## Accession numbers from acidification mesocosms and original in situ samples from coastal and offshore water station at the Pivers Island Coastal Observatory January to February 2017

Website: https://www.bco-dmo.org/dataset/765668 Data Type: Other Field Results Version: 0 Version Date: 2019-04-24

#### Project

» <u>Collaborative Research: Ocean Acidification: microbes as sentinels of adaptive responses to multiple</u> <u>stressors: contrasting estuarine and open ocean environments</u> (OA microbe adaptation)

#### Program

» <u>Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification</u> (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
Johnson, Zackary I.	Duke University	Principal Investigator
Konstantinidis, Kostas	Georgia Institute of Technology (GA Tech)	Co-Principal Investigator
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## Coverage

**Spatial Extent**: N:34.72 **E**:-76.19 **S**:34.04 **W**:-76.67 **Temporal Extent**: 2017-01-19 - 2017-02-12

## **Dataset Description**

This dataset contains Biosample and GenBank Short Read Archive (SRA) sequence accession numbers for metatranscriptomic samples obtained from acidified, warmed and control mesocosms from ocean and coastal waters.

Ocean acidification (OA) is one of the major issues caused by the rising of atmospheric CO2 with immediate effects in the oceans carbonate chemistry. The oceanic microbial communities are key players in the biochemical cycles of the marine ecosystem and are expected to respond to the ocean's changing chemistry in a feedback loop of intertwined microbial mediated processes. While the microbial response and effect to OA is challenging to predict due to the complexity of the system, experimental manipulations under controlled conditions can help us understand the major mechanisms of microbial adaptation to a changing oceanic chemistry. In this work we established replicated mesocosms in order to evaluate the effect of decreasing ph in the microbial community composition and gene expression using metatranscriptomics. We examined the effect of both decreasing ph and increasing temperature, one of the major parameters that is expected to have synergistic effects on the microbial response to OA. We examined the effect of both decreasing ph and increasing temperatures that is expected to have synergistic effects on the major parameters that is expected to have synergistic effects on the

microbial response to OA. Additionally, we established mesocosms with either coastal or ocean waters, with the expectation to observe less gene expression responses from the coastal communities which are adapted to a constantly changing water chemistry in comparison to the more sensitive to change ocean communities. In order to account for the variation in gene expression profiles from environmental samples we established two replicates for each of the following incubations (a) control (b) acidified (-0.3 from the in situ ph) (c) warmed (+3oC from the in situ temperature) (d) warmed and acidified. Each mesocosm was maintained under stable conditions for 5 days, after which we isolated samples for 16S rRNA amplicon sequencing and metatranscriptomic sequencing. Additionally, we sequenced the metatranscriptome of the original coastal and ocean samples in order to obtain a baseline gene expression profile of the in situ communities.

#### Methods & Sampling

In situ or mesocosms water samples were collected within less than 6min using a peristaltic pump. All samples were pre filtered through 3um porosity in line filters, and the bacterial biomass was collected on 0.22 um strive filters. The filters were immediately frozen in liquid nitrogen and store at -80oC until further processing.

Total RNA was extracted from the material collected on the filter using an organic extraction method described previously (Tsementzi et al, 2014; https://doi.org/10.1111/1758-2229.12180). Briefly Lysis buffer (50 mM Tris-HCl, 40 mM EDTA, 0.75 M sucrose) was added to the filters with 1 mg/ml lysozyme and subsequently incubated for 30 min at 37°C. A second 2-h incubation at 55°C was performed after the addition of 1% SDS and 10 mg/ml proteinase K. Acid phenol and chloroform extractions were performed twice on the lysates, and RNA was isolated using filter columns from the mirVANA RNA isolation kit (Ambion), washed twice by following the manufacturer's instructions, and eluted in Tris-EDTA buffer. DNase treatment was performed using the TURBO DNA-free kit (Ambion, Austin, TX). Libraries were prepared from total RNA using the Ribo-Zero rRNA Removal Kit (Bacteria) following the manufacturer;s instructions and without including the rRNA depletion step. The resulting cDNA libraries were sequenced (250-bp single-end reads) using the Illumina HiSeq 2500 sequencer at the Georgia Institute of Technology Genomics Facility.

