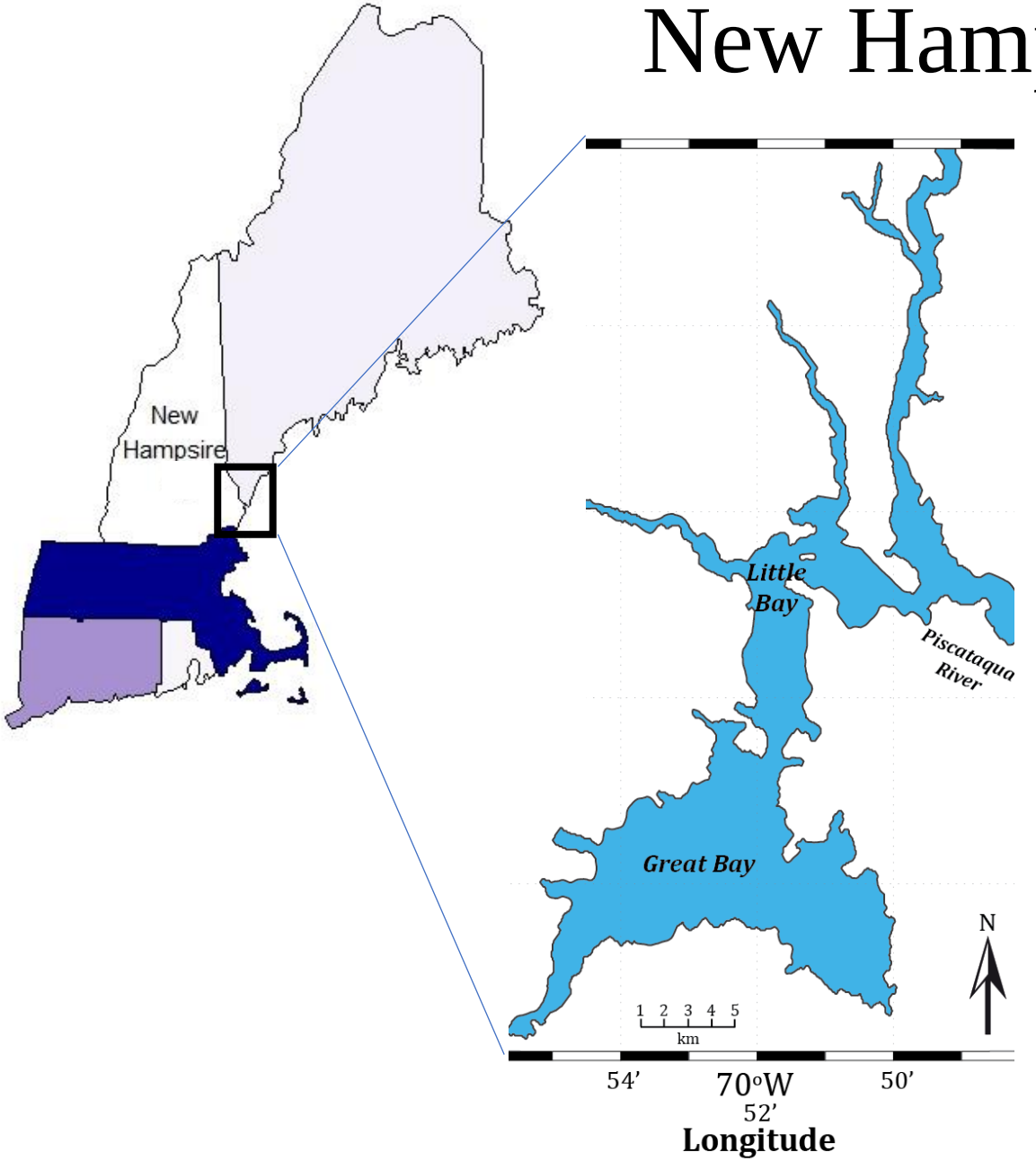
Evolution and Management of *Vibrio parahaemolyticus* Populations in the Northeast USA

