Supplementary Material

# Supplementary Data

# Supplementary Figures and Tables

**Supplementary Table S1**: Criteria used to score behaviors indicative of stress in *Cucumaria frondosa* post-exposure to stress conditions.

|  |  |
| --- | --- |
| Behavior  | Score  |
| Full attachment, no movement, tentacles extended or retracted, no body bloating | 0 |
| Body is ball shaped, podia extended, attached, tentacles extended or retracted | 0.5 |
| Body is ball shaped, podia extended, not attached, tentacles extended or retracted | 0.75 |
| Slow movement | 1 |
| Fast movement | 2 |
| Body is ball shaped, rolling, tentacles sometimes extended but not fluffy | 3 |
| Body is ball shaped with severe body contractions (peristaltic motion) | 3.5  |
| Full Active buoyancy adjustment  | 4 |

**Supplementary Table S2:** Results of two-way ANOVA on density of cellular and hormonal metrics across different treatments (17°C, 5°C, 15 psu, 22 psu).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| TREATMENT | TWO-WAY ANOVA | HOLM-SIDAK | DF | RESIDUAL | STATISTIC | p-VALUE |
| Coelomocytes |
| Air at 17°C | Exposed vs Control | - | 1 | 20 | F = 11.66 | 0.0030 |
| 1 h | 1 | 20 | t = 2.43 | 0.025 |
| 24 h | 1 | 20 | t = 2.40 | 0.026 |
| Time (1 h vs 24 h)  | - | 1 | 20 | F = 1.88 | 0.19 |
| Exposed vs Control x Time | - | 1 | 20 | F = 0.0072 | 0.93 |
| Air at 5°C | Exposed vs Control | - | 1 | 18 | F = 2.97 | 0.0080 |
| 1 h | 1 | 18 | t = 0.80 | 0.44 |
| 24 h | 1 | 18 | t = 3.40 | 0.003 |
| Time (1 h vs 24 h)  | - | 1 | 18 | F = 3.81 | 0.067 |
| Exposure  | 1 | 18 | t = 2.80 | 0.012 |
| Control  | 1 | 18 | t = 0.063 | 0.95 |
| Exposed vs Control x Time  | - | 1 | 18 | F = 3.4 | 0.082 |
| Salinity at 15 psu  | Exposed vs Control | - | 1 | 12 | F = 2.41 | 0.15 |
| Time (1 h vs 24 h)  | - | 1 | 12 | F = 0.050 | 0.83 |
| Exposed vs Control x Time | - | 1 | 12 | F = 4.67 | 0.049 |
| Salinity at 22 psu | Exposed vs Control | - | 1 | 19 | F = 0.38 | 0.54 |
| Time (1 h vs 24 h)  | - | 1 | 19 | F = 0.076 | 0.79 |
| Exposed vs Control x Time  | - | 1 | 19 | F = 2.26 | 0.15 |
| Small aggregates |
| Air at 17°C | Exposed vs Control | - | 1 | 23 | F = 4.38 | 0.048 |
| 1 h | 1 | 23 | t = 2.73 | 0.012 |
| 24 h | 1 | 23 | t = 0.42 | 0.68 |
| Time (1 h vs 24 h)  | - | 1 | 23 | F = 0.082 | 0.78 |
| Exposed vs Control x Time  | - | 1 | 23 | F = 2.093 | 0.16 |
| Air at 5°C | Exposed vs Control | - | 1 | 16 | F = 2.84 | 0.11 |
| Time (1 h vs 24 h)  | - | 1 | 16 | F = 0.0078 | 0.93 |
| Exposed vs Control x Time  | - | 1 | 16 | F = 0.42 | 0.53 |
| Salinity at 15 psu  | Exposed vs Control | - | 1 | 13 | F = 0.084 | 0.78 |
| Time (1 h vs 24 h)  | - | 1 | 13 | F = 0.070 | 0.80  |
| Exposed vs Control x Time  | - | 1 | 13 | F = 1.45 | 0.25 |
| Salinity at 22 psu | Exposed vs Control | - | 1 | 18 | F = 8.343 | 0.010 |
| 1 h  | 1 | 18 | t = 1.64 | 0.12 |
| 24 h  | 1 | 18 | t = 2.39 | 0.028 |
| Time (1 h vs 24 h)  | - | 1 | 18 | F = 0.90 | 0.36 |
| Exposed vs Control x Time  | - | 1 | 18 | F = 6.2 | 0.44 |
| Large aggregates |
| Air at 17°C | Exposed vs Control | - | 1 | 21 | F = 0.43 | 0.52 |
| Time (1 h vs 24 h)  | - | 1 | 21 | F = 1.18 | 0.29 |
| Exposed vs Control x Time  |  | 1 | 21 | F = 0.040 | 0.84 |
| Air at 5°C | Exposed vs Control | - | 1 | 18 | F = 0.11 | 0.75 |
| Time (1 h vs 24 h)  | - | 1 | 18 | F = 4.57 | 0.047 |
| Exposure  | 1 | 18 | t = 1.71 | 0.10 |
| Control | 1 | 18 | t = 1.30 | 0.21 |
| Exposed vs Control x Time | - | 1 | 18 | F = 0.15 | 0.70 |
| Salinity at 15 psu  | Exposed vs Control | - | 1 | 12 | F = 2.41 | 0.15 |
| Time (1 h vs 24 h)  | - | 1 | 12 | F = 0.049 | 0.83 |
| Exposed vs Control x Time | - | 1 | 12 | F = 0.049 | 0.83 |
| Salinity at 22 psu | Exposed vs Control | - | 1 | 17 | F = 0.019 | 0.89 |
| Time (1 h vs 24 h)  | - | 1 | 18 | F = 0.088 | 0.77 |
| Exposed vs Control x Time  | - | 1 | 18 | F = 0.11 | 0.74 |
| Cortisol  |
| Air at 17°C | Exposed vs Control | - | 1 | 5 | F = 4.70 | 0.082 |
| 1 h  | 1 | 5 | t = 3.47 | 0.018 |
| 24 h  | 1 | 5 | t = 0.019 | 0.99 |
| Time (1 h vs 24 h) | - | 1 | 5 | F = 10.65 | 0.022 |
| Exposure | 1 | 5 | t = 3.44 | 0.018 |
| Control | 1 | 5 | t = 0.91 | 0.41 |
| Exposed vs Control x Time |  | 1 | 5 | F = 4.58 | 0.086 |
| Air at 5°C | Exposed vs Control |  | 1 | 5 | F = 1.19 | 0.33 |
| Time (1 h vs 24 h) | - | 1 | 5 | F = 0.44 | 0.54 |
| Exposed vs Control x Time | - | 1 | 5 | F = 0.12 | 0.75 |
| Salinity at 15 psu  | Exposed vs Control | - | 1 | 7 | F = 0.99 | 0.35 |
| Time (1 h vs 24 h) | - | 1 | 7 | F = 0.62 | 0.46 |
| Exposed vs Control x Time | - | 1 | 7 | F = 1.46 | 0.27 |
| Salinity at 22 psu (ranked)  | Exposed vs Control | - | 1 | 4 | F = 1.78 | 0.25 |
| Time (1 h vs 24 h) | - | 1 | 4 | F = 1.78 | 0.25 |
| Exposed vs Control x Time | - | 1 | 4 | F = 1.78 | 0.25 |

