0.13 | 2066.14 ± 21.83 |
| **Average** | **Ambient** | **7.86** ± **0.3** | **---** | **677.91** ± **152** | **7.83** ± **0.08** | **1986.75** ± **23** |
| **Average** | **High** | **7.42** ± **0.08** | **---** | **1365.12** ± **407** | **7.55** ± **0.15** | **2065.02** ± **22** |

**Table S2.** Average *in-situ* parameters and calculated carbonate chemistry values at incubation conditions for the spring experiment, performed in May 2021. Treatment combinations of + temperature are represented at ambient and high (average 902.35 ± 83 , 2170.61 ± 531 ) and 2 discrete temperatures (10, 16) with mean ± 1 SD. Typical nutrient concentrations of seawater source include total P: 2.2 µmol kg−1 and total Si: 2 µmol kg−1. Equilibrium constants for the dissociation of carbonic acid in seawater (K1 and K2) followed Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and the constant for KHSO4 was supplied by Dickson (1990). Sample size (N) varies from 12 to 16 for treatment combinations.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | *In-Situ* Measurements | | Carbonate Chemistry Values | | |
| **Test Temp.**  () | **Test p** | **pH**  (NIST) | **Temperature**  () | **p**  () | **pH**  (Total) | **DIC** |
| 10 | Ambient | 7.94 ± 0.15 | 10.46 ± 0.36 | 932.88 ± 99.95 | 7.70 ± 0.04 | 1946.22 ± 8.61 |
| 10 | High | 7.55 ± 0.08 | 10.68 ± 0.53 | 2111.40 ± 545.02 | 7.39 ± 0.11 | 2038.00 ± 30.50 |
| 16 | Ambient | 7.92 ± 0.02 | 15.53 ± 0.20 | 871.81 ± 65.29 | 7.73 ± 0.03 | 1943.19 ± 11.77 |
| 16 | High | 7.45 ± 0.04 | 15.53 ± 0.26 | 2229.82 ± 516.68 | 7.36 ± 0.10 | 2048.45 ± 20.63 |
| **Average** | **Ambient** | **7.93** ± **0.09** | **---** | **902.35** ± **83** | **7.72 ± 0.04** | **1944.71** ± **10** |
| **Average** | **High** | **7.5** ±**0.06** | **---** | **2170.61** ± **531** | **7.38 ± 0.11** | **2043.23** ± **26** |

**Table S3.** ANOVA results for oxygen consumption rates per embryo () (nmol ) in the winter experiment. Significant values indicated in bold. Arrow indicates the direction of trait effect trend with increasing factor.

|  |  |  |  |
| --- | --- | --- | --- |
| **Factor** | **Df** | **F** | ***p*** |
| **↑** | 1 | 8.08 | **0.012** |
| **Temp ↑** | 3 | 4.90 | **0.013** |
| Temp | 3 | 0.21 | 0.884 |

**Table S4.** LMM results for heart contractions in control embryos and those held at 25 in measurements taken 3 days post- exposure in the spring experiment. Significant values indicated in bold. Arrow indicates the direction of trait effect trend with increasing factor.

|  |  |  |  |
| --- | --- | --- | --- |
| **Factor** | **Df** | **Std. Error** | ***p*** |
| Incubation Temp | 20 | 7.654 | 0.199 |
| Max Temp | 199 | 05.076 | 0.478 |
| **Duration ↑** | 195 | 2.905 | **0.001** |
| **Incubation Temp ↓ Duration ↑** | 198 | 4.115 | **0.053** |

**Table S5.** Winter ANOVA results for time to first hatch (dpf), peak hatch (dpf), total hatch (%), malformation rate of total embryos per replicate (%), malformation rate of total hatchlings (%), and hatching success (%). Significant treatment effects are indicated in bold. Arrow indicates the direction of the trait effect trend with increasing factor.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trait** | **Factor** | **Df** | **F** | ***p*** |
| *Time to First hatch* |  | 1 | 4 | 0.063 |
| **Temp ↓** | 3 | 480 | **<0.001** |
| **Temp ↓** | 3 | 4 | **0.027** |
| *Time to Peak hatch* | **↑** | 1 | 5 | **0.039** |
| **Temp ↓** | 3 | 498.86 | **<0.001** |
| Temp | 3 | 2.86 | 0.069 |
| *Cumulative hatch* |  | 1 | 1.93 | 0.183 |
| Temp | 3 | 0.97 | 0.429 |
| Temp | 3 | 0.77 | 0.526 |
| *Malformation rate*  *(total embryos)* | **↑** | 1 | 12.85 | **0.002** |
| **Temp ↑** | 3 | 6.19 | **0.005** |
| **Temp ↑** | 3 | 3.58 | **0.037** |
| *Malformation rate*  *(total hatch)* |  | 1 | 3.20 | 0.093 |
| **Temp ↑** | 3 | 7 | **0.003** |
| Temp | 3 | 1.70 | 0.207 |
| *Hatching success* |  | 1 | 0.05 | 0.457 |
| **Temp ↓** | 3 | 5.12 | **0.054** |
| Temp | 3 | 0.10 | 0.389 |

**Table S6.** Spring ANOVA results for time to first hatch (dpf), peak hatch (dpf), total hatch (%), malformation rate of total embryos per replicate (%), malformation rate of total hatchlings (%), and hatching success (%). Significant treatment effects are indicated in bold. Arrow indicates the direction of trait effect trend with increasing factor.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trait** | **Factor** | **Df** | **F** | ***p*** |
| *Time to First hatch* |  | 1 | 0.05 | 0.512 |
| **Temp ↓** | 1 | --- | **<0.001** |
| Temp | 1 | 0.05 | 0.512 |
| *Time to Peak hatch* |  | 1 | 0.09 | 0.768 |
| **Temp ↓** | 1 | 87.36 | **<0.001** |
| Temp | 1 | 0.81 | 0.384 |
| *Cumulative hatch* |  | 1 | 2.90 | 0.114 |
| **Temp ↓** | 1 | 14.17 | **0.003** |
| Temp | 1 | 3.60 | 0.082 |
| *Malformation rate*  *(total embryos)* |  | 1 | 0.42 | 0.526 |
| **Temp ↓** | 1 | 6.803 | **0.023** |
| Temp | 1 | 0.75 | 0.402 |
| *Malformation rate*  *(total hatch)* |  | 1 | 1.61 | 0.228 |
| Temp | 1 | 0.051 | 0.825 |
| Temp | 1 | 1.11 | 0.313 |
| *Hatching success* |  | 1 | 3.37 | 0.091 |
| **Temp ↓** | 1 | 5.38 | **0.039** |
| Temp | 1 | 3.12 | 0.103 |

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