Steve Jones*, Cheryl Whistler, Randi Foxall

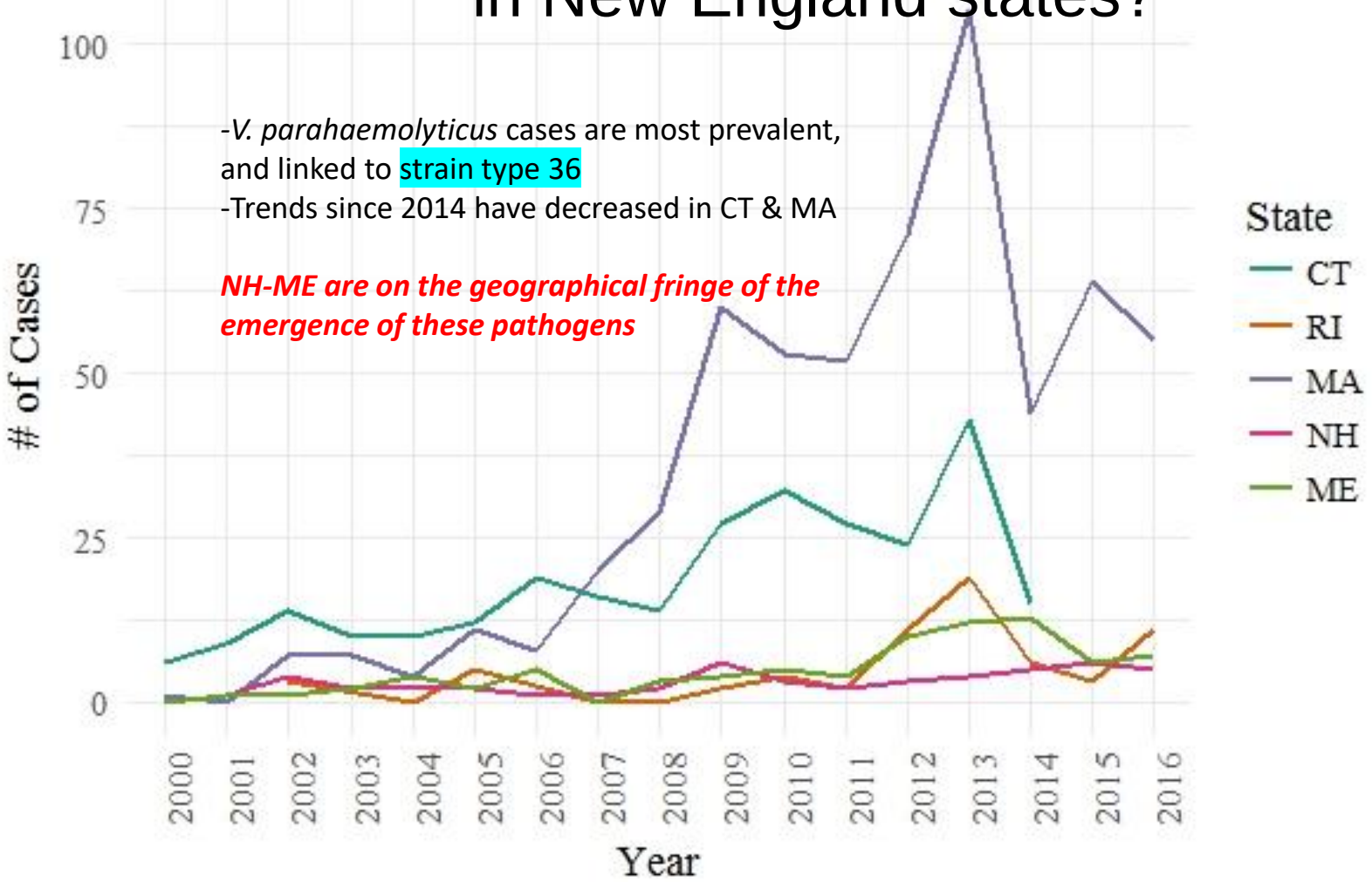
The Great Bay Estuary in New Hampshire and Maine



What is the public health significance of *Vibrio* spp. in New England states?

-*V. parahaemolyticus* cases are most prevalent, and linked to strain type 36
-Trends since 2014 have decreased in CT & MA

