Gene expression data from mussels (Mytilus edulis) subjected to stable versus fluctuating temperatures from laboratory experiments conducted in 2023

Website: https://www.bco-dmo.org/dataset/953794
Data Type: experimental
Version: 1
Version Date: 2025-02-18

Project
» Collaborative Research: Microscale interactions of foundation species with their fluid environment: biological feedbacks alter ecological interactions of mussels (Microscale Mussels)

Contributors	Affiliation	Role
Nishizaki, Michael T.	Carleton College	Principal Investigator
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
We measured gene expression levels of blue mussels (Mytilus edulis Linneaus, 1758) under stable versus fluctuating water temperatures. Mussels were obtained from the intertidal zone at Black Point in Narragansett Bay, RI, USA (41° 24′ 4.4964″ N, 71° 27′ 43.8228″ W) and shipped in chilled coolers overnight to the lab at Carleton College in Northfild MN. Mussels were acclimated in a recirculating seawater (Instant Ocean, Blacksburg, VA, USA) tank system (Aquaneering Inc., San Marcos, CA, USA) for 2-3 weeks at 14-16°C and fed commercial Shellfish Diet 1800 (Reed Mariculture, Campbell, CA, USA) at a rate of 5% dry mussel tissue weight day-1 During our experiment, mussels were maintained for five days under one of three stable temperature treatments (e.g., 15, 20, or 25°C) or under a fluctuating temperature treatment between 15 and 25°C. On each day of the experiment, gill tissue from three mussels per temperature treatment were sampled to test for the expression of stress response (hsp70) and cellular respiration (NADH) related genes.

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Coverage

Location: Lab experiment at Carleton College in Northfild, MN conducted with organisms collected at Black Point in Narragansett Bay, RI
Spatial Extent: **Lat**:41.401249 **Lon**:-71.462173
Temporal Extent: 2023-02-01 - 2023-04-30

Methods & Sampling

Blue mussels (*Mytilus edulis* Linneaus, 1758) were collected from Black Point in Narragansett Bay, RI (41° 24′

4.4964″ N, 71° 27′ 43.8228″ W) and transported in chilled coolers to the lab at Carleton College in Nothfield, MN, USA. Mussels were acclimated in recirculating seawater (Instant Ocean, Blacksburg, VA, USA) for 2-3 weeks at 14°C and fed commercial Shellfish Diet 1800 (Reed Mariculture, Campbell, CA, USA) at a rate of 5% dry mussel tissue weight day^{-1}.

72 adult mussels [initial shell length = 60.11 ± 0.58 mm (± SE)] were divided into four treatment groups and placed into glass aquaria (50 × 25 × 31 cm) filled with 10 L of artificial seawater. Tanks were aerated and temperatures maintained using submersible heating elements (Biotherm 1000 W Titanium Heating Element, Blueline Aquatics, San Antonio, TX, USA). Temperature loggers (HOBO Pro v2; Onset Computer Corporation, Pocasset, MA; temperature sensor precision = 0.01°C.) measured water temperature in each tank every 10 s. Thermal conditions included three stable temperature treatments (14.96 ± 0.14°C, 20.51 ± 0.49°C and, 24.99 ± 0.62°C; mean ± standard deviation) and one fluctuating temperature treatment alternating between 15°C and 25°C every 6 hours (20.15 ± 4.67°C). All mussels were fed commercial Shellfish Diet 1800 (Reed Mariculture, Campbell, CA, USA) at a rate of 5% dry mussel tissue weight. day^{-1}.

