

Monitoring for Vibrio bacteria in Maine Oysters



Bureau of Public Health and Aquaculture
Biotoxin-Water quality lab
Climate Council Meeting
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EXPANDING SHELLFISH INDUSTRY IN MAINE

- AQ jobs doubled from 2016 to 2024
- Shellfish farming is 18.5 times more concentrated in Maine than the national average (Damicis et al., 2023)
- Landings: \$1.2 million in 2011 to just under \$16 million in 2025 (ME DMR 2025).

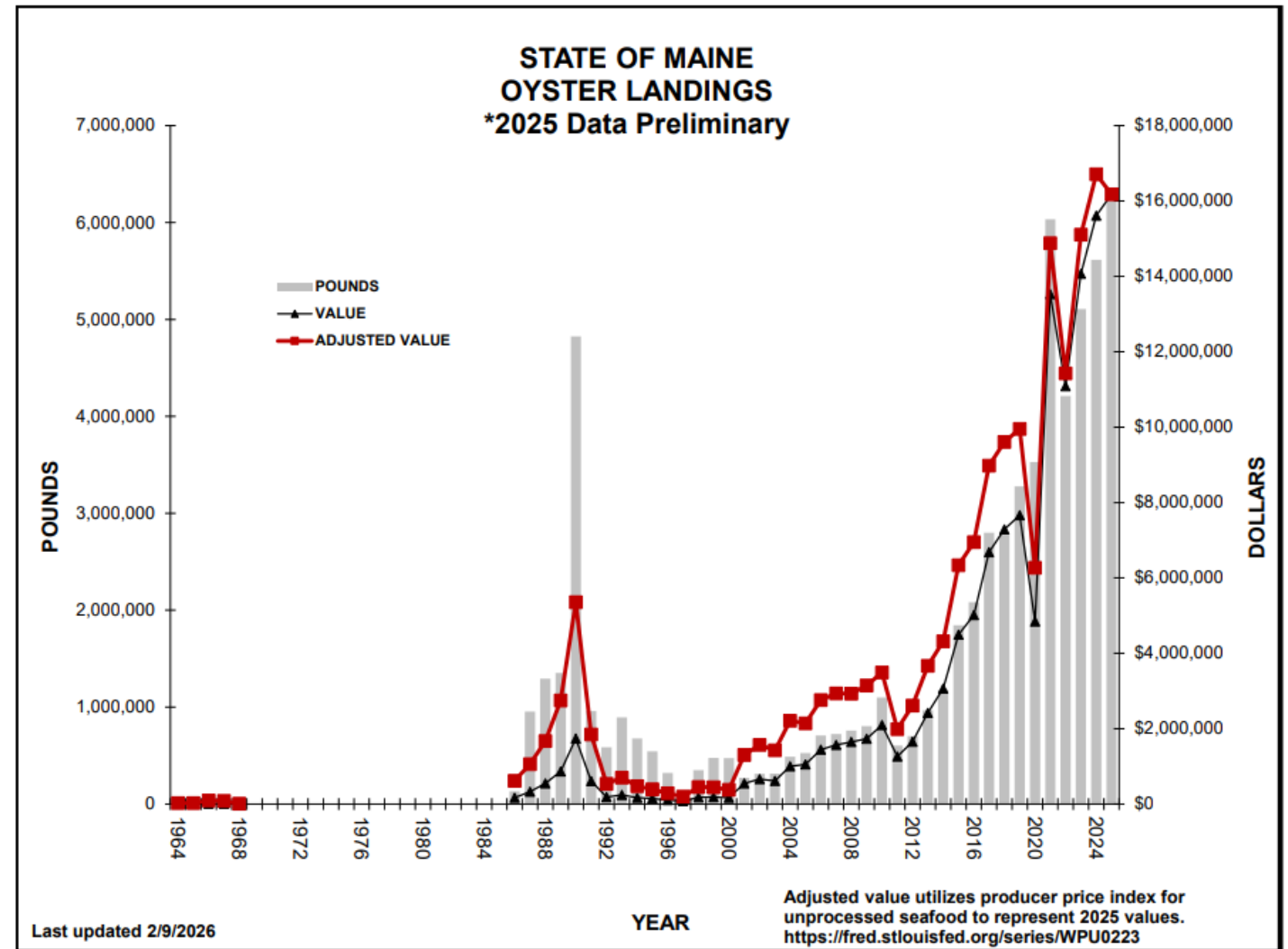


Figure 1. Oyster landing data for the state of maine from 1986-2025

OYSTERS FILTER FEED UNWANTED BACTERIA

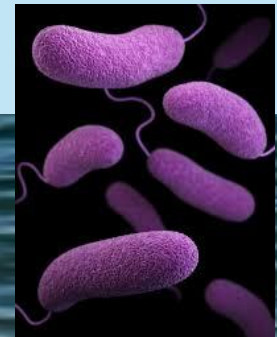
THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*

- Native bivalve mollusk
- Filter feeder
- Filtration rate is a strong function of temperature (8 - 30°C) (Synder et al., 2017)
- Bioaccumulate bacteria 100-fold higher than the surrounding water (Almuhaideb et al 2020, NSSP)
- **Commonly consumed raw**



VIBRIO PARAHAEMOLYTICUS (Vp)