NH-ME are on the geographical fringe of the emergence of these pathogens



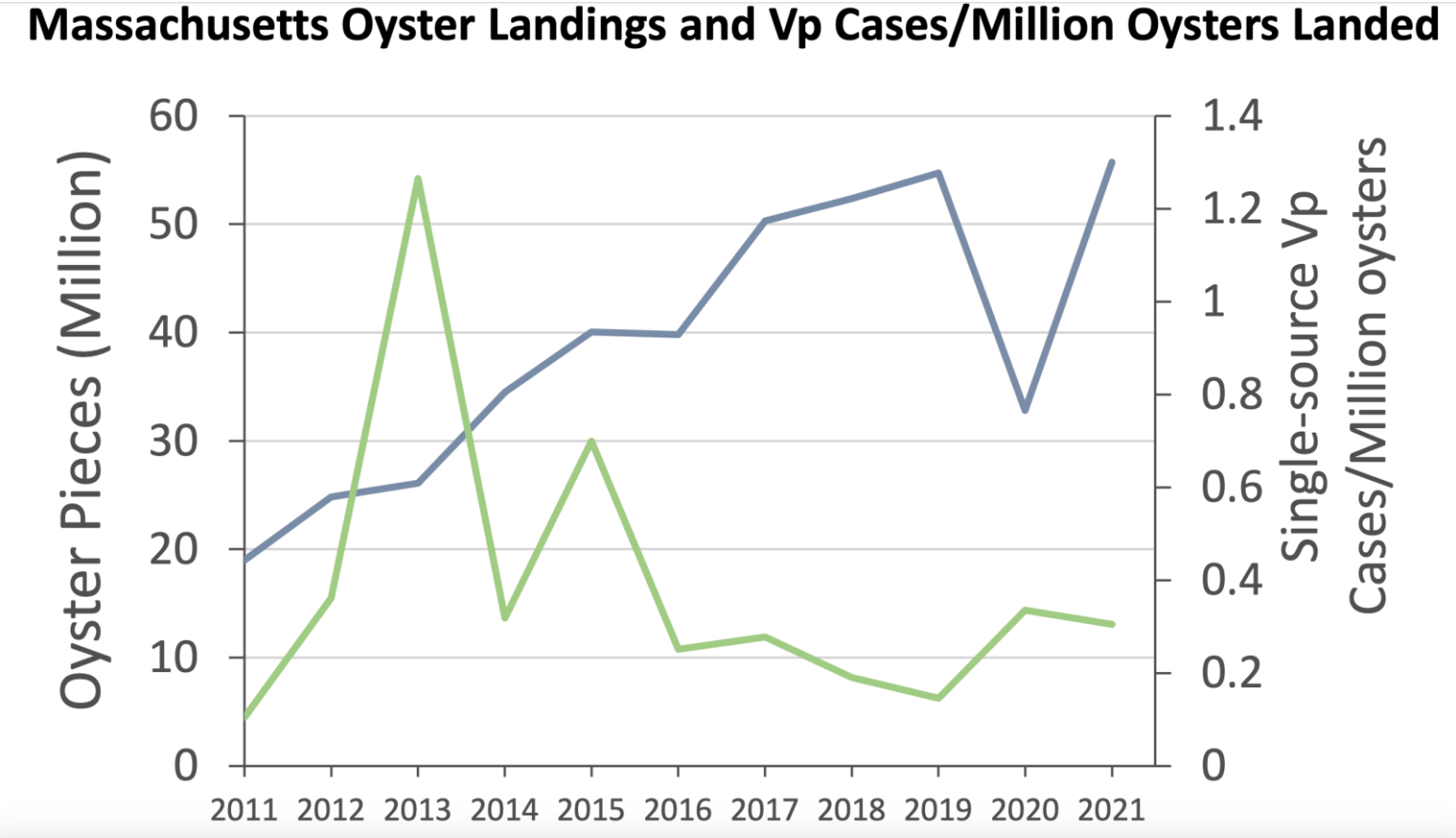
Annual cases of vibriosis in humans for Maine (ME), Massachusetts (MA), New Hampshire (NH), and Connecticut (CT) for 2000 through 2016. Species include *V. parahaemolyticus, V. vulnificus, V. cholerae, V. alginolyticus, V. fluvialis,* and 'unknown'. ***Data from CDC, MA DPH, ME CDC, RI DH.***

Urquhart, E.A., S.H. Jones, J.W. Yu, B.M. Schuster, A.L. Marcinkiewicz, C.A. Whistler and V.S. Cooper. 2016. Environmental conditions associated with Elevated *Vibrio parahaemolyticus* concentrations in Great Bay estuary, New Hampshire. PLoS ONE 11(5): e0155018: doi:10.1371/journal.pone.0155018.

Vibrio parahaemolyticus

- Halophilic bacteria that thrives in warm, brackish water
- Potential 8-9 minute ‘doubling time’ (60-70x increase per hour)
- A small minority of strains are human pathogens that cause inflammatory gastroenteritis & septicemia

--*Typical exposure is via seafood consumption*, *especially raw or slightly cooked bivalve shellfish*--



