Marna Wet Lab Experimental Protocols

iTrack Oysters February 2023

Last updated 2024-09-24 BC IG

Experiment summary:

This experiment was part one of a series of mesocosm experiments and took place at the Marna Wet Lab at Hakai's Quadra Island Ecological Observatory February 22-27, 2023. The purpose of this experiment was to investigate eDNA and eRNA production and degradation under different pCO_2 conditions. Adult Pacific oysters (*Crassostrea gigas*) were chosen as the model organism and exposed to various pCO_2 treatments (520 µatm, 950 µatm, and 1200 µatm) under constant temperature (10°C). eDNA and eRNA samples were collected while oysters were present (Production phase) and after oysters were removed (Degradation phase). A subsample of oysters from each tank were destructively sampled for weight, size, condition/health and gill and gonad RNA at the end of the experiment.

Keywords: Pacific oyster, *Crassostrea gigas,* eDNA, eRNA, pCO2, carbonate chemistry, mesocosms, pH, environmental nucleic acids, ocean acidification

Experiment location:

Marna Wet Lab
Hakai Institute
Quadra Island, BC, Canada

Experiment Timeline:	
Start date:	2023-02-22
End date:	2023-02-27
Acclimation time:	7 days in seatable before start date
Organism exposure time:	30 oysters per mesocosms tank for 30 hrs (Production phase)

Destructive sampling:	All oysters removed from tanks on 2023-02-23 at 12:30. Five oysters from each tank were selected for destructive sampling
eDNA and eRNA sampling:	Production phase 8:00, 10:00, 14:00, and 22:00 on 2023-02-22 and 6:00 and 12:00 on 2023-02-23 Degradation phase 13:00 on 2023-02-23 and 14:00 on 2023-02-24, 2023-02-25, and 2023-02-27
Carbonate chemistry sampling:	2023-02-22 and 2023-02-27
Oysters fed:	2023-02-22 14:30

Study organism:

Small, medium and large *C. gigas,* originally purchased from Fanny Bay Oysters, were collected from the Hakai shellfish raft in Hyacinthe Bay, Quadra Island, BC. Large oysters had an average height (longest possible oyster measurement from umbo) of ~120 mm, medium oysters had an average height of ~90 mm, and small oysters had an average height of ~75 mm. The oysters were scrubbed and scraped to remove barnacles and other organisms. Oysters were kept in outdoor seatables at ambient conditions for a week before they were put into mesocosm tanks. Ten oysters of each size were added to each replicate tank (30 oysters per tank). Control tanks contained no organisms.

Equipment:		
Component	**Make**	**Model**
Mesocosm tanks	Integrated Aqua custom build	
Temperature controller	Dwyer	TSW-250 Dual Stage Temperature Switch

Temperature probe and monitoring	Walchem	600 Series Model WCT600HSNNE-NN with Omega HSRTD-3-100-A-120-E-ROHs temperature probe
pH probe and monitoring	Honeywell	Durafet III pH electrodes
Mass flow controllers	Alicat Scientific	MC-500SCCM-D-DB9S (CO_2), MCR-1000SLPM-D-DB9S (Air)
Burke-o-Lator system with non-dispersive infrared absorbance gas analyzer	Dakunalytics	L1840A LI-COR
Salinity measurement	YSI	Multilab IDS 4010-1
pH probe calibration	YSI	Pro1030
Digital centigram balance (±0.01 g)	OHAUS	SPX2202
Analytical balance (±0.1 mg)	Mettler Toledo	ME204
Vacuum pump	GAST	DOA-P704-AA

Tank description:

Incoming water was pumped from an intake in Hyacinthe Bay from 20 meter depth and moved through a series of filtration devices including disc filters, bag filters (25, 10, 5, 1 µm) and UV. Each 340L acrylic tank had independent temperature and pCO_2 regulation. Temperature was controlled by a Dwyer Dual Stage Temperature Switch, allowing hot or cold glycol to move through a titanium heat exchanger submerged in each tank. Carbonate chemistry was controlled by bubbling in specific mixtures of 99% CO_2 gas and outside air using mass flow controllers.

