Dataset Description: Measuring nitrogen removal rates from oyster aquaculture experiment in Massachusetts

This document provides detailed information about five datasets that were generated through the [2017 - 2020] collaborative research project *Evaluating effectiveness of different oyster aquaculture strategies for nitrogen loading remediation to inform end user decisions to restore water quality*. The project was supported by the National Estuarine Research Reserve System (NERRS) Science Collaborative, which is funded by the National Oceanic and Atmospheric Administration. All Science Collaborative supported projects that collect new data adhere to federal data sharing and archiving requirements.

Data access and archival: Genetic datasets for this project have been archived with the National Center for Biotechnology Information (NCBI) and can be accessed online. Biogeochemistry and oyster data will be archived with the NERRS Centralized Data Management Office and can be downloaded after completing a request form on the NERRS Science Collaborative project page.

Additional information, including some the referenced data, is available in this open access journal article and the supplemental information: Mara et al. 2021. Comparison of Oyster Aquaculture Methods and Their Potential to Q12 Enhance Microbial Nitrogen Removal From Coastal Ecosystems. Frontiers in Marine Science 8:633314. Doi: 10.3389/fmars.2021.633314.

List of Project Datasets
Five related datasets are described in this document

1. Microbial community structure data - iTag Amplicon library
2. Microbial processes data - RNA-Seq Metatranscriptomic sequences
3. Bottom water, porewater and sediment chemistry
4. Gene expression (RT-qPCR) and nitrogen flux measurements
5. Oyster weight data

About the Associated Project

Project title: Evaluating effectiveness of different oyster aquaculture strategies for nitrogen loading remediation to inform end user decisions to restore water quality.
Name of reserve(s) involved in the project: Waquoit Bay National Estuarine Research Reserve, MA
Project period: November 2017 - December 2020
Science Collaborative project page: www.nerrssciencecollaborative.org/project/Rogers17

Project lead and contact information:
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Purpose

Towns along Cape Cod are under pressure to reduce and mitigate nitrogen inputs to improve coastal water quality. Many towns are exploring the use of various shellfish aquaculture systems as a cost effective way to remediate water quality. This project addressed a critical information gap identified by water quality managers and regulators, specifically: how much nitrogen is removed from coastal waters by common oyster aquaculture methods, and what culturing practices should be adopted to maximize benefits for water quality? This information is helping regulators decide how much credit they can give towns for installing shellfish aquaculture as part of their water quality improvement plan, and provide guidance to towns and growers on best practices to maximize water quality benefits.

Abstract

The project team established an experiment that mimicked commercial aquaculture practices and allowed for a robust comparison of nitrogen removal rates from three commonly used gear types: floating bags of oysters, oyster condos suspended in midwater, and bottom cages of oysters. All gear was deployed in the same environmental setting (Waquoit Bay, Falmouth, MA) and maintained by the Town of Falmouth in a manner that a typical grower would follow. The growing systems were maintained for two full growing seasons (2018 and 2019) and compared to a nearby control site. Every two weeks during the growing season, the team conducted a series of measurements to provide a robust estimate of nitrogen fluxes and microbial activity below each of the aquaculture operations. Measurements included: (1) nutrient analyses of sediment, porewater and bottom water samples, (2) genetic sequencing of RNA and DNA extracted from sediment samples to determine the presence and activity level of certain bacteria; and 3) measurements of N2 fluxes from sediment cores placed in flux chambers to measure N2 production rates. All three oyster growing methods enhanced nitrogen removal relative to the control site. However, gene expression data indicate that nitrogen retention may be induced under some gear, particularly after the end of July under bottom cages, and to a lesser extent other gear types.

Experimental Design and Sample Collection for All Datasets

Treatments

We used three commercially available oyster growth methods that result in different proximity of oysters to the sediment.