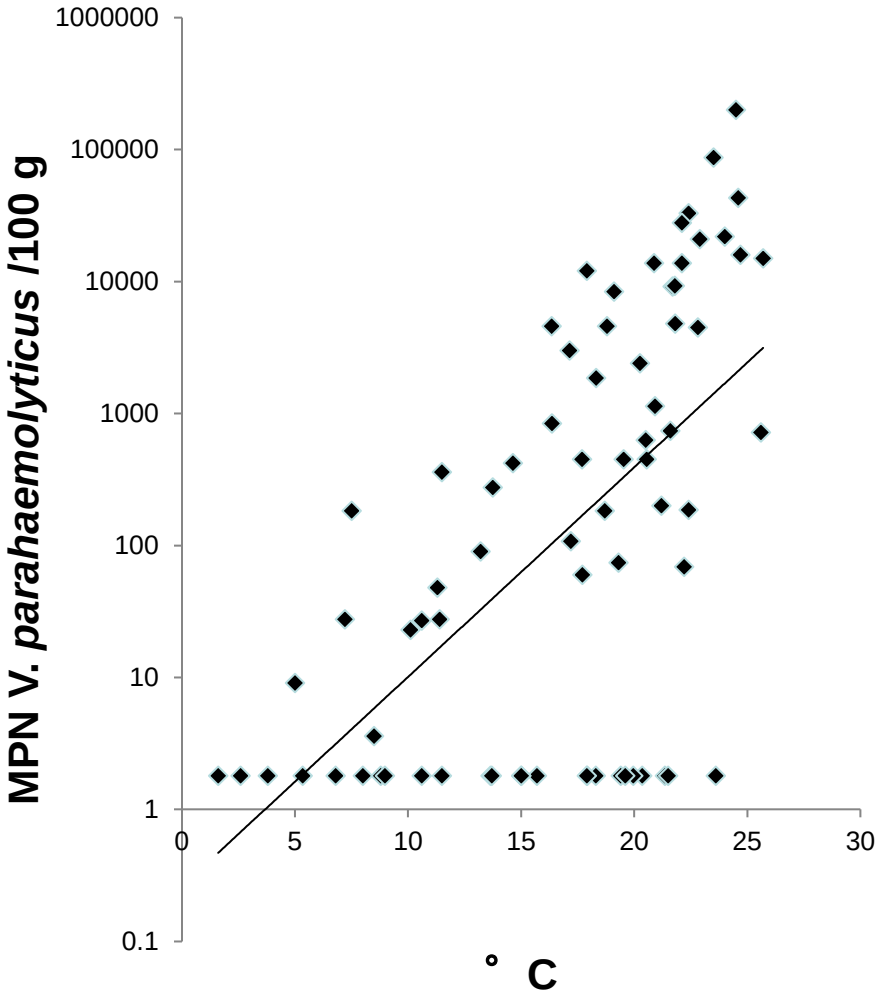
Vp DETECTION AND ENUMERATION METHODS

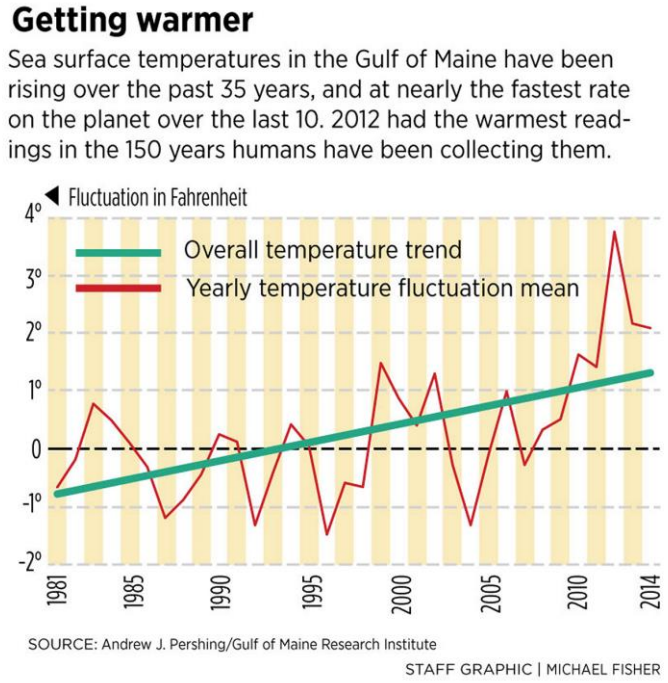
-Vibrios were enriched overnight in a dilution series of alkaline peptone water (APW) tubes containing oyster tissue at 37C, followed by MPN quantification.

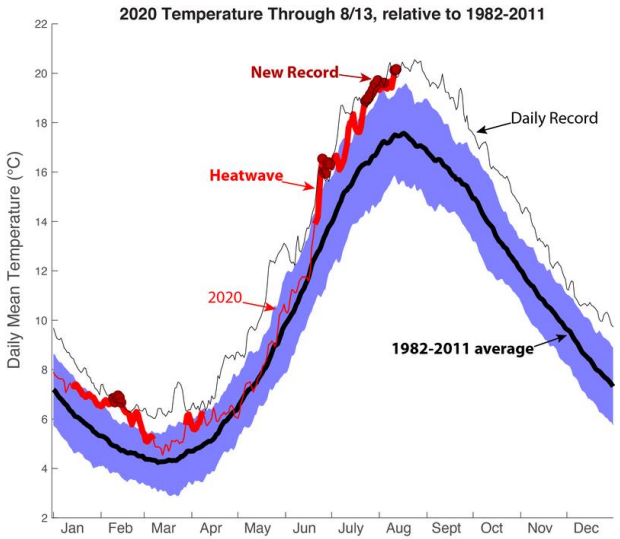
-*Vibrio parahaemolticus* genes *tlh* (total *Vp*), and *tdh* and *trh* (virulence indicators) were measured in oyster samples through an NSSP approved FDA-developed most-probable number quantitative PCR (MPN-qPCR) pipeline (Nordstrom JL, *et al.* (2007).

-Further analysis included determination of the *tdh* alleles and variants with *tdh* 3/6 of most concern.

