# Population changes in Halobacteriovorax cultured with protist & prey

**Website**: https://www.bco-dmo.org/dataset/880924
**Data Type**: experimental
**Version**: 1
**Version Date**: 2022-09-22

**Project**
» Excellence in Research: Assessing the Control by Multiple Micropredators on Bacterial Communities in Estuarine Environments and Characterization of Prey Lysis Products Resulting from Each Predator (Predators of bacteria)


| Contributors | Affiliation | Role |
|---|---|---|
| Williams, Henry Neal | Florida A&M University (FAMU) | Principal Investigator |
| Chen, Huan | Florida State University - National High Magnetic Field Lab (FSU - NHMFL) | Co-Principal Investigator |
| Cobb-Abdullah, Ahkinyala | Virginia Union University (VUU) | Co-Principal Investigator |
| Kranz, Sven | Florida State University (FSU) | Co-Principal Investigator |
| Stukel, Michael | Florida State University (FSU) | Co-Principal Investigator |
| Xue, Jia | Florida A&M University (FAMU) | Contact |
| Soenen, Karen | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |


**Abstract**
Here, we investigate the growth trajectory and predation dynamics of protists, and HBx micro-predators in co-culture with a common prey, V. parahaemolyticus, in a time-series study in marine water microcosms. The microcosms were established with water samples collected from the Apalachicola Bay in northwest Florida, USA and amended with a suspension of prey bacteria. Samples were taken at a high temporal resolution (3-hour intervals for 5 days) to capture detailed measurements and changes in the growth responses of HBx and protists, using both culture- and molecular-based methods. The protists were counted by qPCR and flow cytometry. The HBx were counted by qPCR and a culture plating method

---

## Table of Contents


- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Data Files
- Supplemental Files
- Related Publications
- Parameters
- Instruments
- Project Information
- Funding


---

## Coverage

**Spatial Extent**: **Lat**:30.07401 **Lon**:-84.180243

---

## Dataset Description

These data were published in Williams et al. (2016): Figs 1 and 3.

Data have been published “as is”. Final review by the data submitter was not received after it was imported into the BCO-DMO data system. There were no outstanding questions

### Methods & Sampling

To establish microcosms for the experiment, water samples were collected from the Apalachicola Bay (N 30° 4' 26.436", W 84° 10' 48.874") in northwest Florida, USA. About 20 liters of seawater (SW) were collected in sterile plastic bottles. The samples were transported on ice (4 °C) to the Microbial Ecology Laboratory at Florida A&M University within two hours. To establish the microcosms, water samples were first filtered through 5 µm membrane filters (Whatman Laboratory, NJ) to remove debris and larger organisms. Then 600 ml was dispensed into each of three (A, B, and C) 1-liter flasks to create the various microcosms as shown in Figure 1. For treatment D, 600 ml of SW samples were filtered through 0.8 µm membrane filters to remove protist. One subsample labeled W was unfiltered. All microcosm flasks were shaken at 80 rpm at 25 °C for 120 h. Samples (5 ml) were collected every 3 hours, dispensed in a 96-well microtiter plate and the OD measured at 600 nm using an Absorbance Microplate Reader Q4 (BIO-TEK Instruments Inc., USA). Population changes in *HBx* and *V. parahaemolyticus* were monitored by culture- and qPCR-based methods as described below. The protocol described here is similar to that published by Williams et al. (2016). A key difference is those investigators also filtered the microcosm samples through a 0.1 µm filter to allow viruses to pass into the filtrate as their investigation included monitoring of virus numbers in the various microcosm treatments, and did not include protists.