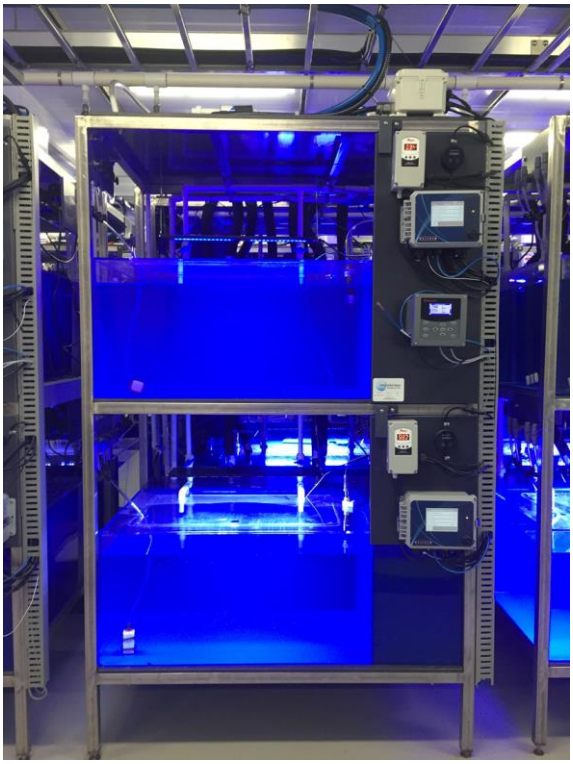

Figure 1. Hakai mesocosm tank set-up

Animal housing description:

Rectangular weight bars (PVC pipe filled with sand) were zip tied at four corners to the bottom of a shellfish basket. Oysters were spread evenly in one shellfish basket and placed on two basket stands in the center of the tank. The basket had holes on all sides and the basket stands had cut-outs to allow water flow. All replicate, spare, and control tanks contained an animal housing unit.