- Bottom Cages (BC) - deployed 1.2 x 0.9 x 0.4 m cages that rested on the bottom and held the oysters 5-6 cm above the sediment. The BC setups allowed oysters to grow on the ocean bottom similar to wild oysters. This treatment covered an area of 80 m2
- Floating Bags (FB) - utilized 0.5 x 0.8 m rigid floating bags with 6 mm2 mesh, attached to foam floats and aligned into a floating system that ensured adequate water circulation. This treatment covered 108 m2.
- The suspended Oyster Gro (OG) method utilized 0.46 x 0.89 m rigid metal wire frames suspended below buoys that held 6 mm2 mesh bags held within. This allowed the oysters to be held about 30 cm below the sea surface and 0.5-2.0 m above the seafloor, depending on the tide. This treatment covered 108 m2.
- Our experimental design also included a control site.

The different oyster methods were first installed in early March 2018 and 2019. Year old oysters were added in late April of each year. Oysters and all gear were removed in October 2018 and 2019 for over wintering. For each growth method initial stocking weights of 1-year old oysters were 2.28 kg of oysters, targeting approximately 225 oysters per bag. This resulted with ~200 kg of oysters initially in the FB, ~205 kg in the OG and BC. BC and OG systems hold 6 bags per unit.

Sample collection

Samples were collected bi-weekly during 2018 (May-September) and 2019 (April-October). We collected 3 sediment cores as biological replicates under each oyster method site and at the control site using a pole coring device (Aquatic Research Instruments, Hope, ID) and polycarbonate core liners (internal diameter, 9.5 cm; total length, 0.5 m). All triplicate cores were collected near the center of each site (avoiding edge effects).The top 3 cm of each core were sectioned immediately (within 5 minutes) in the field and homogenized for RNA, DNA and porewater nutrient analyses. An extra set of 2 sediment cores per study site were collected for the measurements of N2 fluxes using flux chambers. The same number of sediment cores were collected from our control site.

Data collection period: April 2018 - October 2019

Geographic extent: Waquoit Bay, Falmouth Massachusetts (41°34′49”N, 70°31′27”W; Waquoit, MA)

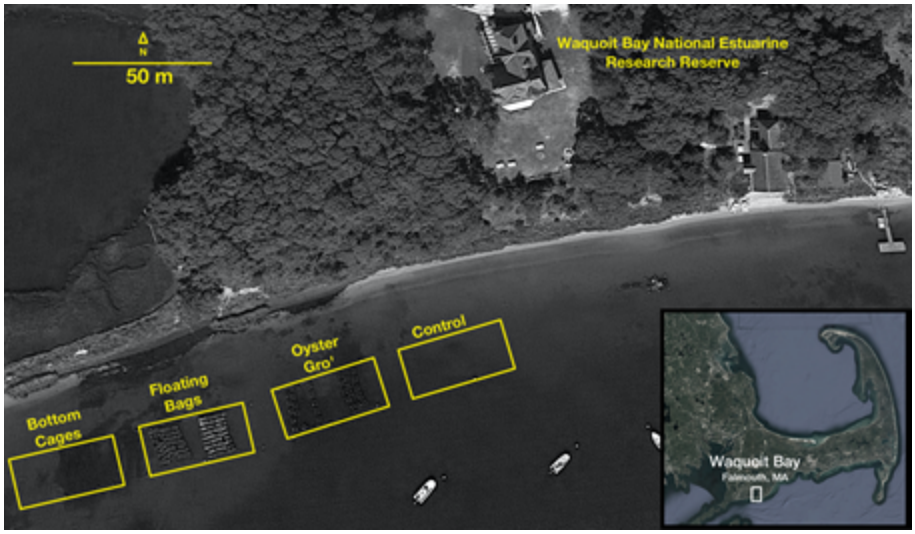

Figure 1. Aerial image of oyster deployments and the sediment sampling sites in Waquoit Bay, MA (41°34′49"N, 70°31′27′W). Within the yellow box from left to right are the bottom cages, the floating bags, and the floating Oyster Gro cages (Image from Google Earth).

About Individual Dataset(s)

1. Microbial community structure - iTag Amplicon library

General description of data:
The microbial community structure was analyzed by sequencing the eDNA (SSU rRNA gene). iTag amplicons are 16S rRNA gene amplicons region V4-V5. The primers used were 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) targeting the hypervariable region 4-5 (V4-V5) of the SSU rRNA gene. This dataset was used to assess the presence of difference groups of microbes.