For each of the four temperature treatments, three replicate mussels were sampled on six consecutive days to extract RNA from gill lamellae tissue (20 mg). Gene expression was halted immediately by immersing each mussel in liquid nitrogen. Tissue was manually homogenized using needle syringes (19 gauge; Becton Dickinson, Franklin Lakes, NJ) and RNA was extracted using Monarch® Total RNA Miniprep Kits according to the manufacturer's protocol (New England Biolabs, Ipswich, MA). After RNA extraction, each sample concentration, integrity, and quality were assessed using both a Qubit 4 Fluorometer using Qubit™ RNA Broad Range Assay Kits and Qubit™ RNA IQ Assay Kits (Thermo Scientific, Wilmington, DE, USA) and a Nanodrop One UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Primers for heat-shock protein 70 (hsp70), NADH-ubiquinone oxidoreductase (NADH), and elongation factor 1 alpha (ELF-a) genes were derived from previous sequences reported for *Mytilus edulis* (sequence information provided below). Forward and reverse primers were designed using Primer3web (v4.1.0) and synthesized by Integrated DNA Technologies (Coralville, IA, USA). We quantified gene expression via one-step quantitative reverse transcription (Luna Universal One-Step RT-qPCR Kit, New England Biolabs, Ipswich, MA) using a QuantStudio 5 qPCR machine (Thermo Scientific, Wilmington, DE, USA). The PCR thermal program consisted of initial denaturation (95°C, 1 minute) followed by 40 cycles of denaturation (95°C, 10 seconds) and extension (60°C, 30 seconds). A melt curve was run to confirm that the products were indeed a single amplicon. For each biological replicate (e.g., each of the 69 mussels sampled), 3 technical replicates were run. C_q values for technical replicates were within 0.5 cycles (Nolan et al., 2006). Expression levels were standardized to elf-a reference gene expression. Amplification data was used to estimate relative expression (RQ = $2^{-\Delta\Delta CT}$). For every temperature treatment, differences in relative gene expression were analyzed between each day compared to day 1 with unpaired Student's t-tests. Analyses were conducted using MATLAB R2024a (MathWorks, Natick, MA, USA).

Species	**Target**	**Forward primer (5'-3')**	**Reverse** primer (5'-3')	**size (bp)**
Mytilus edulis	HSP70	AGA CAC AGG GCG TAC AAG AA	ATG CTG CCA AGA ACC AAG TG	259
HBDQ01298109.1 (BioProject: PRJEB41447)				
Mytilus edulis	ELF-a	CAG GAG ACA ATG TTG GTT TCA A	AAA TTC ATC AAA TCT GGG GAT G	328
AY580270.1				

Organism identifiers:

Taxonomic name used in metadata, common name, Life Science Identifier (LSID)
"Mytilus edulis (Linneaus, 1758)", blue mussel, urn:lsid:marinespecies.org:taxname:140480

Data Processing Description

Expression levels were standardized to a housekeeper gene, elf-a. Amplification data were used to estimate relative expression between temperature treatments. For every temperature treatment, differences in relative gene expression were analyzed between each day compared to day 1 and reported as Relative Quantification (RQ) = $2^{-\Delta\Delta CT}$. Analyses were conducted using the Relative Quantification app on the ThermoConnect platform (https://www.thermofisher.com/us/en/home/digital-science/thermo-fisher-connect.html).

BCO-DMO Processing Description

* Data table within submitted file "Mytilus_edulis_hsp70.csv" was imported into the BCO-DMO data system for this dataset. Values "nd" imported as missing data values. Table will appear as Data File: 953794_v1_mussel-hsp70.csv (along with other download format options).

Missing Data Identifiers:
* In the BCO-DMO data system missing data identifiers are displayed according to the format of data you access. For example, in csv files it will be blank (null) values. In Matlab .mat files it will be NaN values. When viewing data online at BCO-DMO, the missing value will be shown as blank (null) values.

* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

* Date converted to ISO 8601 format

* LSIDs added for taxonomic names used in the metadata using the world register of marine species on 2025-02-18. Exact match to name used.

* Geospaital bounds added for organism source location in RI as indicated was the relevant location for this dataset. "Lab experiment at Carleton College in Northfild, MN conducted with organisms collected at Black Point in Narragansett Bay, RI "
41° 24′ 4.4964″ N, 71° 27′ 43.8228″ W converted to decimal degrees for entry: 41.401249,-71.462173

Problem Description

There are no measurements included for date_local:2024-02-24, Day:1,Temperature:25

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Data Files

File
Mytilus edulis qPCR experiment filename: 953794_v1_mussel-hsp70.csv (Comma Separated Values (.csv), 4.40 KB) MD5:22e5f29f575beaadbb3ef37a298538b8 Primary data file for dataset ID 953794, version 1. Data related to experiment measuring gene expression responses to stable versus fluctuating water temperatures in Mytilus edulis mussels.

