Data collected from an experiment on the carbonate system of oyster extrapallial fluid during May to September 2017

Website: https://www.bco-dmo.org/dataset/785155
Data Type: Other Field Results, experimental
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Project
» Collaborative Research: Does ocean acidification induce a methylation response that affects the fitness of the next generation in oysters? (Epigenetics to Ocean)

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Coverage

Temporal Extent: 2017-05-09 - 2017-09-15

Methods & Sampling

Prior to the start of the experiment, extrapallial fluid extraction ports were inserted into the shells of all oysters. To do this, a 3-mm hole was drilled into the right valve of each oyster. This hole was drilled approximately 1 cm from the umbo along the central band. Drill sites were flushed with seawater during drilling to minimize friction and overheating. A lear-lock was sealed into place using epoxy, and capped to create an extraction port. Following this procedure, oysters were returned to their sea tables for at least 7 days prior to being assigned to an experimental tank.

Extrapallial fluid was extracted immediately prior to tissue sampling. Oysters were removed from their tanks and patted dry before extrapallial fluid pH was measured. The cap was removed from the port, and all extrapallial fluid was extracted using a syringe. The port was then re-sealed and oysters were returned to their treatment tanks.

Extrapallial fluid pH was immediately measured following extraction using an Orion 91'10DJWP Double Junction Micro pH probe standardized with pH 7 and 10 NBS buffers. Extrapallial fluid samples were stored and refrigerated in 2 mL microcentrifuge tubes for later analysis of DIC. DIC was analyzed on a subset of individuals using an Apollo SciTech AS-C2 dissolved inorganic carbon analyzer standardized against a Dickson CRM. The salinity of the extrapallial fluid was measured using a Mettler Toledo InLab Expert Pro-ISM conductivity probe standardized against a Dickson CRM.

Twenty nine oysters were excluded from tissue sampling and set aside for measurements of respiration rate and EPF chemistry at the end of the experimental period. Respiration rate was measured by placing oysters in

sealed air-tight containers (height = 70 mm, diameter = 130 mm, volume = 1100 mL) filled with air-equilibrated seawater. A PreSens SP-PSt-NAU oxygen sensor dot was glued to the inside of the lid of each container to record oxygen concentration during measurements. An egg-crate platform inside each container separated the oyster from a stir-bar. Containers were sealed underwater to remove air bubbles. Containers were placed in a temperature-controlled water bath held at treatment temperature. This water bath was placed on top of six stir plates, so that six replicate measurements could be done simultaneously (5 oysters, and one negative control containing just seawater). Stir plates were set so that stir bars rotated at a speed of 136 - 150 resolutions per minute. Oysters were left for an acclimation time of 15 minutes, after which an air saturation reading was obtained every 15 minutes using a PreSens Fibox 4 oxygen meter. Three readings were obtained per container at each time point and an average value was taken. Respiration measurements were conducted until oxygen percentage had declined from 15% of the initial oxygen content recorded.

To understand rates of EPF recharge, the EPF was re-extracted from these 29 oysters at one, two, four, eight, twelve and twenty-four hour intervals. The order of these time periods was randomized to minimize the coupled effects of time-period and any sequential stress caused by re-extracting the EPF.

Problem Report: DIC could not be measured for all oysters, as measurement requires at least 0.5 mL, and some oysters did not contain enough extrapallial fluid.

Data Processing Description

To process respiration data, a linear model of air saturation as a function of time was created to generate respiration rate slopes. Plots were generated for each regression to ensure linearity of the slope. If slope R2 was less than 0.95, the plots were inspected visually for abnormal respiration patterns (i.e. oxygen concentration not changing or increasing). Bivalves respire anaerobically when shells are closed, therefore anaerobic portions of plots were removed. Plots were re-generated, and any with an R2 value of less than 0.9 were removed from analysis due to poor measurement quality. An air consumption rate per hour was calculated from each regression. This rate was then normalized to dry tissue weight (g).