- Naturally occurring (Sal 10-34 psu)
- Northeast detection in 1970
(Bartlet & Slanetz 1971)
- Some strains of *Vp* are pathogenic to humans, causing gastroenteritis (Vibriosis):
 - Nausea, diarrhea, and vomiting
 - Wound infections
 - Rare cases, septicemia



VIBRIOSIS CASES INCREASING WITH WARMING WATERS IN THE GOM

- Recent rapid warming due to regime shift in 2010 is correlated with increased vibriosis cases (Goncalves Neto et al., 2021, Friedland et al., 2020, Urqhaurt et al 2016)
- SST was the dominant factor associated with an increasing trend of *Vp* in Great Bay Estuary oysters (Hartwick et al., 2019)
- Vibriosis cases doubled in 2023
- Warming waters is beneficial for both oysters and *Vp*

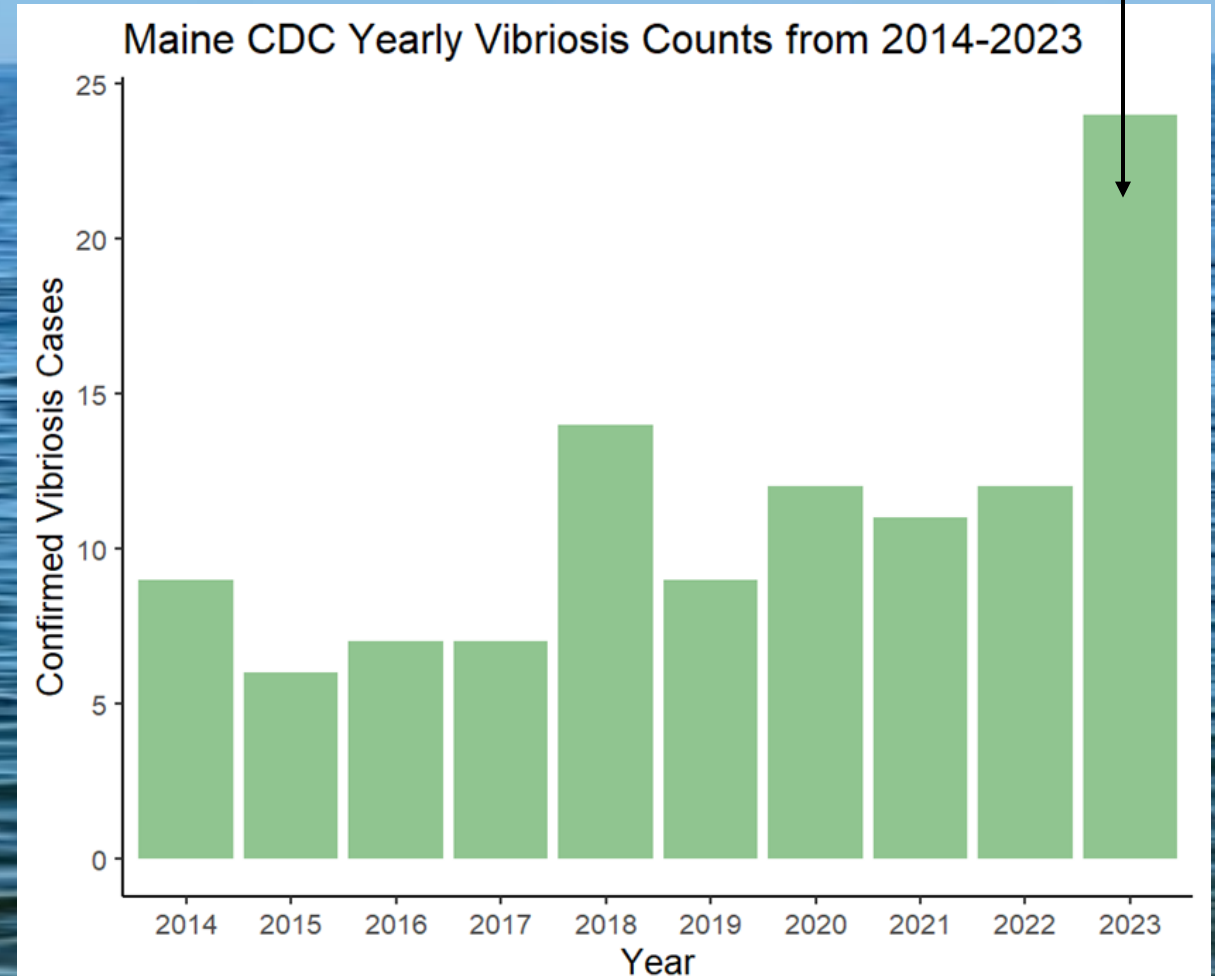
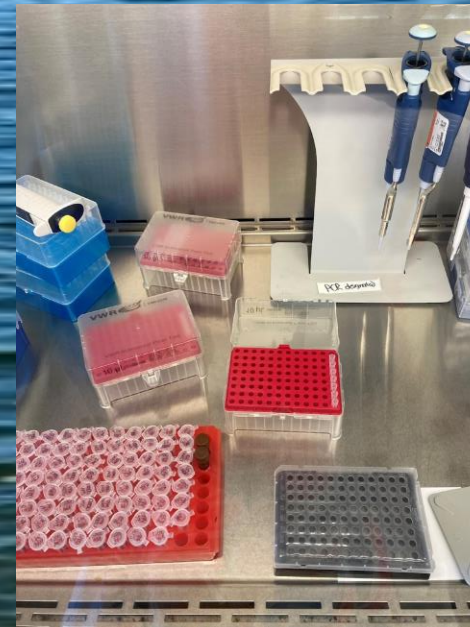
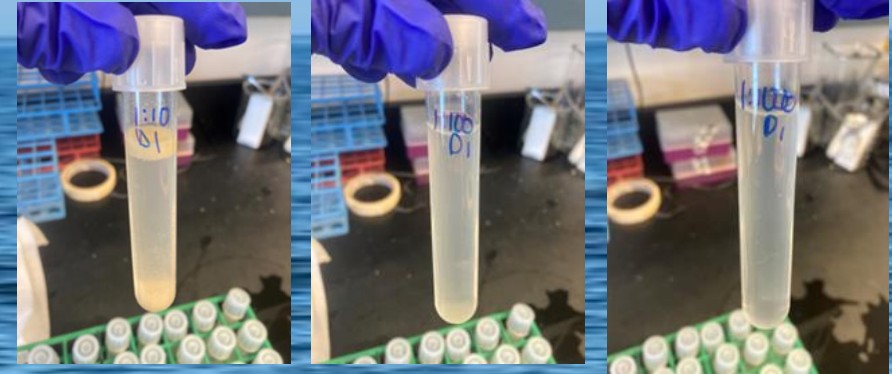