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Supplemental Files

File
Experimental water temperatures filename: Mytilus_edulis_water_temperatures.csv (Comma Separated Values (.csv), 2.28 MB) MD5:be2e9ce001a47ff50231276ff5f37e1e Water temperatures used in Mytilus edulis qPCR experiment. ISO_Datetime_CST = Date and time according to Central Standard Time 15C = Water temperatures measured in the 15°C stable temperature treatment. Precision of HOBO temperature sensor = 0.01°C. 20C = Water temperatures measured in the 20°C stable temperature treatment. Precision of HOBO temperature sensor = 0.01°C. 25C = Water temperatures measured in the 25°C stable temperature treatment. Precision of HOBO temperature sensor = 0.01°C. 15-25C =Water temperatures measured in the 15-25°C fluctuating temperature treatment. Precision of HOBO temperature sensor = 0.01°C. nd = no data

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Related Publications

MathWorks (2024), MATLAB version R2024a Documentation, The Mathworks, Inc. Retrieved from https://www.mathworks.com/help/releases/R2024a/index.html
Software

Reed Mariculture. (2025) “SHELLFISH DIET 1800®.” Reed Mariculture. Retrieved Feb 18th, 2025 from https://reedmariculture.com/products/shellfish-diet.
Methods

Thermo Fisher Scientific Inc. (n.d.) Thermo Fisher Connect Platform. Retrieved from https://www.thermofisher.com/us/en/home/digital-science/thermo-fisher-connect.html
Methods

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Related Datasets

IsRelatedTo

European Bioinformatics Institute (2020). Environmental heat stress in Greenland blue mussels (Mytilus edulis). NCBI:BioProject: PRJEB41447. [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/bioproject/PRJEB41447

European Bioinformatics Institute (2020). TSA: Mytilus edulis, Cluster-74039.155504, transcribed RNA sequence. NCBI:Genbank accession: HBDQ01298109.1 [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/nuccore/HBDQ01298109.1

Peterson, K. J., Lyons, J. B., Nowak, K. S., Takacs, C. M., Wargo, M. J., & McPeek, M. A. (2020). (2005). Mytilus edulis elongation factor 1 alpha mRNA, partial cds. NCBI:Genbank accession: AY580270.1 [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/nuccore/AY580270.1

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Parameters

Parameter	Description	Units
Species	Scientific name of mussel species tested (Genus species). "Mytilus edulis" = " Mytilus edulis (Linneaus, 1758)", blue mussels, urn:lsid:marinespecies.org:taxname:140480	unitless
date_local	date of sampling (ISO 8601, local time zone)	unitless
Day	day of experiment	unitless
Temperature	target water temperature used in experiment (integer or range provided).	unitless
QUBIT_QUANT	RNA concentration in measured via fluorometry (Qubit)	nanograms per microliter (ng/uL)
QUBIT_QUAL	RNA integrity and quality number (IQ) represents the percentage of large (e.g., >100 bp) to small RNA molecules in the sample (Qubit). Reported on a scale of 1–10, with 1 denoting a significantly degraded sample and 10 representing high-quality.	unitless
NANO_QUANT	RNA concentration measured by the UV-Vis spectrophotometry (Nanodrop).	nanograms per microliter (ng/uL)
A260_to_280	A260/280. Measure of RNA purity reporting the ratio of absorbance at 260 nm versus 280 nm (Nanodrop).	unitless
delta_delta_Ct	Measure of differences (e.g., fold change) in gene expression. See "Methods & Sampling" section for more details.	unitless

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Instruments

Dataset-specific Instrument Name	Qubit 4 Fluorometer (Thermo Fisher Scientific, Wilmington, DE, USA)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	
Generic Instrument Name	Immersion heater
Dataset-specific Description	submersible heating elements (Biotherm 1000 W Titanium Heating Element, Blueline Aquatics, San Antonio, TX, USA)
Generic Instrument Description	Submersible heating element for water tanks and aquaria.

Dataset-specific Instrument Name	QuantStudio 5 qPCR machine (Thermo Scientific, Wilmington, DE, USA)
Generic Instrument Name	qPCR Thermal Cycler
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

Dataset-specific Instrument Name	HOBO Tidbit MX2203 (Onset Computer Corporation, Bourne, MA)
Generic Instrument Name	Temperature Logger
Generic Instrument Description	Records temperature data over a period of time.