**Supplementary Table S3:** Number of free coelomocytes (separated by cell type) per mL of fluid in the Polian vesicle fluid of sea cucumber measured across different treatments (17°C, 5°C, 15 psu 22 psu).

|  |  |
| --- | --- |
| TREATMENT | Coelomocyte types  |
|  |  |  | **Phagocytes** | **Fusiform cells** | **Morula cells** | **Crystal cells** |
| 17°C air | *1 h* | *Control* | 366667 | 8333 | 0 | 0 |
|  |  | *Exposed* | 500000 | 20000 | 15000 | 10000 |
|  | *24 h* | *Control* | 300000 | 11667 | 0 | 0 |
|  |  | *Exposed* | 950000 | 25000 | 41667 | 0 |
| 5°C air | *1 h* | *Control* | 657143 | 5556 | 22222 | 5556 |
|  |  | *Exposed* | 600000 | 5556 | 33333 | 11111 |
|  | *24 h* | *Control* | 470000 | 16667 | 16667 | 0 |
|  |  | *Exposed* | 883333 | 66667 | 58333 | 33333 |
| 15 psu | *1 h* | *Control* | 533500 | 22083.33 | 0 | 0 |
|  |  | *Exposed* | 644000 | 29000 | 16875 | 0 |
|  | *24 h* | *Control* | 438125 | 19350 | 13250 | 13500 |
|  |  | *Exposed* | 566875 | 0 | 0 | 0 |
| 22 psu | *1 h* | *Control* |

|  |
| --- |
| 353929 |

 | 0 | 0 | 0 |
|  |  | *Exposed* |

|  |
| --- |
| 408107 |

 | 5964 | 14286 | 4750 |
|  | *24 h* | *Control* | 389500 | 3750 | 2083 | 0 |
|  |  | Exposed | 459500 | 4000 | 6000 | 2000 |



**Figure S1:** Experimental tank setup: **A)** Random distribution of tanks/treatments in the experimental setup. **B)** Tanks used for controls and exposures to low salinity received flow at the desired salinity (35 and 22/15 psu, respectively). CM = camera, IF = inflow, OF = outflow, WL = waterline. **C)** Tanks used for exposure to 17°C air were empty and exposed to ambient temperature. **D)** Tanks used for exposure to 5°C air were empty and placed on ice to achieve the desired temperature. All tanks held one sea cucumber and empty tanks had a lid.



Supplementary Figure S2: Different coelomocyte types found in *C. frondosa.* A) Inactive (bladder) phagocyte (scale bar = 5 µm). B) Two active (petaloid) phagocytes (scale bar = 10 µm). C) Morula cell (scale bar = 5 µm). D) Active (filopodial) phagocyte (scale bar = 10 µm). E) Progenitor cell (scale bar = 5 µm). F) Crystal cell (scale bar = 9 µm). G) Fusiform cell (scale bar = 4 µm). H) A cluster of hemocytes (scale bar = 8 µm).



Supplementary Figure S3: Frequency of cloacal openings (min-1) in *C. frondosa* at various time points before exposure (time 0 h, negative control), after the 1-h exposure (time 1 h) and over a subsequent recovery period of 23 h (until time 24 h; positive control). A) Comparison between controls and individuals exposed to 17°C and 5°C air. B) Comparison between controls and individuals exposed to 15 and 22 psu salinity. Data shown as mean ± SD (n = 5-11).



Supplementary Figure S4: Force of attachment of *C. frondosa* measured in Newtons (N) at various time points before exposure (-0.5 h, negative control), immediately following exposure (1 h, positive control) and after 23 h recovery post exposure (24 h, positive control). The treatments correspond to **A)** 17°C air, **B)** 5°C air, **C)** 15 psu salinity, and **D)** 22 psu salinity. Data shown as mean ± SD (n = 4-15).