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Parameters

Parameter	Description	Units
ID	Unique identifier for each oyster	unitless
shelf	Shelf containing the experimental tank	unitless
tank	Replicate tank within each shelf	unitless
respiration	Respiration rate (/hour/mg of dry tissue/mL of water)	mg O2
timepoint_hrs	Time since last extrapallial fluid extraction	hours
timepoint_order	Order in which extrapallial fluid extractions occurred	unitless
sample_date	The date at which EPF was sampled; format: yyyymmdd	unitless
sample_time	The time in eastern standard time at which the EPF was sampled; format: hhmmEST (EST = Eastern Standard Time)	unitless
EPF_pH	Extrapallial fluid pH on the NBS scale	pH units
EPF_DIC	Extrapallial fluid dissolved inorganic carbon	umol/kg
EPF_salinity	Extrapallial fluid salinity	ppt
notes	Any notes taken during sampling	unitless
ISO_DateTime_UTC	Sampling date and time in ISO 8601 format; converted from EST to UTC; format: yyyy-mm-ddTHH:MM:SS	unitless

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Instruments

Dataset-specific Instrument Name	Mettler Toledo InLab Expert Pro-ISM conductivity probe
Generic Instrument Name	Conductivity Meter
Generic Instrument Description	Conductivity Meter - An electrical conductivity meter (EC meter) measures the electrical conductivity in a solution. Commonly used in hydroponics, aquaculture and freshwater systems to monitor the amount of nutrients, salts or impurities in the water.

Dataset-specific Instrument Name	Apollo SciTech AS-C2 dissolved inorganic carbon analyzer
Generic Instrument Name	Inorganic Carbon Analyzer
Generic Instrument Description	Instruments measuring carbonate in sediments and inorganic carbon (including DIC) in the water column.

Dataset-specific Instrument Name	PreSens SP-PSt-NAU oxygen sensor dot
Generic Instrument Name	Oxygen Sensor
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	Orion Double Junction Micro pH probe
Generic Instrument Name	pH Sensor
Dataset-specific Description	Orion 91'10DJWP Double Junction Micro pH probe
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

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Project Information

Collaborative Research: Does ocean acidification induce a methylation response that affects the fitness of the next generation in oysters? (Epigenetics to Ocean)

Coverage: Coastal Massachusetts near Nahant: 42°25'06"N 70°54'14"W

NSF Award Abstract:
Marine ecosystems worldwide are threatened by ocean acidification, a process caused by the unprecedented rate at which carbon dioxide is increasing in the atmosphere. Since ocean change is predicted to be rapid, extreme, and widespread, marine species may face an "adapt-or-die" scenario. However, modifications to the DNA sequence may be induced in response to a stress like ocean acidification and then inherited. Such "epigenetic" modifications may hold the key to population viability under global climate change, but they have been understudied. The aim of this research is to characterize the role of DNA methylation, a heritable epigenetic system, in the response of Eastern oysters (Crassostrea virginica) to ocean acidification. The intellectual merit lies in the integrative approach, which will characterize the role of DNA methylation in the intergenerational response of oysters to ocean acidification. These interdisciplinary data, spanning from molecular to organismal levels, will provide insight into mechanisms that underlie the capacity of marine invertebrates to respond to ocean acidification and lay the foundation for future transgenerational studies. Ocean acidification currently threatens marine species worldwide and has already caused significant losses in aquaculture, especially in Crassostrea species. This research has broader impacts for breeding, aquaculture, and the economy. Under the investigators' "Epigenetics to Ocean" (E2O) training program, the investigators will build STEM talent in bioinformatics and biogeochemistry, expose girls in low-income school districts to careers in genomics, and advance the field through open science and reproducibility.

This research will specifically test if intermittent exposure to low pH induces a methylation response with downstream beneficial effects for biomineralization. These methylation states could be inherited and confer a fitness advantage to larvae that possess them. Phase 1 of the project will use an exposure experiment to determine the degree to which DNA methylation is altered and regulates the response to OA. Data from this experiment will be used to test the hypotheses that (i) DNA methylation, induced in the tissue of shell formation (i.e., mantle tissue), is correlated with changes in transcription and regulation of pallial fluid pH (calcifying fluid pH, measured by microelectrode), and (ii) that methylation changes induced in the mantle tissue are also induced in the germline --indicating that such changes are potentially heritable. Phase 2 of the project will use a pair-mated cross experiment to test the hypothesis that parental exposure to OA alters larval traits (calcification rate, shell structure, and polymorph mineralogy). Larvae will be generated from parents exposed to OA or control seawater, and then raised under control or OA conditions. Results will be used to (i) characterize inheritance of induced methylation states, (ii) estimate the variance in larval traits explained by genotype, non-genetic maternal/paternal effects, adult OA exposure, larval OA exposure, and parental methylome, and (iii) test the hypothesis that adult exposure alters the heritability (a quantity that predicts evolutionary response) of larval traits. Since the effects of epigenetic phenomena on estimates of heritability are highly debated, the results would advance understanding of this important issue. Because the investigators could discover that DNA methylation is a mechanism for heritable plastic responses to OA, knowledge of this mechanism would significantly improve and potentially transform predictive models for how organisms respond to global change.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635423

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