**TITLE:**

Cellular-molecular response to acute hypercapnia mismatch

Mitochondrial and transcriptional underpinnings of hypercapnic change in conditioned and naïve cohorts

Mitochondrial and transcriptional memory response to hypercapnic seawater

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**ABSTRACT**

**INTRODUCTION**

**METHODS**

***Animals***

Parental Bay scallops reared under low pCO2 (400 uatm) and moderately elevated pCO2 (800 uatm) since 4 hours post-fertilization were spawned to produce a second generation. Similarly, embryos from both parental cohorts were exposed to matched paternal pCO2 condition at four hours post-fertilization. At settlement, offspring from low pCO2 condition were split and a third larval rearing treatment was added as a severe pCO2 condition (1200), resulting in three Bay scallop cohorts from two parental lines. Scallops were reared under continuous pCO2 conditions until adulthood for this study. Scallops were fed a live mixed algae throughout their live history and composed of differing proportions of *Isochrysis, Chaetoceros,* and *Tetraselmis*.

***Feeding***

Fed continuous mixed-algae diet of Isocrysis, Chaetocerous, and Tetraselmis – occasionally varied in proportion of algae constituents when available. Seawater flow rate always 1400 mL per minute. Fed 6 mL per minute of algae culture – head tanks composed of 20L Iso, 12 L chaet and 12 L Tetraselmis during the experiment, prior to the experiment approximated the same ratio of species. We have PBR data where relative density == 1 is 10 million isocrysis cells, and 1/10th (1 = 1 million cells) for Tetraselmis. We also have chaetocerous counts that Katyenne has measured daily during the first week of the exposure period, relate these values to the relative density of the PBR to back track the cell counts. The mass culture Ply tank has been measured in past months when sufficiently dense via packed cell volume to feed, this value tends to be consistent.

***Re-exposure challenge***

For clarity, the following experimental description represents rearing chemistry as pCO2 ‘history’ and the two-week experiment as pCO2 ‘challenge’. Thirty adult Bay scallops from each pCO2 (N = 90 total scallops, pCO2history) were tagged two weeks prior to a re-exposure challenge and positioned in nine tanks with flow-through seawater (*N*=3 tanks pCO2 treatment-1). To initiate the experiment, twenty scallops were removed from their pCO2 history (6-7 tank-1) and positioned, in a full reciprocal fashion, within each pCO2 level (N= 10 individuals *p*CO2\_history x *p*CO2\_challenge-1) for two weeks. Scallops were destructively sampled for hemolymph extraction and gill and adductor tissue at 24 hours and 14 days of exposure, each time point removed 45 scallops (*N*=5 individuals *p*CO2\_history x *p*CO2\_challenge-1).

***Hemolymph extraction and flow cytometry***

00 ul of hemolymph was extracted from the adductor muscle of 45 adult scallops (spawned August 2022) on May 2nd and May 16th 2023 ( ~1.5 years in age).

***Tissue homogenate assays***

Cellular spectrophotometric assays

***Gene expression***

***Seawater chemistry***

***Data analysis***

**RESULTS**

**DISCUSSION**

**CONCLUSION**

*Notes:*

F2 scallops conditioned to 400, 800 and 1200 uatm all their life. Important, the cohort of F2 scallops under 1200 uatm was conditioned post-set from those that were reared as larvae under 400 – these were divided into both downwellers at setting stage under 400 and 1200 uatm to have a high pCO2 cohort. Therefore the 400 and 800 uatm cohort was conditioned since embryogenesis whereas the high pCO2 cohort was raised under 400 uatm to st and thereafter under 1200 uatm

90 Adult F2s were tagged March 23rd

* #s 1-30 = 400 uatm (pH 8)
* #s 31-60 = 900 uatm (pH 7.5)
* #s 61-90 = 1200 uatm (pH 7)
* Note: these ‘pH’ calls are not real, just used in our system and in communication for this multigenerational project for ease

Adults were always on drum filter seawater in the basement until 4/16/2023when moved to raw water.

Raw water quantified on 4/18/2023 for algae and read ~11-18 cells mL-1

As of 4/18/2023 we are feeding the same diet at 6 mL minute to each bin. We are discussing the need to supplement algae differently as our algae stock is limited and the F3 larave will soon move downstairs. We may supplement with algae paste if needed alongside our mixed live algal diet

Experiment timeline

* April 26th – pre experiment chemistry full carbonate chem
* May 1st – move animals to treatments, the color indicates the cohort and tag color
  + #s 1-10 = 400 x 400 uatm MATCHED!
  + #s 11-20 = 400 x 800 uatm
  + #s 21-30 = 400 x 1200 uatm
  + #s 31-40 = 800 x 400 uatm
  + #s 41-50 = 800 x 800 uatm MATCHED
  + #s 51-60 = 800 x 1200 uatm

* + #s 61-70 = 1200 x 400 uatm
  + #s 71-80 = 1200 x 800 uatm
  + #s 81-90 = 1200 x 1200 uatm MATCHED
  + Design: there are nine buckets each with the

**Pre experiment**

|  |  |  |  |
| --- | --- | --- | --- |
| **400 uatm** | 1-10 | 11-20 | 21-30 |
| **800 uatm** | 31-40 | 41-50 | 51-60 |
| **1200 uatm** | 61-70 | 71-80 | 81-90 |

**Experiment – EACH TANK HAS 10 INDIVIDUALS!**

|  |  |  |  |
| --- | --- | --- | --- |
| **400 uatm** | 1-3 (3)  31-34 (4)  61-63 (3) | 4-6 (3)  35-37 (3)  64-67 (4) | 7-10 (4)  38 -40(3)  68-70 (3) |
| **800 uatm** | 11-14 (4)  41-43 (3)  71-73 (3) | 15-17 (3)  44-47 (4)  74-76 (3) | 18-20 (3)  48-50 (3)  77-80 (4) |
| **1200 uatm** | 21-23 (3)  51-53 (3)  81-84 (4) | 24-27 (4)  54-56 (3)  85-87 (3) | 28-30 (3)  57-60 (4)  88-90 (3) |

28-30 (3); 57-60 (4); 88-90 (3)

Materials to prepare

* 90 labeled tubes for hemolymph