### Enumeration of *HBx* and *V. parahaemolyticus* by culture-dependent method

At 3-h intervals, 1 ml was collected from all microcosms (A, B, D, and W) and 10-fold dilutions of these prepared in sterile SW.  One tenth (0.1) ml taken from three of these dilutions (10-6, 10-7, 10-8) was inoculated on seawater yeast extract (SWYE) agar in duplicate to obtain countable numbers of CFU of *V. parahaemolyticus*. All plates were incubated at 28°C ± 0.5°C for 24 hours, and colony-forming units (CFU) were counted. To culture *HBx* requires a prey bacterium suspension. The suspension was prepared by flooding a SWYE agar plate with an overnight-grown culture of *Vibrio parahaemolyticus* with 5 ml of autoclaved SW. The fluid remained undisturbed on the surface of the plate for 5 minutes. The culture was then suspended in the fluid using s circular motion with a sterile L-shaped spreader. The resulting suspension was transferred into a 15 ml tube and used as the stock prey suspension. One ml of the prey stock suspension was inoculated into tubes along with samples (5, 1, 0.1 ml) from each of the microcosms. In cases where the sample volume was less than 5 ml, autoclaved SW was added to bring the final sample volume to 5 ml). The suspension was added to top agar, and the mixture mixed mechanically and overlaid onto the surface of bottom agar in agar plates. The plates were cultured at room temperature for 3 to 5 days and examined daily for plaques typical of *HBx*, Plaque –forming units (PFU).were counted. The daily counts of *HBx* and *V. parahaemolyticus* were plotted and changes over the course of the experiment were observed and compared with counts of protist during the same time period.

### DNA extraction from water samples for qPCR

For DNA extraction, a 1 ml sample was collected from each microcosm at each time interval, and 250 µl was used for DNA extraction. Genomic DNA was isolated using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer’s instructions and the DNA was quantified with a NanoDrop ND-1000 UV spectrophotometer (Thermo Scientific, Wilmington, USA). The DNA samples were stored at -20 °C prior to use.

### Data Processing Description

### Quantitative PCR assays

TaqMan based quantitative PCR assay for *V. parahaemolyticus* and SYBR green based qPCR assays for *HBx* and presumptive protist were conducted (Table 1) . All qPCR assays were performed using the BioRad Real-Time PCR system. For TaqMan based qPCR, the reaction mixture (20 µl) contained 1x PerfeCTa qCPR ToughMix

(Quanta Biosciences, Beverly, MA), 0.5 µM of each primer, and 2.5 µl of the template DNA. QPCR reactions were performed in duplicate and amplification protocols consisted with a hold at 95 °C for 2 min, followed by 45 cycles of 94 °C 5s, 59 °C 45 s. SYBR green-based qPCR reaction mixture (15 µl) contained 1x SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), 0.5 µM of each primer, and 2.5 µl of the template DNA. The amplification conditions for *HBx* consisted of a hold at 94°C for 2 min, followed by 45 cycles of 94°C 30s, 62°C 10 s, and 72°C 10 s. The amplification conditions for presumptive protist consisted of a hold at 94°C for 2 min, followed by 30 cycles of 94°C 30s, 55°C 30 s, and 72°C 45 s.

A calibration curve with concentrations spanning the range from 10 to 106 gene copies per reaction, with two replicates, was prepared. Duplicate no-template controls (NTC) were included in each run. The amplification efficiencies (AE) were calculated based on the equation: AE= 10(-1/slope) –1. A summary of qPCR target organisms, primer/ probe name, and sequences are detailed in Table 1.

About the lower limit of detection (LLOD): Theoretically the LLOD for qPCR is 3 gene copies per reaction. In our experiment, we use the lowest point of our standard curve as the lower limit of quantification (LLOQ) which is 10 gene copies per reaction. The LLOD can also be calculated based on the methods mentioned in these two papers: Staley et al. (2012) and Xue et al (2019): which is the inverse log of five times the difference between the Y-intercept and the upper 99% confidence interval of the intercept divided by the slope based on the standard curve.

The LLOD for flow cytometry is 2 cells per ml.