Figure 2. Vibriosis Cases by Year, Maine 2014-2023

BASELINE VP DATA IS NECESSARY FOR ILLNES RESPONSE

- Section II Model Ordinance – Chapter II. Risk Assessment and Risk Management @0.2.(10)
 - Collect and analyze samples to ensure that **TDH** does not exceed **10 MPN g⁻¹** and **TRH** does not exceed **10 MPN g⁻¹**;
 - OR** other such values as determined appropriate by the Authority **based on studies**.



Proposal No. 15-111

Proposal Subject

MPN-Real-Time PCR for Pathogenic *V. p.*

Specific NSSP

Section IV. Guidance

Guide Reference

Chapter II. Growing Areas 11 Approved NSSP Laboratory Tests

Text of Proposal/
Requested Action

This method was developed by Jessica Jones (FDA Gulf Coast Seafood Laboratory) and is being submitted by the ISSC Executive Board. The Executive Board granted interim approval to this method on March 13, 2013. The Executive Board is submitting this proposal to comply with Article V, Section 1, of the ISSC Constitution, Bylaws, and Procedures.

Submitted by method developer Jessica Jones (FDA Gulf Coast Seafood Laboratory)

5. Approved Methods for Vibrio Enumeration

	Vibrio Indicator Type:	Application: PMP Sample Type: Standard	Application: <u>Seawater</u>
EIA ¹	<i>Vibrio vulnificus</i> (P+) / <i>Vibrio vulnificus</i> (P+)	X	
MPN ²	<i>Vibrio vulnificus</i> (P+) / <i>Vibrio vulnificus</i> (P+)	X	
SYBR Green 1	<i>Vibrio vulnificus</i> (P+)	X	
QPCR-MPN	<i>Vibrio parahaemolyticus</i> (P+) / <i>Vibrio parahaemolyticus</i> (P+)	X	
PCR	<i>Vibrio parahaemolyticus</i> (P+) / <i>Vibrio parahaemolyticus</i> (P+)	X	
MPN-Real Time	<i>Vibrio parahaemolyticus</i> (P+) / <i>Vibrio parahaemolyticus</i> (P+)	X	
MPN-Real Time	<i>Vibrio parahaemolyticus</i> (P+) / <i>Vibrio parahaemolyticus</i> (P+)	X	
MPN-Real Time	<i>Vibrio parahaemolyticus</i> (P+) / <i>Vibrio parahaemolyticus</i> (P+)	X	

Footnotes:

¹ EIA procedure of Tamplin, et al. as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992.

² MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or by the DNA-alkaline phosphatase labeled gene probe (vvhA).

³ MPN format with confirmation by biochemical analysis, gene probe methodology as listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.

⁴ PCR methods as they are listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.

⁵ *Vibrio vulnificus*, ISSC Summary of Actions 2009: Proposal 09-113, Page 123

⁶ *MPN-Real Time PCR method for the *Vibrio* and *Vibrio* for total *V. parahaemolyticus* as described in Kasper et al., 2011.*

Public Health
Significance

The current NSSP method for enumeration of *Vibrio* requires a minimum of four days from receipt of sample to results reporting. Currently, there is no NSSP approved method for enumeration of *Vibrio*. At the 2013 conference, proposal 13-102 was adopted which requires testing for the presence of *Vibrio* and *Vibrio* prior to reopening of growing areas closed as a result of *Vibrio* illnesses (Chapter II @ 01.F(5)). This proposed MPN real-time PCR method provides results in as little as 24h from receipt of sample. Availability of this more rapid method will facilitate reopening decision making.

Cost Information

This method costs ~\$120 per sample for laboratory consumables, supplies, and reagents.