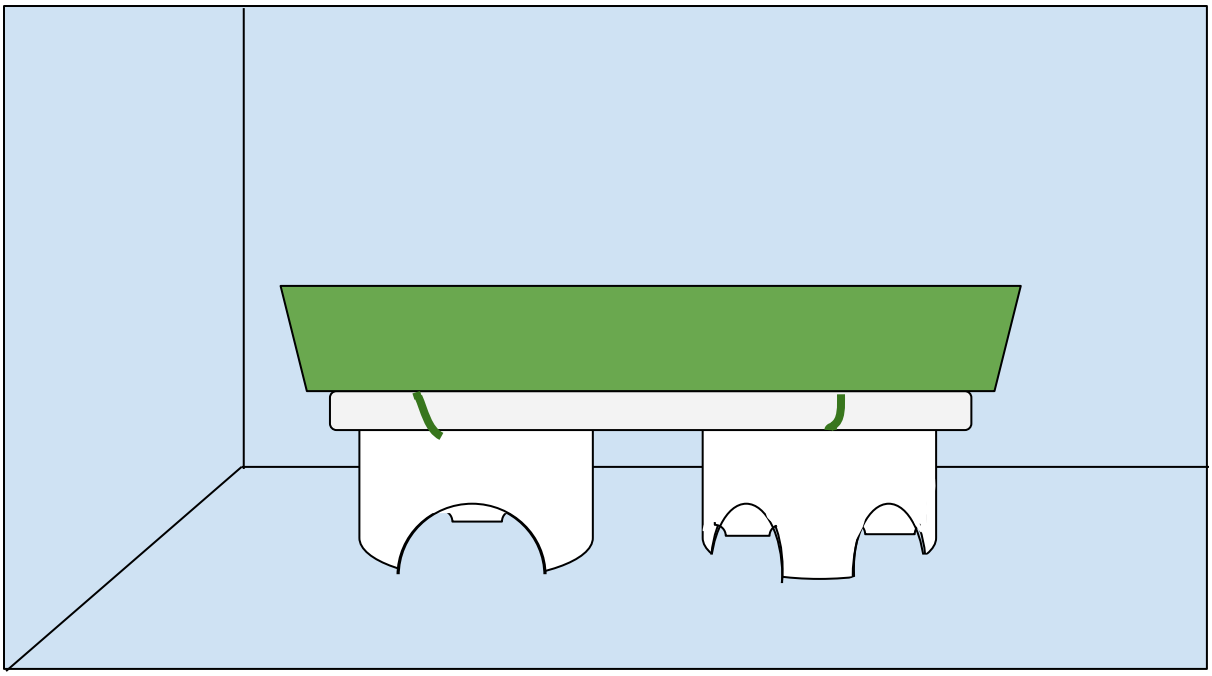

Figure 2. Diagram of Shellfish basket set-up

Tank Conditions:				
Air flow rate: 7.5 LPM Water flow rate: 0.5 LPM UV on/off: ON Salinity: 29.3 - 29.6				
Treatment name	pCO_2 (µatm) target/range	Temperature (°C) target	Static or dynamic pCO_2	Replicates
Ambient	520	10	Static	4
Mid	950	10	Static	4
High	1200	10	Static	4
Total tanks: 12				

Animal husbandry (cleaning, feeding etc.):

Oysters were given 10 mL of concentrated Tisochrysis lutea paste (Instant Algae ISO 1800) per tank once at the beginning of the experiment. For sample types 1-5 (see Data Collection and Analysis section below), five oysters from each replicate tank were destructively sampled. At least one oyster of each size was sampled per replicate and the size of the remaining two were randomly selected.

Data Collection and Analysis
General QA/QC procedures: Data was recorded on customized blank data sheets. The data was later recorded into a digital spreadsheet and a different person compared the data in the spreadsheet to the paper copy to make sure values were entered correctly. Each oyster was kept in a numbered weigh boat before and after each sampling type to ensure the oyster had the correct ID for imaging, destructive sampling, and weighing from start to finish.
1. Sample/data type: Height/length/width measurements Method description: Each sampled oyster was given a unique ID number to make a complete health assessment. Height, length, and width were measured with calipers on unopened oysters to the nearest millimeter. Height is the longest measurement from the umbo to the furthest growth end of the shell, length is the longest measurement from one side of the shell to the other perpendicular to the height, and width is the longest measurement from the top of the flat shell to the bottom of the cupped shell perpendicular to height and length (Figure 4. Galtsoff, 1964).

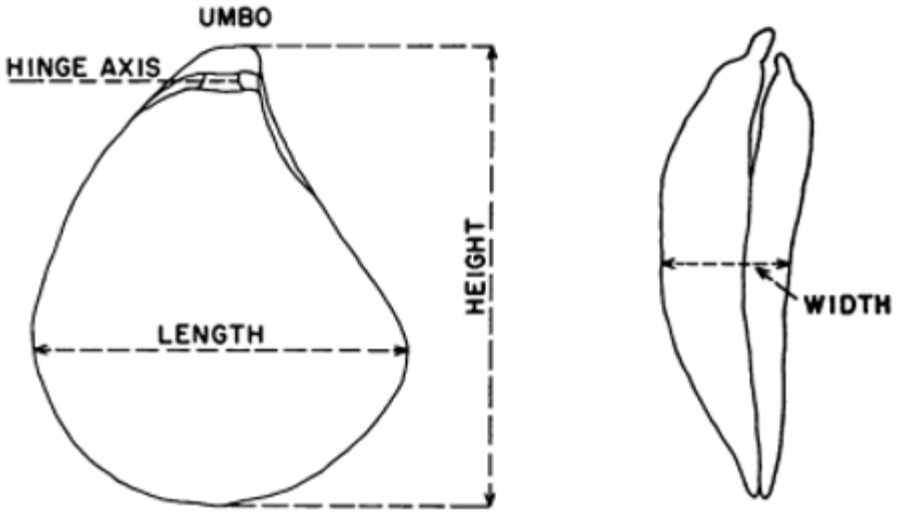

Figure 4. Length, Width, and Height measurements (Galtsoff, 1964)