[ table of contents | back to top ]

## Data Files

| File |
| --- |
| **protist_predation.csv** (Comma Separated Values (.csv), 9.40 KB) MD5:5d8153538f38359c03927dd5f0cfd69e<br>Primary datafile for dataset 880924 |

[ table of contents | back to top ]

## Supplemental Files

| File |
| --- |
| **experimental_design.jpg** (JPEG Image (.jpg), 111.47 KB) MD5:8a150cea0b982f1ad491c2367aaf88f9<br>Supplemental file for dataset 880924. Schematic flow chart of the experimental design to establish the various microcosms. |
| **qpcr_assays.jpg** (JPEG Image (.jpg), 137.43 KB) MD5:9accbaf8d66d971c96214a2cc9fe1ac2<br>Supplemental file for dataset 880924. A summary of qPCR target organisms, primer/ probe name, and sequences. |

[ table of contents | back to top ]

## Related Publications

Euringer, K., & Lueders, T. (2008). An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. Journal of Microbiological Methods, 75(2), 262–268. https://doi.org/10.1016/j.mimet.2008.06.012
*Methods*

Nordstrom, J. L., Vickery, M. C. L., Blackstone, G. M., Murray, S. L., & DePaola, A. (2007). Development of a Multiplex Real-Time PCR Assay with an Internal Amplification Control for the Detection of Total and Pathogenic Vibrio parahaemolyticus Bacteria in Oysters. Applied and Environmental Microbiology, 73(18), 5840–5847. https://doi.org/10.1128/aem.00460-07 https://doi.org/10.1128/AEM.00460-07
*Methods*

Staley, C., Gordon, K. V., Schoen, M. E., & Harwood, V. J. (2012). Performance of Two Quantitative PCR Methods for Microbial Source Tracking of Human Sewage and Implications for Microbial Risk Assessment in Recreational Waters. Applied and Environmental Microbiology, 78(20), 7317–7326. https://doi.org/10.1128/aem.01430-12 https://doi.org/10.1128/AEM.01430-12
*Methods*

Williams, H. N., Lymperopoulou, D. S., Athar, R., Chauhan, A., Dickerson, T. L., Chen, H., Laws, E., Berhane, T.-K., Flowers, A. R., Bradley, N., Young, S., Blackwood, D., Murray, J., Mustapha, O., Blackwell, C., Tung, Y., & Noble, R. T. (2015). Halobacteriovorax, an underestimated predator on bacteria: potential impact relative to viruses on bacterial mortality. The ISME Journal, 10(2), 491–499. https://doi.org/10.1038/ismej.2015.129
*Results*

Xue, J., & Feng, Y. (2019). Comparison of microbial source tracking efficacy for detection of cattle fecal contamination by quantitative PCR. Science of The Total Environment, 686, 1104–1112. https://doi.org/10.1016/j.scitotenv.2019.06.091
*Methods*

Zheng, G., Wang, C., Williams, H. N., & Pineiro, S. A. (2008). Development and evaluation of a quantitative real-time PCR assay for the detection of saltwater Bacteriovorax. Environmental Microbiology, 10(10), 2515–2526. https://doi.org/10.1111/j.1462-2920.2008.01676.x
*Methods*

[ table of contents | back to top ]

## Parameters

| Parameter | Description | Units |
|---|---|---|
| Treatment | Experimental Conditions: 'Sw Fil. 5 + V.p.': sea water (SW) passed through 0.5µm filter to remove larger particles but retaining microbes (protists, bacteria, viruses) with V. parahaemolyticus (V.p.) added as prey bacterium. 'Auto SW + V.p.': Autoclaved sterilized SW amended with V.p.'SW no V.p.': SW unfiltered with no V.p. added.SW Fil. 0.8 + V.p.'- SW passed through a 0.8µm filter to remove most protists and amended with V.p.'Fil. 0.2 + V.p.': SW passed through a 0.2 µm filter to select for viruses and amended with V.p.'Natural + V.p.': Natural SW unfiltered amended with V.p. | unitless |
| Time | Time since expt start | hours |
| Optical_Density | Optical Density | OD units |
| Protists | Protist Abundance | counts per ml |
| Vibrio | Vibrio Abundance | gene copies per reaction |
| Halobacteriovorax | Halobacteriovorax (Hbx) Abundance | gene copies per reaction |

[ table of contents | back to top ]

