Management of Long-Term and Seasonally Changing Pathogenic *Vibrio spp.* Populations in the Northeast U.S.

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Pathogenic *Vibrio* species are halophilic bacteria that thrive in warm, brackish water. A small minority of strains are human pathogens that cause inflammatory gastroenteritis, skin infections, and septicemia. Typical exposure is via seafood consumption, especially raw or slightly cooked bivalve shellfish.

RESEARCH GOALS

1. Track the occurrence and spatial/temporal trends for concentrations of for pathogenic *Vibrio* species in oysters in critical harvest areas.
2. Demonstrate and optimize pre- and post-harvest management strategies that are effective for reducing health risks associated with shellfish consumption

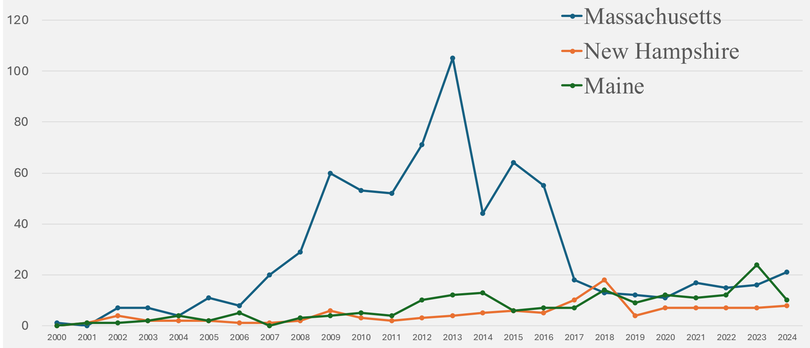
BACKGROUND

- Pathogenic *Vibrio* species have recently become a significant public health issue in the Northeast US coastal waters. Over the past 20 years, shellfish-borne illnesses from Vibrios, especially *V. parahaemolyticus* (Vp), *V. vulnificus* (Vv), and *V. cholerae* (Vc) have significantly increased.

- Long-term tracking and forecasting the population dynamics of potentially pathogenic Vibrios in important shellfish harvest areas is critical to inform risk assessment and for developing strategies to effectively mitigate human illness potential and expansion.

HUMAN ILLNESSES

- *The annual reported vibriosis cases* in states bordering the Gulf of Maine have increased since 2000. In Massachusetts there was a large increase peaking in 2013 when outbreaks occurred then declines in response to effective management strategies.
- The historic relatively low vibriosis rates for Maine and New Hampshire have increased over the past 10 years.



STUDY AREAS

Great Bay Estuary-NH

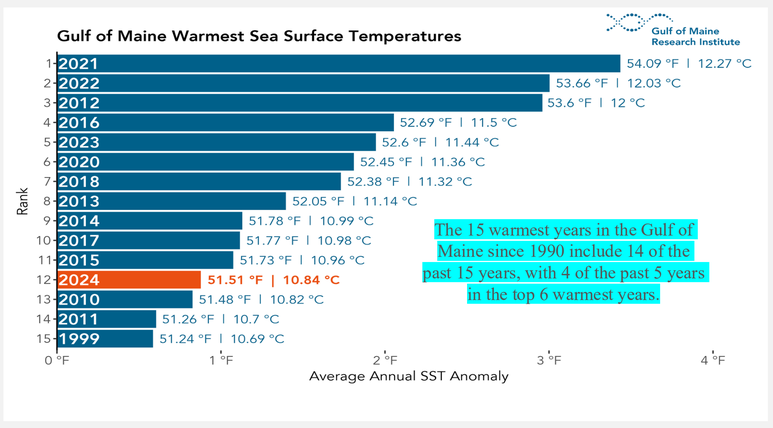
- Two sample sites:
  1. Nannie Island/Woodman Point
  2. Oyster River
- Oysters were collected monthly from April to December, 2007 to 2025
- Water temperature obtained from nearby buoys, 2007 to 2025 (*Great Bay NOAA/National Estuarine Research Reserve System-Wide Monitoring Program)*

Gulf of Maine

Study areas in Maine (ME), Massachusetts (MA) and Connecticut (CT)

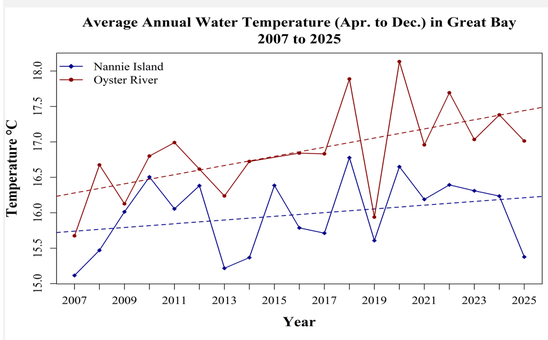
STUDY AREA SEA SURFACE TEMPERATURE (SST) TRENDS

Gulf of Maine



The 15 warmest years in the Gulf of Maine since 1990 include 14 of the past 15 years, with 4 of the past 5 years in the top 6 warmest years.