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DMR MONITORING BEGAN IN 2023

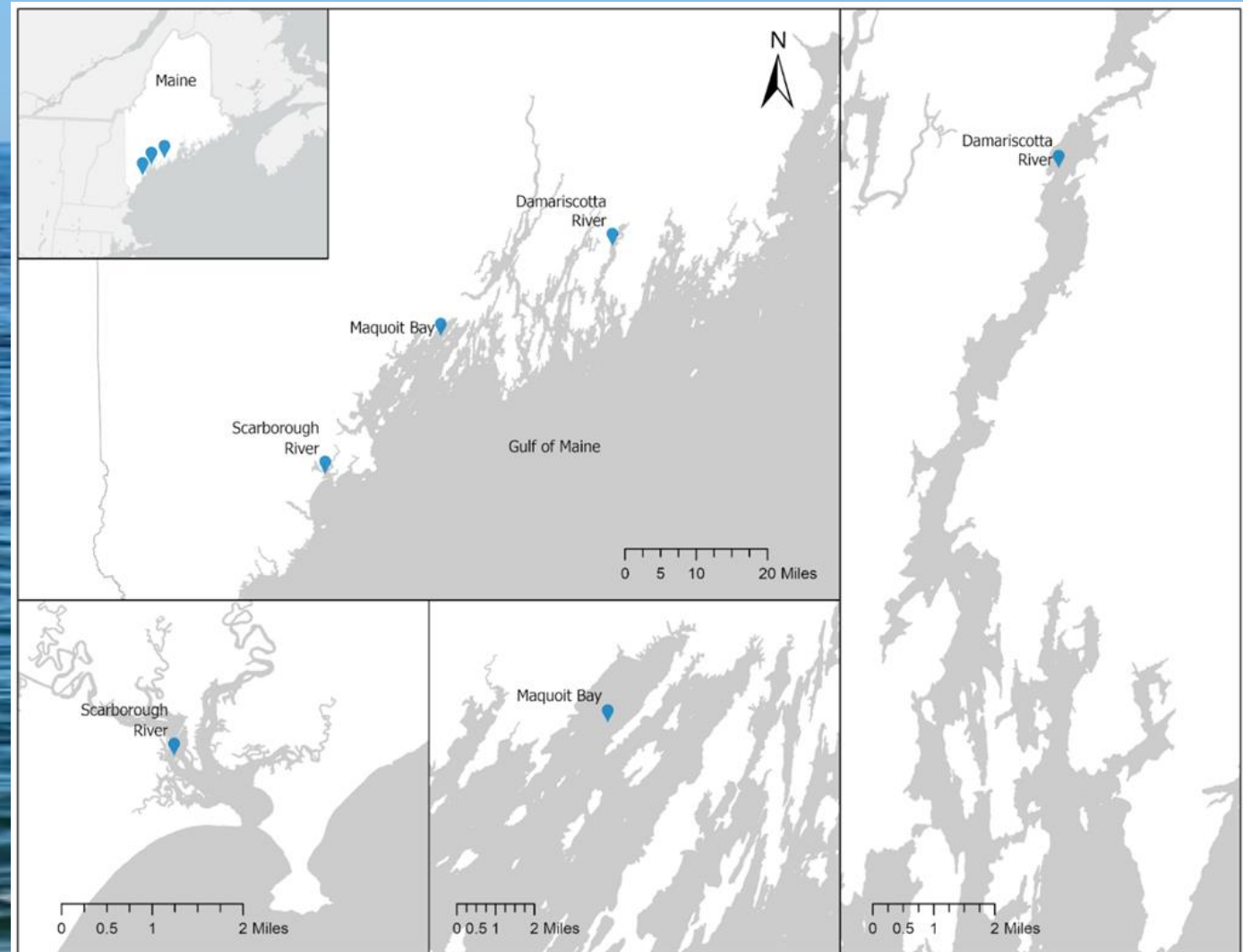


Figure 3. Sampling locations for *vibrio parahaemolyticus* monitoring in 2023.

COLLECTING ABUNDANCE DATA

- Detecting total and pathogenic Vp
 - TLH – total Vp
 - TDH- gene found in pathogenic strains
 - TRH – gene found in pathogenic strains
- Could not detect peak abundance due to current methodologies
- This bacterial abundance is natural and detecting trh/tdh means the strain is “**potentially pathogenic**”
- This bacteria in normal quantities concerns immunocompromised individuals