## Instruments

| Dataset-specific Instrument Name | CytoFLEX Flow Cytometer |
|---|---|
| Generic Instrument Name | Flow Cytometer |
| Dataset-specific Description | CytoFLEX Flow Cytometer (Beckman Coulter Life Sciences Headquarters, Indianapolis, IN 46268, USA) |
| Generic Instrument Description | Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm) |

| Dataset-specific Instrument Name | Absorbance Microplate Reader Q4 |
|---|---|
| Generic Instrument Name | plate reader |
| Dataset-specific Description | Absorbance Microplate Reader Q4 (BIO-TEK Instruments Inc., USA) |
| Generic Instrument Description | Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23. |

| Dataset-specific Instrument Name | CFX96 Touch Deep Well Real-Time PCR Detection System |
|---|---|
| Generic Instrument Name | qPCR Thermal Cycler |
| Dataset-specific Description | CFX96 Touch Deep Well Real-Time PCR Detection System (Bio-Rad, Hercules, CA 94547, USA) |
| 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 | NanoDrop ND-1000 UV spectrophotometer |
|---|---|
| Generic Instrument Name | Spectrophotometer |
| Dataset-specific Description | NanoDrop ND-1000 UV spectrophotometer (Thermo Scientific, Wilmington, USA) |
| Generic Instrument Description | An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples. |

[ table of contents | back to top ]

## Project Information

**Excellence in Research: Assessing the Control by Multiple Micropredators on Bacterial Communities in Estuarine Environments and Characterization of Prey Lysis Products Resulting from Each Predator (Predators of bacteria)**

NSF Award Abstract:

Microbes are the most abundant organisms on Earth and play an important role as degraders, cycling nutrients in the environment. Too many or too few bacteria may disrupt a sensitive ecological balance and proper functioning of environmental processes such as carbon, nitrogen and phosphorus cycles. The abundance of bacteria populations in any given environment is controlled by various biological, chemical and physical mechanisms. Among the biological agents are microscopic predators, or micropredators, of bacteria. The most studied of these are protists, viruses that infect bacteria, and a group of bacteria collectively known as the Bdellovibrio and like organisms (BALOs). These micropredators prey upon certain bacteria to obtain required nutrients or other cellular material for their replication. In the process, cellular products from the prey bacteria are released into the environment and utilized as nutrients by other microbes. Although the micropredators co-occur, and likely interact, in nature, most experimental studies have investigated their activities individually, rather than collectively. As a result, little is known about their collective role in controlling bacteria populations and the cycling of nutrients. The goal of the proposed research is to address this gap in knowledge by investigating all three as a collective group under simulated natural conditions representing a range of temperature, salinity and abundance of prey. This project is conducted at two Historically Black Universities (HBCUs) with strong records of training and mentoring students and postdocs from underrepresented populations in science. The project benefits up to 100 students by providing unique and meaningful educational and research training experiences at the undergraduate and graduate student levels and for early-career scientist. Specific activities include courses on scientific writing and presenting results at annual project workshops as well as national and international scientific meetings. Graduate students are being trained in modern advanced methodologies in chemistry and microbiology. There is an ongoing assessment module to document education and training outcomes.

Up to now, the two mainly accepted mechanisms of mortality in bacterial populations are heterotrophic protist grazing and viral infection. Increasingly, it has become evident that an understudied group of predatory bacteria, BALOs, can also contribute to bacterial mortality. Yet, the mechanisms underlying the dynamics of BALO-prey interactions are poorly understood, as are the interactions among the micropredators, BALOs, protists and bacterial viruses. Ultimately, these processes may have contrasting influences on the structure and functioning of the microbial loop, including impacting higher trophic levels and biogeochemical cycles. The investigators hypothesize that environmental factors significantly influence how mortality in bacterial populations is partitioned among the micropredators. To test this hypothesis researchers are (1) investigating the interactions amongst the micropredators, (2) examining the molecular-level composition and dynamics of dissolved organic matter as the result of the different mortality processes by the NMR/ FT-ICR mass spectrometry (MS) hybrid approach, and (3) modeling these tri-trophic dynamics. Intellectual Merit: Results from this research will define a new mechanistic understanding of mortality dynamics that influence the microbial loop and oceanic biogeochemical cycles.

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.

[ table of contents | back to top ]

---

## Funding

| Funding Source | Award |
| --- | --- |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1948758 |

[ table of contents | back to top ]