Great Bay Estuary



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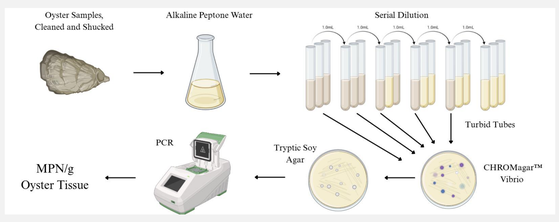
VIBRIO DETECTION & DATA ANALYSIS METHODS



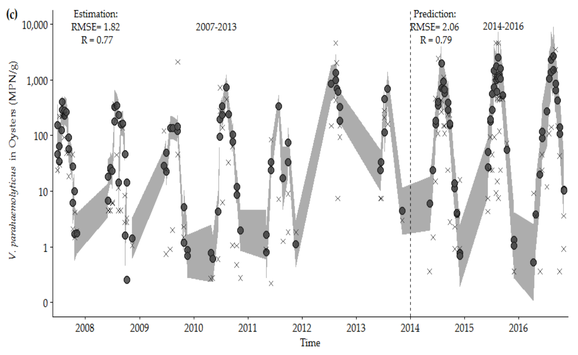
- Oysters were enriched with alkaline peptone water at 37°C that was streaked onto CHROMagar™ Vibrio, and colonies were transferred onto tryptic soy agar. PCR of boiled colony lysates was used to confirm species ID and pathogenicity markers to determine the concentration (MPN/gr oyster tissue) of targeted species and strains in each sample.
- MPN/g values for *Vibrio* spp. were log transformed and trends of average annual concentrations were assessed using one-way ANCOVAs. Generalized linear models with a negative binomial distribution were fit to determine environmental predictors of *Vibrio* concentrations in oysters.

RESULTS: LONG-TERM SURVEILLANCE OF VIBRIO SPECIES

Estimates of *V. parahaemolyticus* concentrations (closed circle) with observed (x's) *V. parahaemolyticus* concentrations for a harmonic regression model for training (2007-2013) and test (2014-2016) periods.

Hybrid Harmonic Model

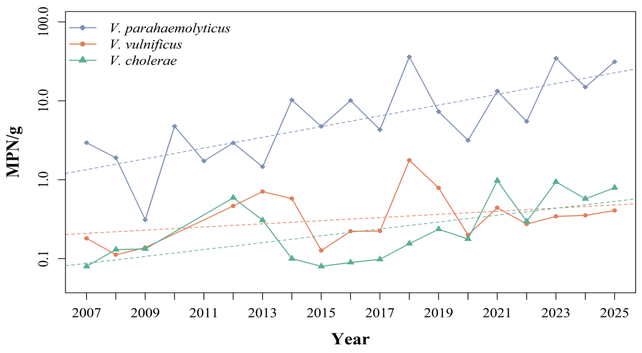
$$\log(\mathrm{Vp}) = \beta_0 + \beta_1\,\mathrm{Temp} + \beta_2\,\mathrm{pH} + \beta_l t + \beta_s \sin(2\pi\omega t) + \beta_c \cos(2\pi\omega t)$$



DON (mg/L)
NH4 (mg N/L)
NO3 1 NO2 (mg N/L)
NPOC (mg/L)
PC (mg/L)
PN (mg/L)
PO4 (mg P/L)
TDN (mg/liter)
Chlorophyll a (mg/L)
Dissolved oxygen (mg/L)
pH
Pheophytin (mg/L)
Salinity (ppt)
Water temperature (°C)
Total suspended solids(mg/L)
Turbidity (NTU)

Water temperature, pH, trend and seasonality terms were significant factors for determining trends for Vp concentrations.