Water temperature & *V. parahaemolyticus* levels in oysters in New Hampshire: 2007-13



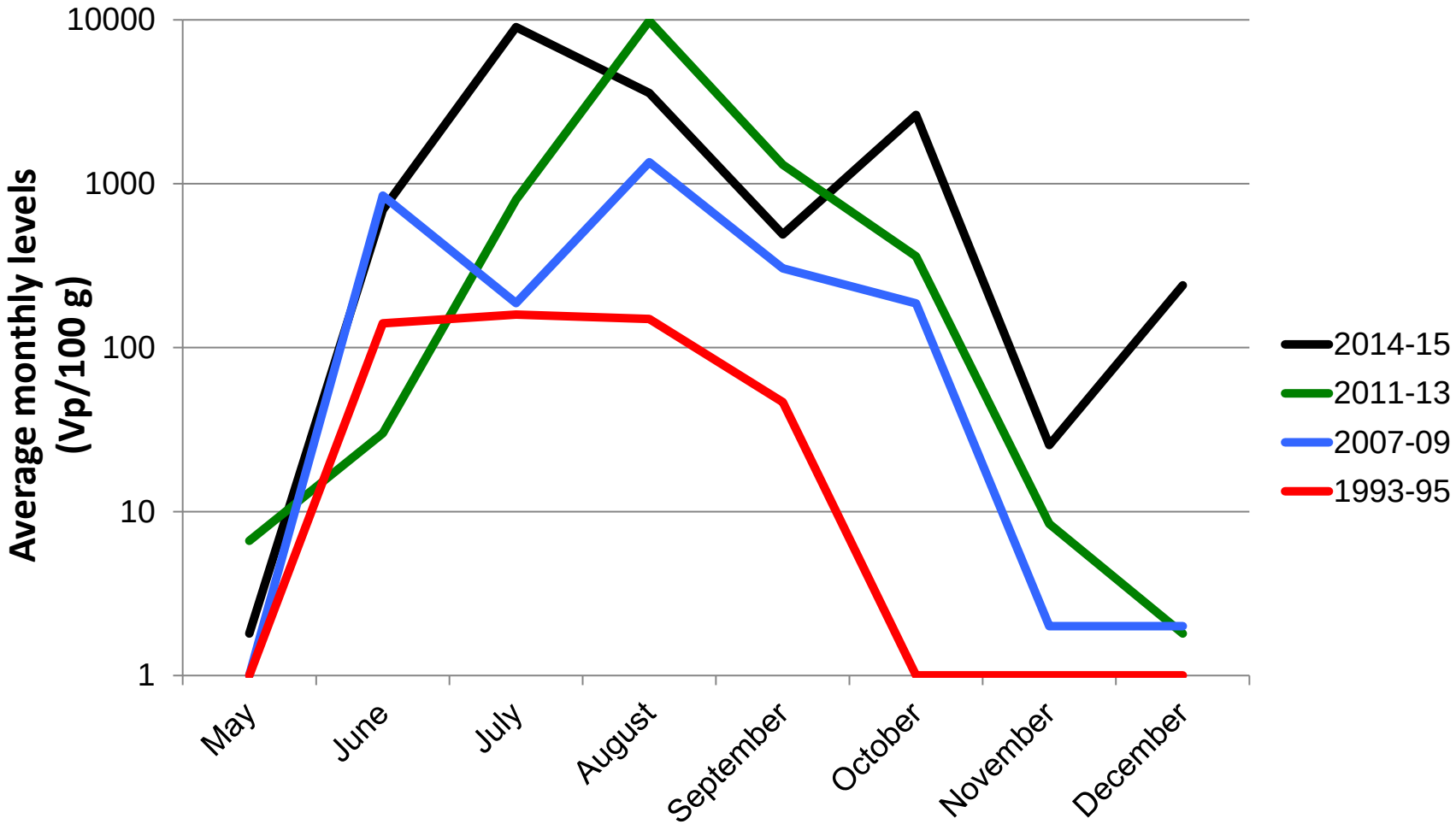


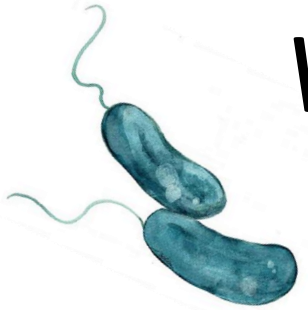


Annual cycle of sea surface temperatures in the Gulf of Maine.
Pershing 2020. 2020 Gulf of Maine Warming Update. GMRI.