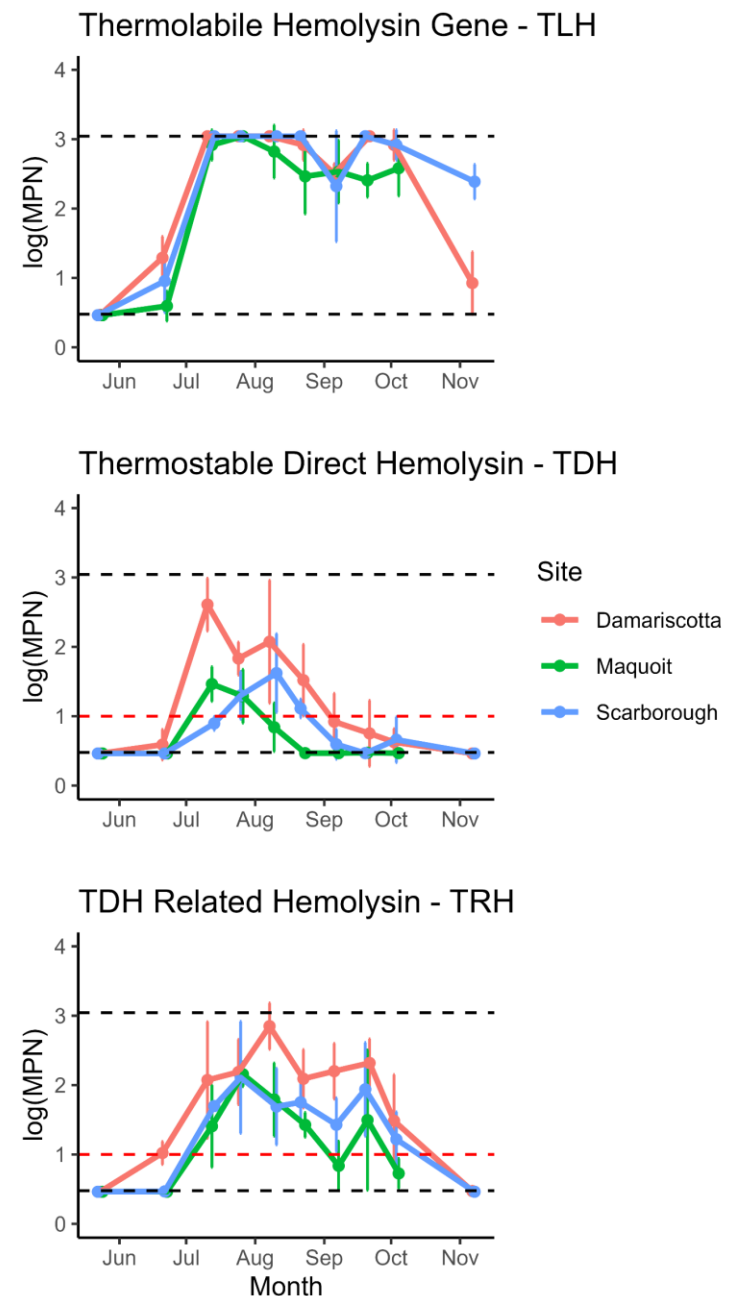


Figure 5. Abundance graph for 3 Vp target genes

PATHOGENIC VIBRIO RESPONDS TO TEMPERATURE

Significant positive relationship seen between pathogenic gene and temperature

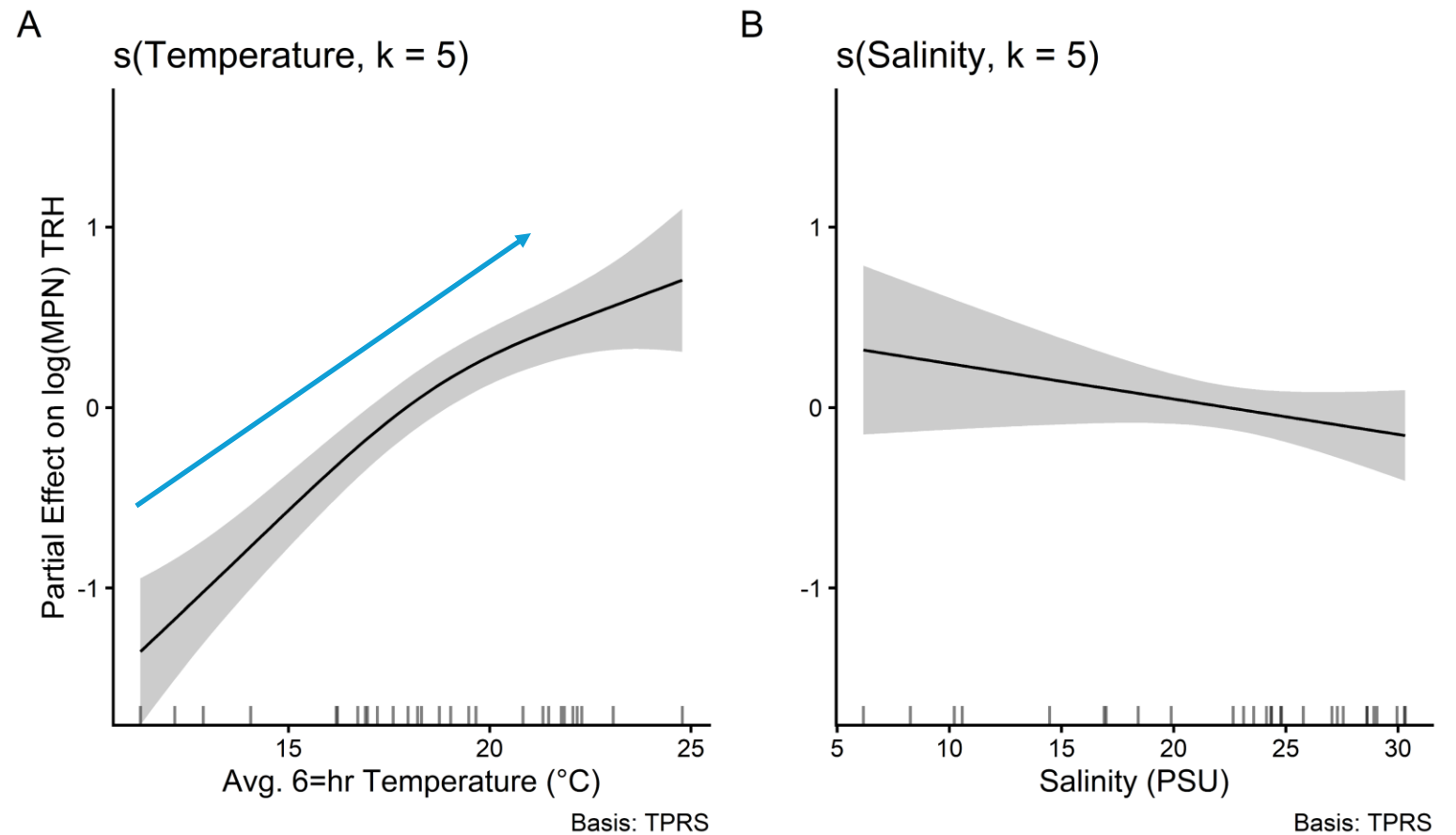