Average Annual *Vibrio* spp. Concentrations, 2007 to 2025

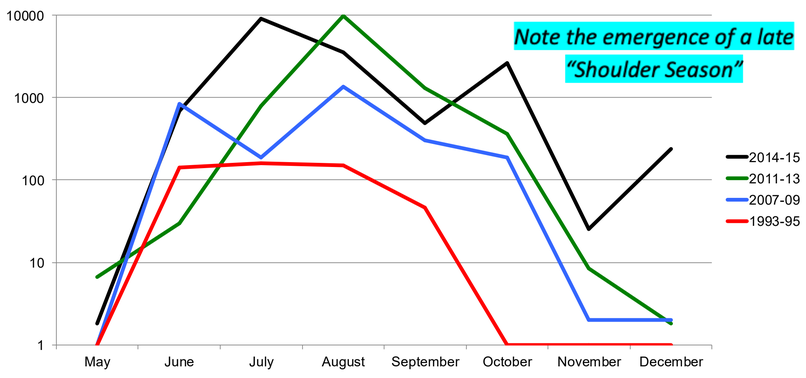


The concentrations of all 3 targeted Vibrio species significantly increased from 2007 to 2025.

Species	Site	Time	Temperature	Salinity	pH
V. parahaemolyticus	<0.001	0.0027	<0.001	<0.001	<0.001
V. vulnificus	0.0044	0.015	<0.001	0.53	0.016
V. cholerae	0.98	0.14	<0.001	0.052	0.93

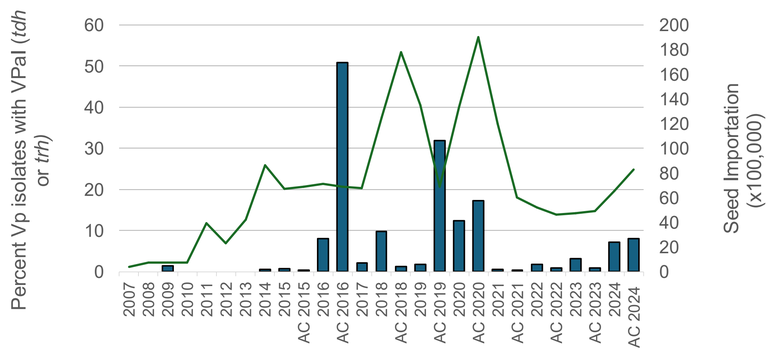
Water temperature and pH, along with site, are significant factors associated with increasing Vp and Vv populations, though only water temperature was significant for Vc.

3-year average monthly *V. parahaemolyticus* levels (Vp/100g) in Great Bay (NH) oysters: 1993 to 2015



Vp populations expanded throughout the late spring, summer and early fall, and expanded into late fall from the mid 1990s through to the mid 2010's and remain that way into the present.

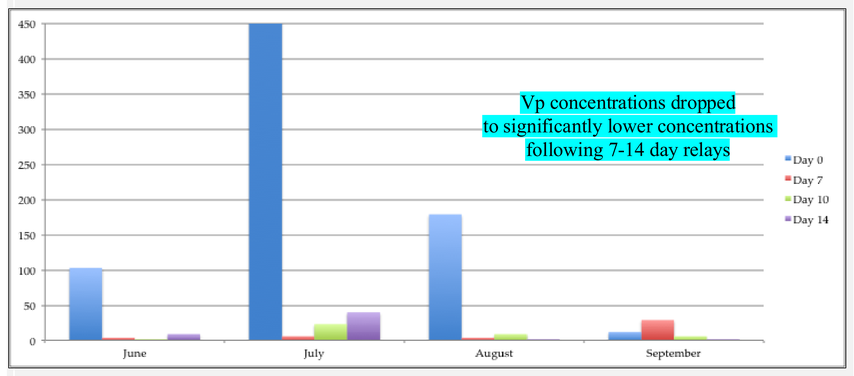
Prevalence of Vp strains with pathogenic potential* in aquaculture (AC) and wild oysters in NH. (* contain *trh* and/or *tdh* genes)