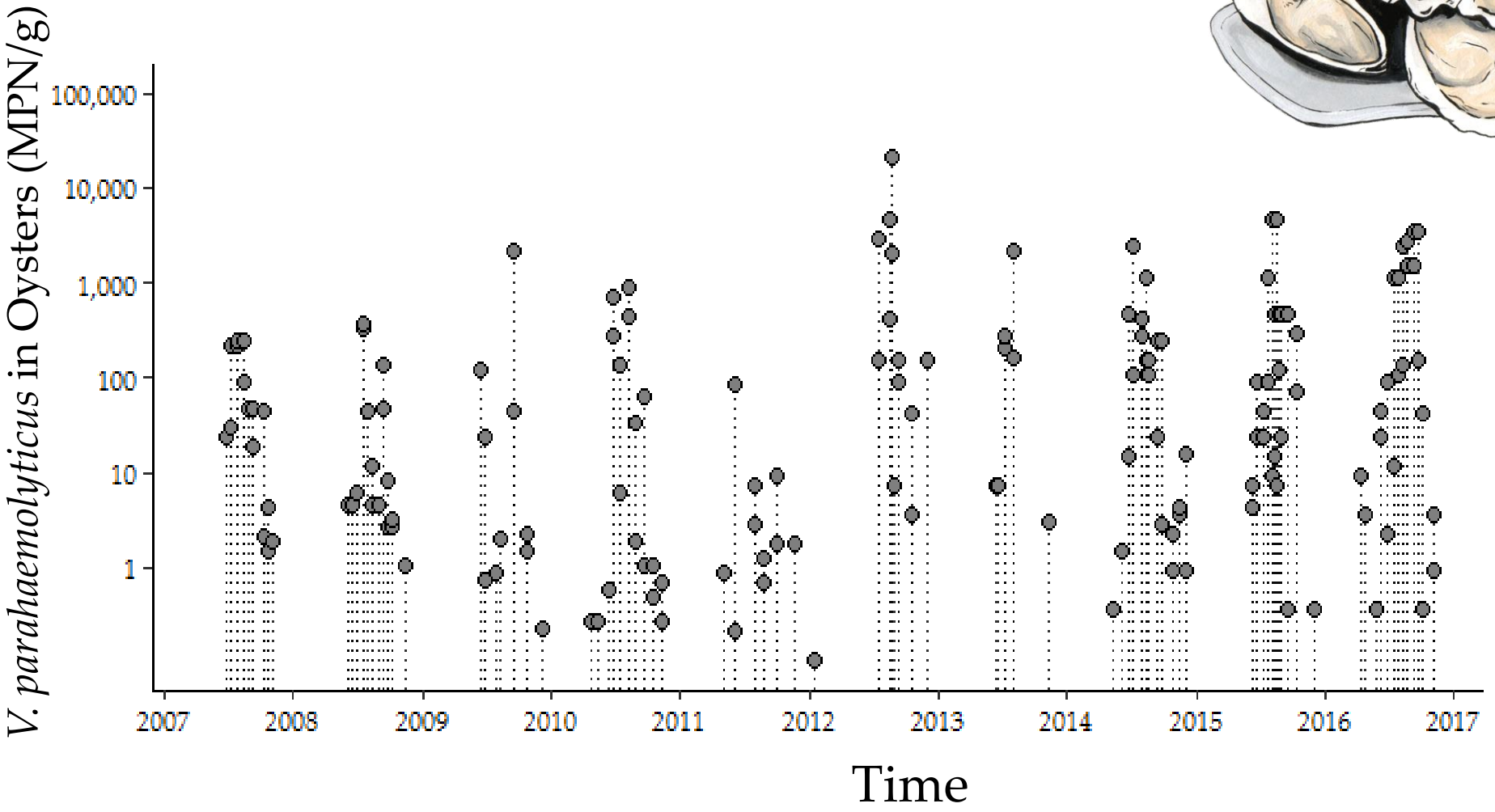
The Gulf of Maine is warming

Levels *of V. parahaemolyticus* in New Hampshire oysters are rising and persisting as human health risks increase