Sampling frequency: Once at end of experiment

QA/QC procedure: While measuring, the recorder said the measurement back to the measurer to ensure they heard correctly. After sampling was completed, a second person checked the accuracy and completeness of the data before filing the datasheet.

2. Sample/data type: Weight

   Method description: Total oyster weight, oyster tissue wet weight, and oyster tissue dry weight were recorded. Total oyster weight was measured on unopened oysters using a digital centigram balance. Oyster tissue wet weight was measured on an analytical balance after scraping all tissue from the shell into a labelled preweighed aluminum weigh boat. Oyster tissue was dried in their pre-weighed aluminum weigh boat in an isotemp drying oven (Fisher Scientific 6903) for 24-48 hours. Oyster tissue dry weight was then re-weighed on the analytical balance.

   Sampling frequency: Once at end of experiment

   QA/QC procedure: While weighing total oyster weight, measurements and oyster number were said to the recorder who repeated the number back to the weigher to ensure the correct number was recorded. For the wet weight, the number on the weigh boat was double checked before weighing and the weigh boat weight was checked against its pre-weigh weight to ensure two weigh boats were not stacked. For dry

weights, the number on the weighboat was double checked and wet weight was compared to dry weights to ensure the numbers make sense.

3. Sample/data type: Oyster health
   Method description: Oyster health was determined by ability to close valves, tissue condition, mantle recession, conchiolin deposits, lesions, and digestive gland colouration. Ability to close valves was scored an N for normal, a W for weak, or an X for dead when opening the oyster with an oyster knife. Tissue condition was scored a 1 if the tissue is watery and emaciated, a 3 if the tissue was fat and plump, and a 2 if it was in between. Mantle recession was scored a 0 if there was no mantle recession, a 1 if there was minor recession where the condition appears acute or recent, or a 2 if the condition is moderate to severe. Conchiolin deposits were scored a 0 for no deposits, a 1 for a less than 25% coverage of shell surface area, a 2 for more than 25% coverage, or a 3 for extensive coverage suggestive of brown ring disease. Lesions were scored a 0 if there were none, a 1 if there were green to yellow pustules or abscesses, a 2 if there were brown lesions or colourless ulcerated area, a 3 for nodules in the gonad, or a 4 for other lesions. Digestive gland colouration was scored a 0 if the gland was a normal dark colour with green to brownish red hues, a 1 if the gland was slightly pale with olive green to brown hues, or a 2 if the gland was extremely pale with gray to beige hues.
   Sampling frequency: Once at the end of the experiment
   QA/QC procedure: One person would quantify the assessments to be consistent.

4. Sample/data type: Images
   Method description: Images of the oysters were taken with a digital camera. The cupped shell containing the otherwise unaltered oyster, the flat shell, a ruler for scale, and the oyster ID number were placed on a blank sheet of Rite in the Rain paper.
   Sampling frequency: Once at the end of the experiment
   QA/QC procedure: The oyster was kept in the numbered weigh boat before and after taking the image to ensure the oyster had the correct ID for imaging, destructive sampling, and weighing

5. Sample/data type: Genomics

Method description: A 5-10 mm^3 cube of gill tissue was preserved in a 1.5 mL eppi tube filled with 1mL of RNAlater and a 5-10 mm^3 cube of gonad tissue was preserved in a 1.5 mL eppi tube filled with 1 mL of RNAlater for each oyster. Tools were sterilized after each use by immersing the working end in 0.5% hypochlorite solution for two minutes before rinsing in smart2pure water, dipping in 95% ethanol and flaming with an ethanol lamp. Tissues in RNAlater were stored in a 4 ^oC fridge for 24 hours before moving to a -20 ^oC freezer.