RESULTS: MANAGEMENT STRATEGIES TO REDUCE HUMAN HEALTH RISKS

Post-Harvest Management Strategies: RELAY

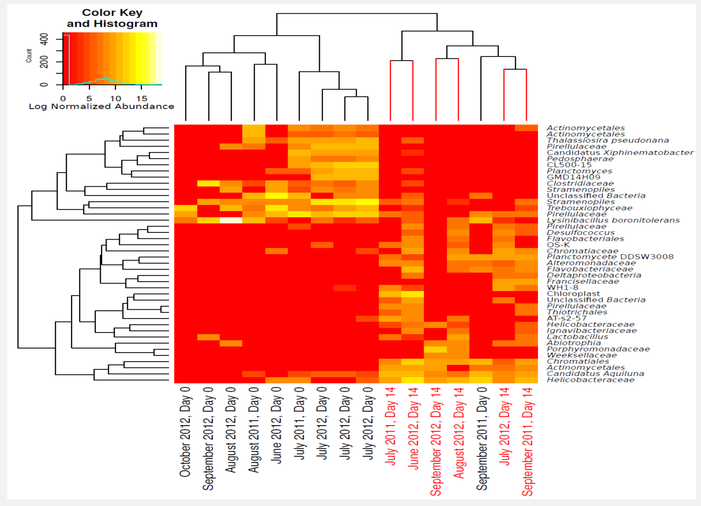
***V. parahaemolyticus* concentrations (MPN/g) in freshly harvested (Day 0) and relayed (Days 7,10,14) oysters from a harvest areas with elevated Vp concentrations and lower salinity to a relay site with low Vp concentration & elevated salinity**



The relative abundance and relatedness between OTUs associated with fresh (Day 0) and relayed (Day 14-in red) oyster and water samples during 2011-12.

The heat map (below) shows taxa associations among statistically different samples. Yellow-orange-red color intensity indicates sequence relative abundance (see key). The dendrograms show hierarchical clustering of OTUs (left) and samples (top). The hierarchal clustering highlights the distribution patterns of the OTUs that differed significantly between Day 0 and 14 samples.

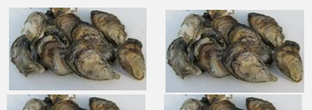
→ *Oyster microbiome community profiles change when oysters are exposed to different environmental conditions* (i.e., Relay) *that also reduce Vp concentrations*.



Post-Harvest Management Strategies: RESUBMERGENCE

---Water level---

Exposed oysters



Control oysters (Spatially separated from exposed oysters)

Summary Results for 4-Years of Re-submergence Field Trials in Massachusetts, New Hampshire & Maine

Location / Trial conditions	Year	Trial	Abuse effect* Log10 Vp conc inc	Recovery time† Days	
MASSACHUSETTS	2019	1	1.2	4	
Subtidal bottom culture/48 h air exposure-Duxbury		2	2.1	2	
Subtidal bottom culture/48 h air exposure-Katama Bay		3	2.7	2	
Subtidal bottom culture/48 h air exposure-Plymouth	2020	1	2.7	4	
Intertidal bottom culture/48 h air exposure-Plymouth		1	3	4	
NEW HAMPSHIRE	2019	1	1.2	0	
Bottom culture/3 h air exposure		2	0.4	0	
		3	1.8	≤4	
	2020	1	1.5	0	
		2	1.3	2	
		3	1.4	0	
		4	0.9	2	
	2021	1	0.4	0	
		2	0.5	3	
	2022	1	0.6	0	
MAINE	2019	1	2.9	≤7	2-7 days
Surface culture/48 h air exposure		2	1.3	2	
	2020	1	1.2	1	
		2	1.3	>4 days did not recover	4 d trial
		3	1	1	

*the increase in Vp concentration in oysters following temperature abuse and before re-submergence

†Days required for Vp concentrations in abused oysters to be not significantly higher than in control oysters

- The recovery times for all trials were less than 7 days
- The abuse effect was lower and recovery times generally more rapid in NH with 3 h exposure compared to in ME & MA where exposure time was 48 h.
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CONCLUSIONS

- The long-term trends for total and potentially pathogenic *Vibrio* species populations in the Northeast US oysters reflect continued increases in concentrations, pathogenicity potential, and season occurrence.
- Continued modeling of total *Vp* levels in oysters, along with synoptic population genomics studies, continue to inform regionally effective *Vp* management strategies for reducing human health risks from shellfish consumption.
- New Hampshire is a key study area for addressing the regional Vibrio (*Vp, V. vulnificus, V. cholerae,*) issue.

ONGOING AND FUTURE STUDIES

Ongoing studies focus on:

- microbial communities associated with offshore, integrated multi-trophic aquaculture (IMTA) operations,
- strategies to reduce potential human health issues associated with importing seed oysters to local farms from areas where vibriosis has occurred;
- The prevalence of emergent Vibrio species of concern, i.e., *V. fluvialis* and *V. alginolyticus*