Vibrio parahaemolyticus in GBE oysters 2007-2016

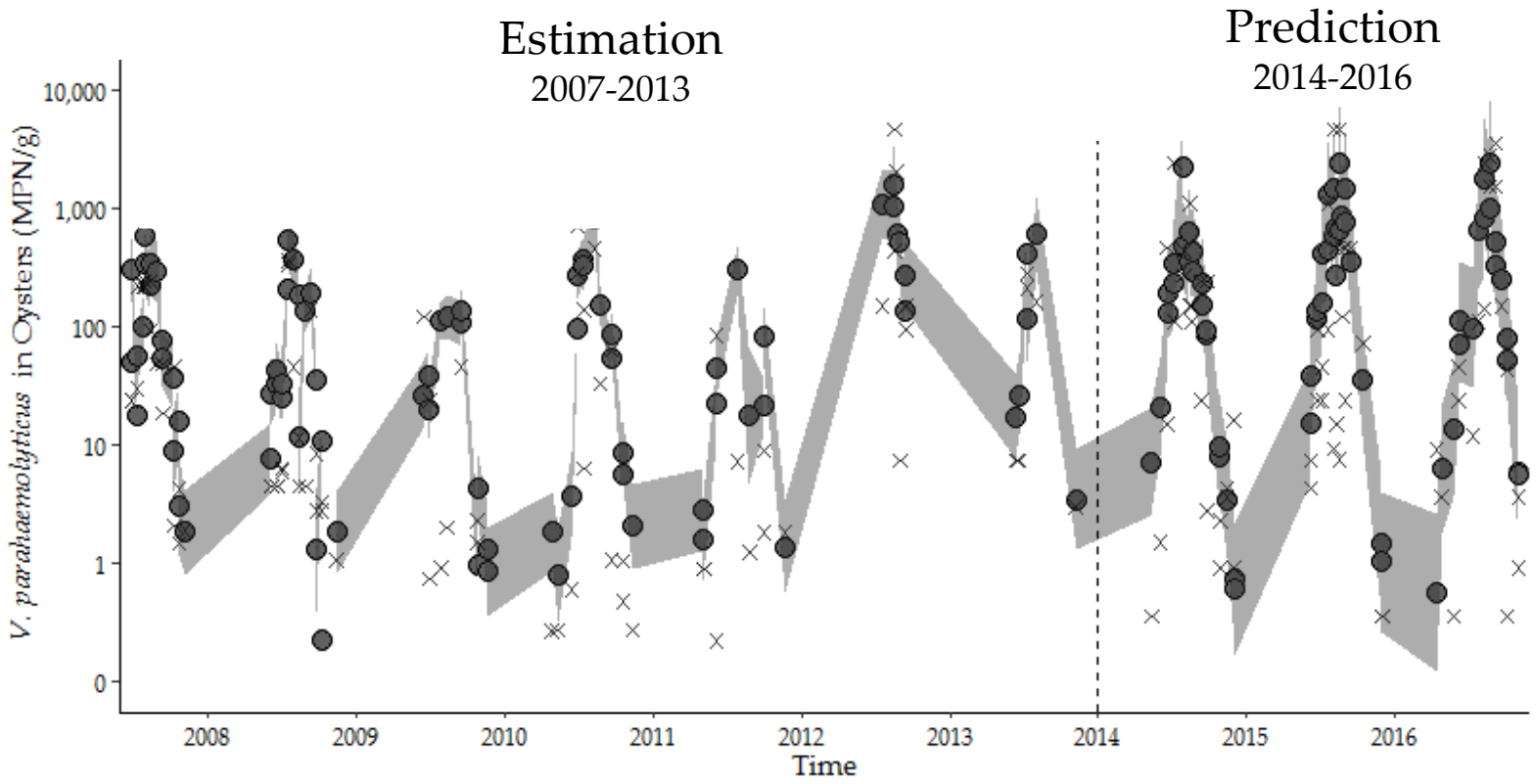


Water Temperature
Salinity
pH
Turbidity
Chlorophyll-*a*
TDN
Rainfall
(Plankton)

Forecasting *V. parahaemolyticus* concentrations in Great Bay estuary oysters

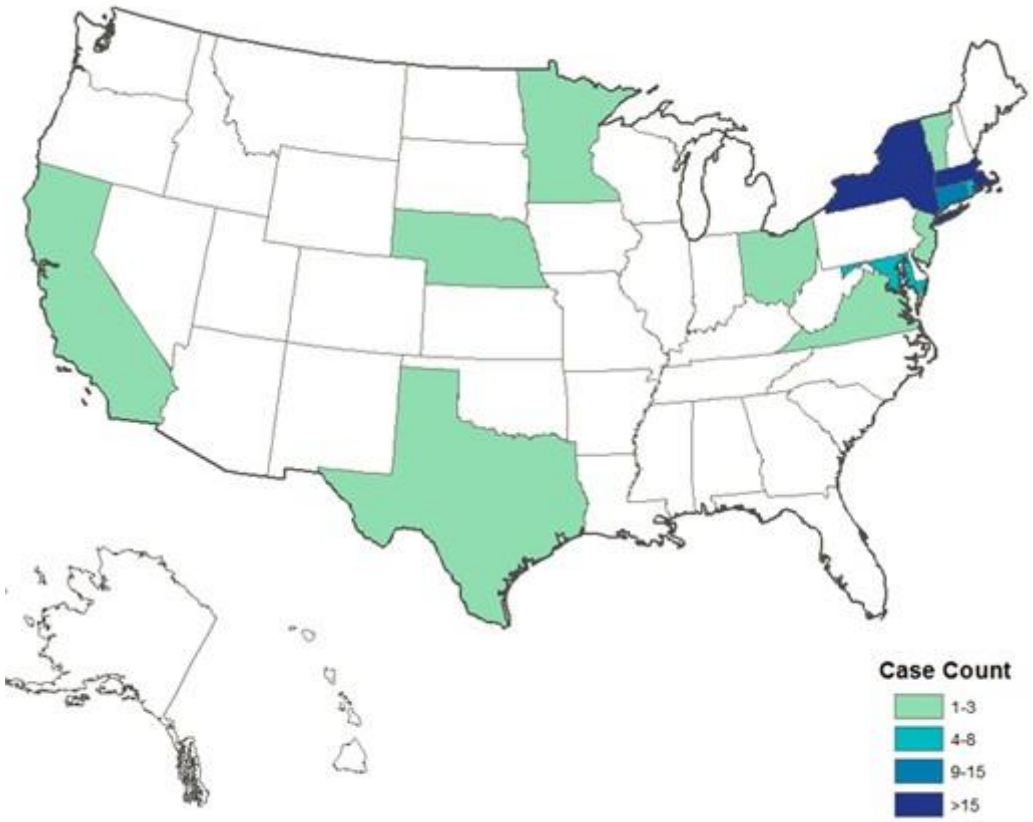
→ **Hybrid Harmonic Model**: Water temperature, pH, trend and seasonality terms