Search keywords: iTag, 16S hypervariable

More about the data:

- We extracted 0.5 g of sediment for each sample collected in 2018 in a UV-sterilized clean hood using the PowerSoil® DNA Isolation Kit (MOBIO) following manufacturer's instructions.
- Bacterial and archaeal SSU rRNA gene fragments were PCR amplified and sequenced at Georgia Genomics and Bioinformatics Core (Univ. of Georgia). The primers used were 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) targeting the hypervariable region 4-5 (V4-V5) of the SSU rRNA gene (Parada et al., 2016).
- Paired reads were quality checked and trimmed using FastQC (v. 0.11.7).
- We analyzed the sediment microbiome data using the QIIME2 platform (Bolyen et al., 2019), and used the DADA2 plugin provided in the QIIME2 pipeline to denoise and optimize the merging of the forward and reverse reads.
- Taxonomy was assigned to amplicon sequence variants (ASVs) using the scikit-learn multinomial naive Bayes classifier (q2-feature-classifier plugin; Bokulich et al., 2018) with the SILVA v132 database as the reference (Quast et al., 2013).

Data Access and Archival:
Sequences were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRR13101827-SRR13101797 and SAMN16838263-SAMN16838385) database under project BioProject ID PRJNA679576.

File format:
See NCBI Guide on RNA and DNA datasets: **https://www.ncbi.nlm.nih.gov/guide/dna-rna/**

2. Microbial processes - RNA-Seq Metatranscriptomic sequences

General description of data:
This dataset includes Metatranscriptomic data collected at Waquoit Bay, Falmouth, MA. Data was generated using a paired end Illumina Hiseq methodology.

More about the data:

- Biweekly samples were taken both years to follow the activities of the microbial processes of denitrification, anammox, DNRA and sulfur-oxidation genes using RT-qPCR.
- We extracted 2 g of sediment for each sample in a UV-sterilized clean hood using the RNeasy PowerSoil Total RNA Isolation Kit (Qiagen, USA) following the manufacturer's protocol.
- The metatranscriptome libraries were prepared only for selected samples collected from both the control site and the different oyster treatments in May (May_23), July (July_17) and September (Sep_25) 2018 and were stored at -80°C until sequencing using 150 bp paired-end Illumina NextSeq 550 (Georgia Genomics and Bioinformatics Core; Univ. of Georgia).
- To remove traces of contaminating DNA we treated our RNA extracts twice (2x30 min) with TURBO DNA-free™ (Invitrogen, USA) as suggested by the manufacturer. To confirm absence of DNA from the RNA solutions we ran a PCR reaction using the bacterial primers BACT 1369F and PROK 1541F targeting the 16S rRNA. Each 25 µL reaction contained 0.5 U µL−1 Taq DNA Polymerase (Thermo Fisher Scientific), 1× Taq DNA Polymerase reaction buffer (Thermo Fisher Scientific), 0.4 mM dNTPs (Thermo Scientific dNTP Mix) and 4 µM of each primer (final concentrations). These reactions were performed at 94oC for 5 min, followed by 35 cycles of 94oC (30s), 55oC (30s) and 72oC (45s).

Search keywords: Metatranscription, RNA-seq, Illumina pair-end

File format:
See: NCBI Guide on RNA and DNA datasets: **https://www.ncbi.nlm.nih.gov/guide/dna-rna/**

Data Access and Archival
Sequences were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRR13101827-SRR13101797 and SAMN16838263-SAMN16838385) database under project BioProject ID PRJNA679576.

3. Bottom water, porewater and sediment chemistry data

General description of data:
This dataset includes nutrient analyses of bottom water, porewater and sediment below each of the aquaculture treatments (see Experimental Design, above). This data was used to evaluate patterns of nitrogen removal connected to shellfish aquaculture.