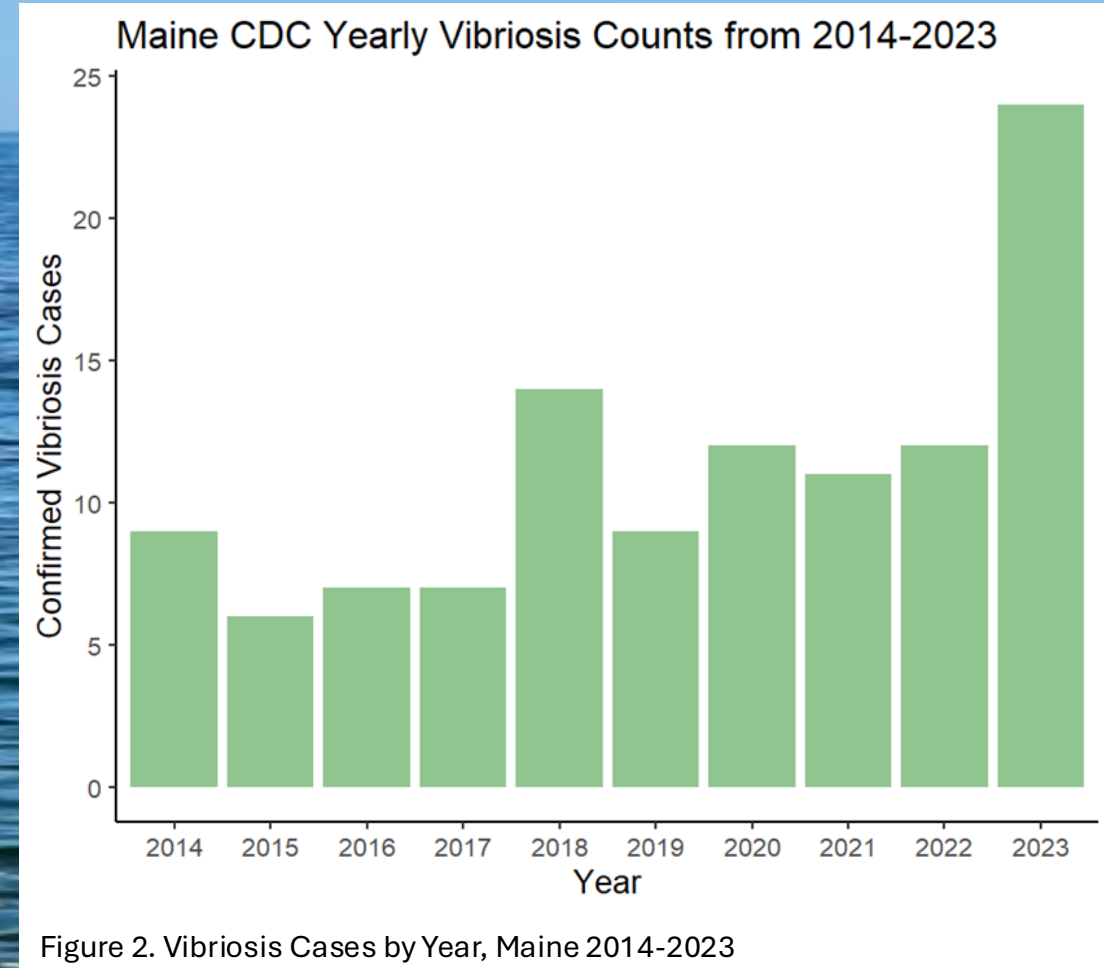


Figure 6. A.) Generalized additive model response curve for pathogenic target gene TRH and sea surface temperature. B.) Generalized additive model response curve for pathogenic target gene TDH and sea surface salinity.