Dataset-specific Instrument Name	HOBO Tidbit MX2203 loggers (Onset Computer Corporation, Bourne, MA)
Generic Instrument Name	Temperature Logger
Generic Instrument Description	Records temperature data over a period of time.

Dataset-specific Instrument Name	Nanodrop One UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA)
Generic Instrument Name	Thermo Scientific NanoDrop spectrophotometer
Generic Instrument Description	Thermo Scientific NanoDrop spectrophotometers provide microvolume quantification and purity assessments of DNA, RNA, and protein samples. NanoDrop spectrophotometers work on the principle of ultraviolet-visible spectrum (UV-Vis) absorbance. The range consists of the NanoDrop One/OneC UV-Vis Spectrophotometers, NanoDrop Eight UV-Vis Spectrophotometer and NanoDrop Lite Plus UV Spectrophotometer.

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Project Information

Collaborative Research: Microscale interactions of foundation species with their fluid environment: biological feedbacks alter ecological interactions of mussels (Microscale Mussels)

Coverage: University of Washington Friday Harbor Laboratories

NSF Award Abstract:
The project investigates how the metabolic activity of dense aggregations of marine organisms alter the water chemistry of their interstitial spaces, and how these microscale alterations feedback to affect the organisms' interactions in coastal ecosystems. The research team focuses on bivalve mussels, foundation species that form dense 'beds' typically known for facilitating other species by ameliorating harsh flow conditions. This

ability can become a liability, however, if flow is not sufficient to flush the interstitial spaces and steep, metabolically-driven concentration gradients develop. The research evaluates whether corrosive chemical microclimates (such as low oxygen or low pH) are most extreme in low flow, high temperature conditions, especially for dense aggregations of mussels with large biomass and/or high respiration rates, and if they negatively impact mussel beds and the diverse biological communities they support. The research addresses a global societal concern, the impact of anthropogenic climate change on coastal marine ecosystems, and has potential applications to aquaculture and biofouling industries by informing adaptation strategies to “future-proof” mussel farms in the face of climate change and improved antifouling practices for ships, moorings, and industrial cooling systems. The project forges new collaborations with investigators from three campuses and integrates research and education through interdisciplinary training of a diverse group of graduate, undergraduate and high school students. STEM education and environmental stewardship is promoted by the development of a K-12 level science curriculum module and a hand’s-on public exhibit of bivalve biology at a local shellfish farm. Research findings are disseminated in a variety of forums, including peer-reviewed scientific publications and research presentations at regional, national and international meetings.

The research team develops a framework that links environmental conditions measured at a coarse scale (100m-100km; e.g., most environmental observatories) and ecological processes at the organismal scale (1 cm – 10 m). Specifically, the project investigates how aggregations of foundation species impact flow through interstitial spaces, and how this ultimately impacts water chemistry immediately adjacent to the organisms. The research focuses on mytilid mussels, with the expectation that the aggregation alters the flow and chemical transport in two ways, one by creating a physical resistance, which reduces the exchange, and the other by enhancing the exchange due to their incurrent/excurrent pumping. These metabolically-driven feedbacks are expected to be strongest in densely packed, high biomass aggregations and under certain ambient environmental conditions, namely low flow and elevated temperature, and can lead to a range of negative ecological impacts that could not be predicted directly from coarse scale measures of ambient seawater chemistry or temperature. The team develops computational fluid dynamic (CFD) models to predict interstitial flows and concentration gradients of dissolved oxygen and pH within mussel beds. The CFD model incorporates mussel behavior and physiological activity (filtration, gaping, respiration) based on published values as well as new empirical work. Model predictions are compared to flow and concentration gradients measured in mussel aggregations in the laboratory and field. Finally, the team conducts several short-term experiments to quantify some of the potential negative ecological impacts of corrosive interstitial water chemistry on mussel aggregations, such as reduced growth, increased dislodgement, increased predation risk, and reduced biodiversity. Because the model is based on fluid dynamic principles and functional traits, the framework is readily adaptable to other species that form dense assemblages, thereby providing a useful tool for predicting the ability of foundation species to persist and provide desirable ecosystem services under current and future multidimensional climate scenarios.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2050129

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