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## **Related Publications**

Tsementzi, D., Poretsky, R., Rodriguez-R, L. M., Luo, C., & Konstantinidis, K. T. (2014). Evaluation of metatranscriptomic protocols and application to the study of freshwater microbial communities. Environmental Microbiology Reports, 6(6), 640–655. doi:<u>10.1111/1758-2229.12180</u> *Methods* 

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#### Parameters

Parameter	Description	Units
biosample_accession	biosample_accession	unitless
sample_name	sample_name	unitless
sample_title	sample_title	unitless
bioproject_accession	bioproject_accession	unitless
organism	organism	unitless

host	host	unitless
isolation_source	isolation_source	unitless
collection_date	collection_date	unitless
geo_loc_name	geo_loc_name	unitless
lat_lon	lat_lon	unitless
samp_mat_process	samp_mat_process	unitless
samp_size	samp_size	unitless
source_material_id	source_material_id	unitless
description	description	unitless
library_ID	library_ID	unitless
title	title	unitless
library_strategy	library_strategy	unitless
library_source	library_source	unitless
library_selection	library_selection	unitless
library_layout	library_layout	unitless
platform	platform	unitless
instrument_model	instrument_model	unitless
design_description	design_description	unitless

filetype	filetype	unitless
filename	filename	unitless
filename2	filename2	unitless
filename3	filename3	unitless
filename4	filename4	unitless
assembly	assembly	unitless
ISO_datetime_UTC	ISO_datetime_UTC	yyyy-MM-dd'T'HH:mm:ss'Z'
lat	latitude with north positive	degrees
lon	longitude with east positive	degrees

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#### Instruments

Dataset- specific Instrument Name	Illumina HiSeq 2500 sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset- specific Description	The resulting cDNA libraries were sequenced (250-bp single-end reads) using the Illumina HiSeq 2500 sequencer at the Georgia Institute of Technology Genomics Facility.
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

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## **Project Information**

Collaborative Research: Ocean Acidification: microbes as sentinels of adaptive responses to multiple stressors: contrasting estuarine and open ocean environments (OA microbe adaptation)

#### Extracted from the NSF award abstract:

This collaborative project by Duke University and Georgia Institute of Technology researchers will combine oceanographic and advanced molecular techniques to characterize the adaptive responses of microbial communities to multiple stressors associated with OA. In particular, microbial communities from estuarine and coastal ecosystems as well as open ocean waters will be incubated under conditions of increased acidity or temperature or both, and their activities will be measured and quantified.

Preliminary data from time-series observations of a coastal temperate estuary shows that pH, temperature and other stressors vary over multiple space and time scales, and this variability is relatively higher than that observed in open ocean waters. Based on this evidence, the guiding hypothesis of this work is that microbes in coastal ecosystems are better adapted to ocean acidification as well as multiple stressors compared to similar microbes from the open ocean. To quantify the adaptive genetic, physiological and biogeochemical responses of microbes to OA, the team's specific goals are to: (1) characterize complex natural microbial community responses to multiple stressors using factorial mesocosm manipulations, (2) assemble a detailed view of genomic and physiological (including transcriptional) adaptations to OA at the single species level using cultured model marine microbes (e.g. Prochlorococcus, Synechococcus, Vibrio) identified as responsive to stressors in whole community mesocosm experiments, and (3) assess the power of model microbial strains and mesocosm experiments to predict microbial community responses to natural OA variability in a temporally dynamic, temperate estuary and along a trophic/pH gradient from the Neuse-Pamlico Sound to the Sargasso Sea. By comparing an estuarine ecosystem to its open ocean counterpart, this study will assess the sensitivity of microbial structure and function in response to ocean acidification.

This project is associated with **Pivers Island Coastal Observatory**.

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## **Program Information**

# Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm\_summ.jsp?pims\_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (<u>https://www.nsf.gov/funding/pgm\_summ.jsp?</u> <u>pims\_id=504707</u>).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

#### Solicitations issued under this program:

<u>NSF 10-530</u>, FY 2010-FY2011 <u>NSF 12-500</u>, FY 2012 <u>NSF 12-600</u>, FY 2013 <u>NSF 13-586</u>, FY 2014 NSF 13-586 was the final solicitation that will be released for this program.

#### **PI Meetings:**

<u>1st U.S. Ocean Acidification PI Meeting</u>(March 22-24, 2011, Woods Hole, MA) <u>2nd U.S. Ocean Acidification PI Meeting</u>(Sept. 18-20, 2013, Washington, DC) 3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

#### NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

<u>Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification</u> <u>This Way Comes - US National Science Foundation (NSF)</u>

<u>Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New</u> <u>Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)</u>

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

<u>Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show</u> <u>How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)</u>

<u>Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation</u> <u>research grants</u>

<u>Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover</u> answers questions about ocean acidification. - US National Science Foundation (NSF)

<u>Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly</u> resistant to ocean acidification - US National Science Foundation (NSF)

<u>Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards</u> \$11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

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## Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1416673</u>

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