MAINE VIBRIO REGULATIONS CHANGED IN 2024

Vibrio regulations in place from June 1st- October 15th

- 2023 regulations
 - 6 growing areas subject to regulations
- First Vibrio related outbreak in 2023
- 2024 regulations
 - statewide vibrio control
 - All oysters in Maine
 - Hard clams from the New Meadows River
 - Time/temp requirements for harvesters and primary dealers
 - Mandatory vibrio training and vibrio permits



EXPANDING VP MONITORING 2025

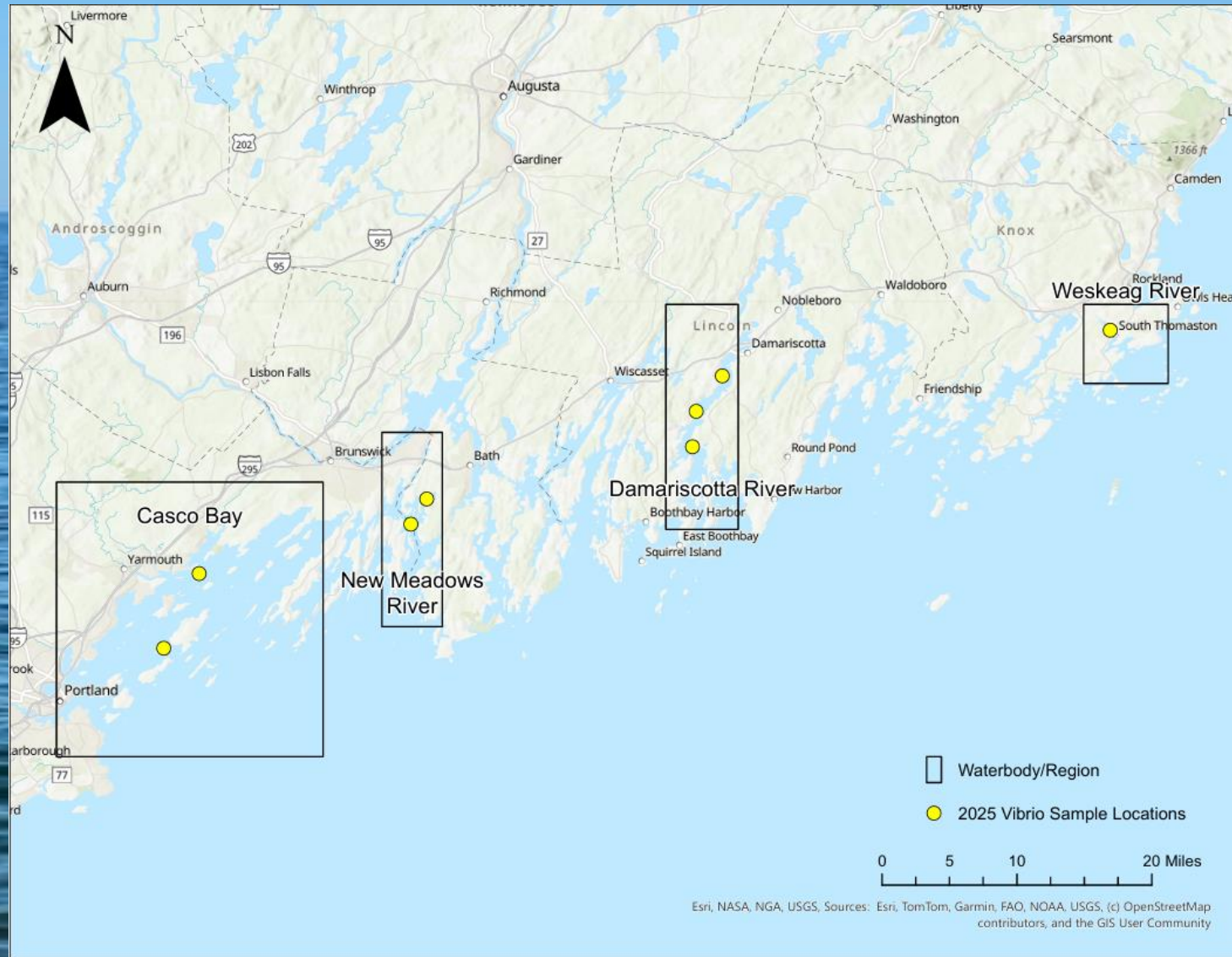


Figure 7. 2025 Vibrio site locations

WHAT CHANGED IN 2025

- Sampled more areas
- Increased dilutions to determine peak abundance
- 3 sites in 2023
↓
- 8 sites in 2025
- Partnership with HETL for WGS
- Detected other *Vibrio* species
- Strain confirmation
- More pathogenicity data



2025 SUMMARY

- Processed 174 samples (2,088 oysters)
- 155 *Vibrio* isolates were submitted for Whole Genome Sequencing (WGS)
- WGS detected:
 - *Vibrio parahaemolyticus*
 - *Vibrio anguillarum*
 - *Vibrio alginolyticus*
 - *Vibrio antiquarius*
 - *Vibrio cholera*
- Abundance data of 2025 Vp is still being analyzed, results coming soon.

		1	2	3	4	5	6	7	8	9	10
M01230988	1	0	17851	17805	17832	18229	17073	19531	17331	17330	18682
M01231165	2	17851	0	17314	17423	18018	17809	19772	17676	17675	18825
M01232768	3	17805	17314	0	17116	17909	17808	19749	17716	17715	18725
M01232771	4	17832	17423	17116	0	18009	17828	19709	17656	17657	18617
M01233463	5	18229	18018	17909	18009	0	18110	19848	18159	18160	18801
M01233464	6	17073	17809	17808	17828	18110	0	19576	17243	17242	18541
M01233465	7	19531	19772	19749	19709	19848	19576	0	19655	19654	18888
M01233987	8	17331	17676	17716	17656	18159	17243	19655	0	1	18518
M01233990	9	17330	17675	17715	17657	18160	17242	19654	1	0	18517
M01233991	10	18682	18825	18725	18617	18801	18541	18888	18518	18517	0