Sampling frequency: Once at the end of the experiment

6. Sample/data type: eDNA and eRNA

   Method description: For each replicate tank and control tank, 1000 mL of tank water was collected for each eDNA and eRNA. For each water sample for eDNA and eRNA, the water was filtered through Nalgene Analytical Test Filter Funnels with 0.45 µm Cellulose Nitrate Filters [CAT No. 145-2045] using a vacuum pump. The pump is allowed to run for an additional minute after all water passes through to dry the filter. Filters were folded in half with filtered side in then in half again before placing in a coin envelope within in a small sealable plastic bag with colour indicating silica beads.

   Sampling frequency: 2, 4, 8, 16, 24, 30 hours after placing oysters in tanks and 1 hour, 1 day, 2 days, and 4 days after removing oysters from tanks

7. Sample/data type: Carbonate Chemistry

   Method description: Water from tanks were collected in 350 mL amber coloured glass bottles using a siphon. Samples were preserved with 200 µL of saturated $HgCl_2$ and capped with a crimped sealed polyurethane-lined metal cap then inverted three times to mix sample and check quality of the seal. Bottles were analyzed for salinity using a YSI Multilab IDS 4010-1 as well as TCO_2 and PCO_2 using a non-dispersive infrared absorbance gas analyser housed within a Burke-o-Lator system. TCO_2 and PCO_2 were validated with certified reference materials (CRM) provided by A. Dickson (Scripps Institute of Oceanography) and certified gas standards respectively. The estimated uncertainty for both values is less than 1% (Evans et al, 2019, 2022).

   Data analysis: Temperature, salinity, TCO_2 and pCO_2 measurements during analysis were used as inputs in the R package 'seacarb' (Gattuso et al., 2021) to calculate *in situ* (i.e., in tank) pCO_2, pH_T, total alkalinity, calcite and aragonite saturation using carbonic acid dissociation constants from Lueker et al., 2000, calcite and aragonite

solubility constants from Mucci, 1983, bisulphate dissociation constant from Dickson et al., 1990, and the boron/chlorinity ratio from Uppstrom, 1974.
Sampling frequency: Once at start and end of experiment
QA/QC procedure: The raw data and calculated carbonate chemistry data was checked and recalculated by someone different than the technician that ran the original analysis. Any discrepancies were flagged, discussed and corrected, producing a first QC'ed coarse dataset. Coarsed QC'ed data was reviewed by Iria Gimenez and samples were flagged if values were outside of expected range for further evaluation. Only samples that were determined to be outside of expected values due to collection, preservation, or analytical issues and thus not representative of the actual conditions of the mesocosm tank were removed from the finalized full QC'ed dataset. The summary table was produced with a custom R script, and 5 cells within the table were manually checked using excel formulas.

8. Sample/data type: Temperature and pH data (sensor network)
   Method description: Temperature and pH data was measured using Walchem and Honeywell probes, respectively, throughout the experiment to monitor tank treatments.
   Data analysis: Temperature and pH data was downloaded and run through an R script to reformat the data and remove any faulty data identified during QC procedures.
   Sampling frequency: Recorded every minute and reported in five minute averages continuously
   QA/QC procedure: Data was visually inspected and compared with Daily Observation notes to identify any data that was faulty (e.g., a probe failure) and not representative of real temperature and pH conditions. This data was removed from the dataset.

Please contact Iria Gimenez (Iria.Gimenez@hakai.org), Kate Rolheiser (Kate.Rolheiser@hakai.org) or *Brenna Collicutt (Brenna.Collicutt@hakai.org) with any questions.*