Search keywords:
Nitrate, Ammonia, Sedimentary Carbon and Nitrogen

More about the dataset:
- Sediment cores were collected near the center of each site, directly under each aquaculture treatment. The top 3 cm of each core were sectioned immediately (within 5 min) in the field and homogenized for RNA, DNA, and porewater nutrient analyses.

- Bottom water samples were filtered through a 0.45 µm sterivex filter (Fisher Scientific, United States) and frozen until analysis.
  Porewater and bottom water were analyzed for nitrate, nitrite, and ammonium.
- Sediments were oven-dried (80"C), ground with a mortar and pestle (<250 µm), weighed and encapsulated in Al cups, and analyzed for total carbon and total nitrogen using a Micro-Dumas (flash) combustion method at the Stable Isotope Ecology Laboratory at the University of Georgia.
- Additional information on methods is available in this open access journal article and the supplemental information: Mara et al. 2021. Comparison of Oyster Aquaculture Methods and Their Potential to Enhance Microbial Nitrogen Removal From Coastal Ecosystems. Frontiers in Marine Science 8:633314. Doi: 10.3389/fmars.2021.633314.

File format:
Excel spreadsheet. Additional data documentation is provided within the spreadsheet.

Data Access and Archival:
This dataset will be archived with the NERRS Centralized Data Management Office and can be downloaded after completing a request form on the NERRS Science Collaborative project page (www.nerrssciencecollaborative.org/project/Rogers17) Prior to being available publicly, potential users can contact the project lead to discuss their applications of the data - Dan Rogers: drogers2@stonehill.edu

4. Gene expression (RT-qPCR) and Nitrogen flux measurements

General description of data:
This dataset includes the results of flux measurements taken from cores placed in flux chambers. These measurements were used to calculate the N_2 production as an indicator of denitrification rates. Gene expression of denitrification, anammox, DNRA, and sulfur-oxidation marker genes were determined with two-step quantitative RT-qPCR. This gene expression data can be used to assess the importance and activity levels for different microbial processes

Search keywords:
Denitrification, N2 production, flux chambers, RT-qPCR, gene expression

More about the data:
- Replicate sediment cores were collected to measure N_2 fluxes. Collected cores from each site were submerged in a cooler containing site water and allowed to equilibrate for at least 1 h. After equilibration, cores were sealed with a cap that contained a magnetic stir bar and a sensor that allowed real time monitoring of oxygen concentrations.
- Cores were incubated in the dark until 20%–25% of the oxygen in the overlying waters had been consumed. Then the overlying water from the core was collected into exetainers with no headspace and analyzed for NOx and ammonium. N_2/Ar was measured on a membrane inlet mass spectrometer.

- Excess N_2 production was calculated as production above background, scaled to water volume and normalized to incubation time. All results for experimental site cores were compared to N_2 production from the control cores.
- Gene expression data was obtained from the sediment collected synoptically with the sediment chemistry. Samples were frozen in liquid nitrogen in the field. RNA was extracted, transcribed and quantified as described in Mara et al. 2021. Comparison of Oyster Aquaculture Methods and Their Potential to Enhance Microbial Nitrogen Removal From Coastal Ecosystems. Frontiers in Marine Science 8:633314. Doi: 10.3389/fmars.2021.633314.
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File format:
Excel spreadsheet. Additional data documentation is provided within the spreadsheet.

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5. Oyster weight data

General description of data:
This dataset includes measurements of the weight of oysters used in the three aquaculture treatments for this project. This dataset aided in the calculations of nitrogen removal rates.

Search keywords:
Eastern oysters, *Crassostrea virginica*, aquaculture, shellfish, oyster farms

More about the data:
- Oyster weights were measured within each of the cages or growing bags on a monthly basis during the growing season in 2018 and 2019

File format:
Excel file

Data Access and Archival:
This dataset will be archived with the NERRS Centralized Data Management Office and can be downloaded after completing a request form on the NERRS Science Collaborative project page (www.nerrssciencecollaborative.org/project/Rogers17) Prior to being available publicly, potential users can contact the project lead to discuss their applications of the data - Dan Rogers: drogers2@stonehill.edu