Figure 8. Vp SNP matrix from WGS

CONTINUING MONITORING 2026

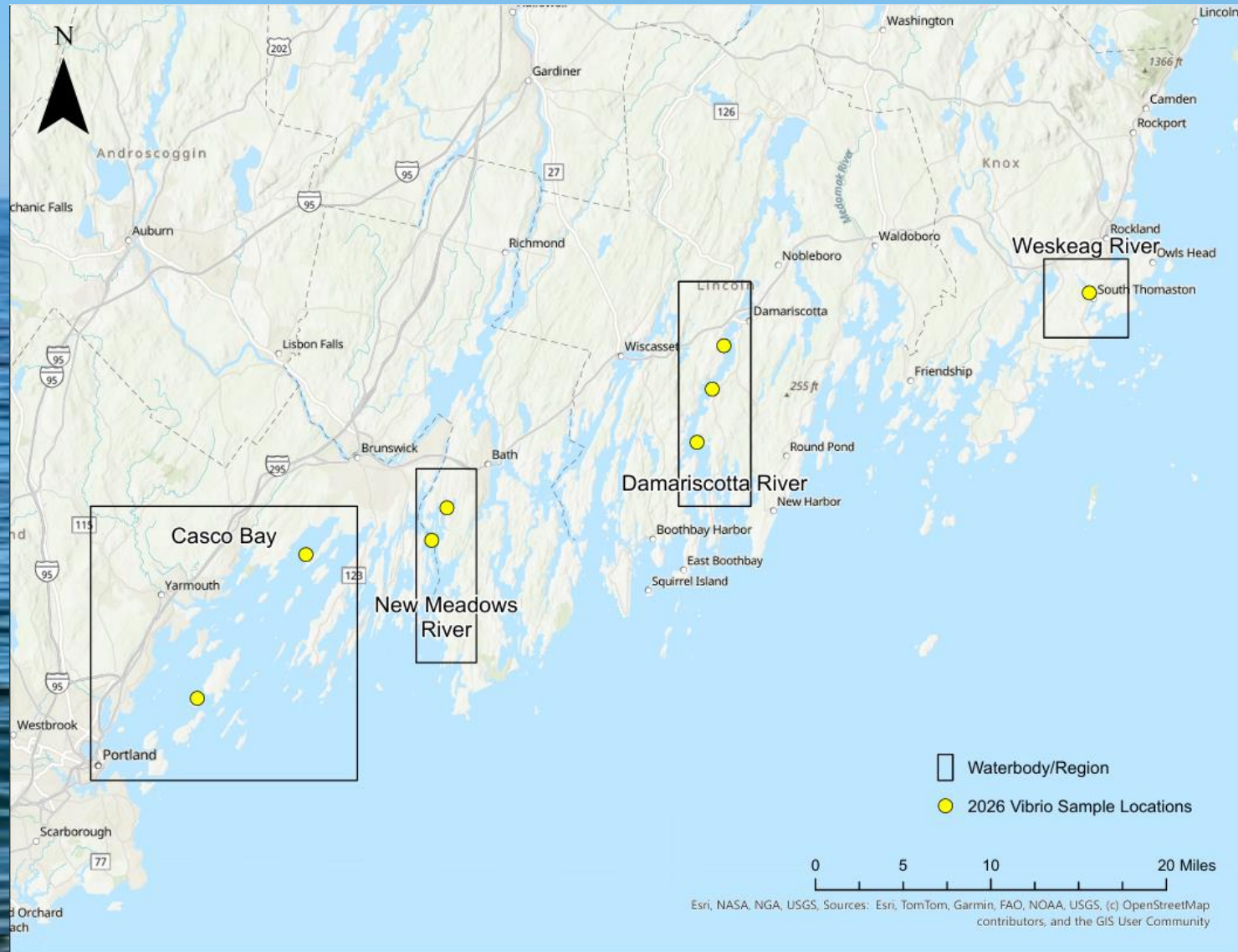


Figure 9. 2026 Vibrio site locations.

2026 PROCESSING

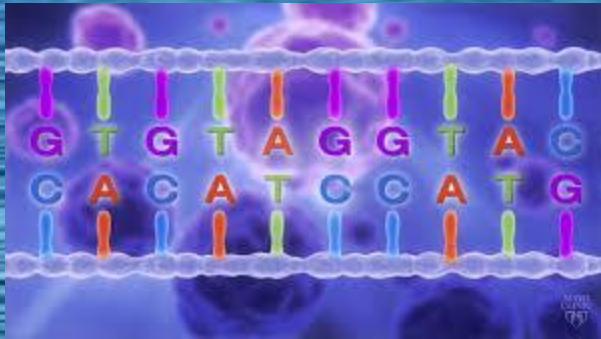


IMPORTANCE

- Prepared for future illness outbreaks
 - Baseline abundance data
 - Vibrio strain confirmation for distinct areas – traceback
 - Quicker reopening
- 3 years of comparable Vp data for better understanding of climate changes impact on Maine's shellfish industry



QUESTIONS?



This work is completed by the hard work of: Amy Webb, Julia Neumann, Tyler Spillane, Mariah Pagliaro, Nathan Alexander, and the Health and Environmental Testing Lab
Contact me for more questions: aidan.u.Lurgio@maine.gov