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



Hartwick et al. 2019. *Int. J. Environ. Res. Public Health*, *16*(22), 4341;https://doi.org/10.3390/ijerph16224341

A major factor in increased illnesses in the Northeast was the introduction of Pacific endemic ST36



http://www.cdc.gov/vibrio/investigations/vibriop-09-13/map.html

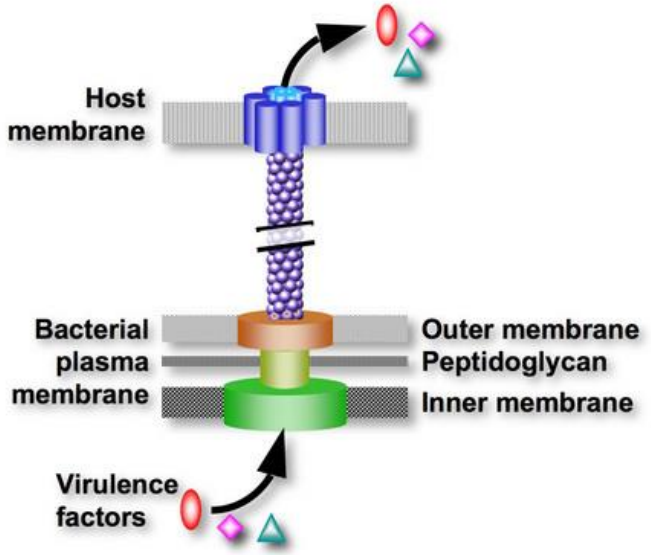
- In 2012, cases reported in both LIS and Spain were caused by Pacific native ST36
- In 2013, 104 cases in 13 US states were caused by ST36 and traced to several Northeast harvest areas
- ST36 has now established resident populations in the Atlantic
- In the PNW ST36 underwent multiple diversification events, genome reduction, and population replacement prior to dispersal

Martinez-Urtaza et al. mBio 2017; doi:10.1128/mBio.01425-17

Genomics is a forensic tool to understand the evolution and spread of invasive pathogenic strains into the region

tdh and *trh* hemolysins

M. Nishibuchi



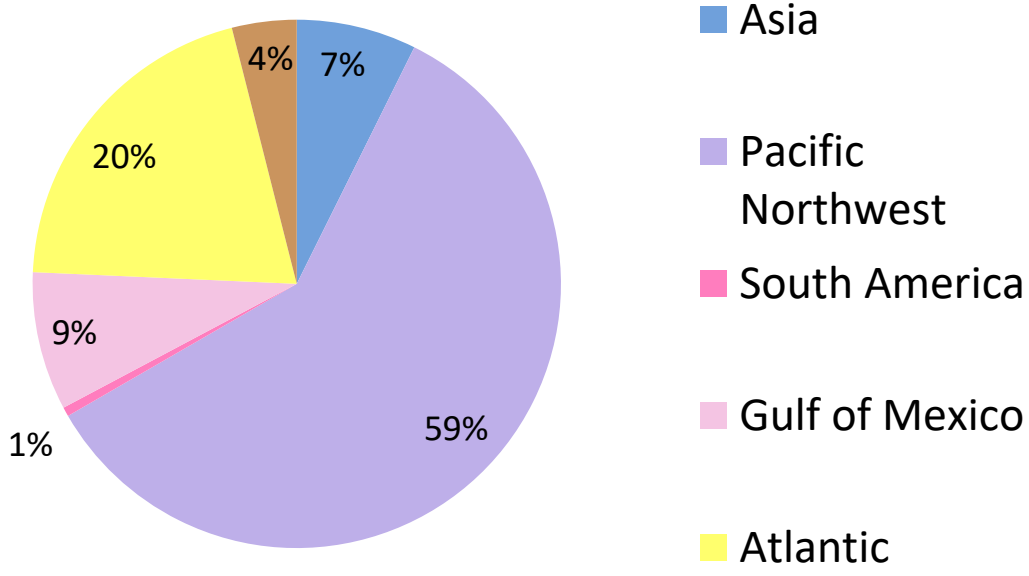
The Type III secretion system

http://carbon.bio.ku.edu/research.html

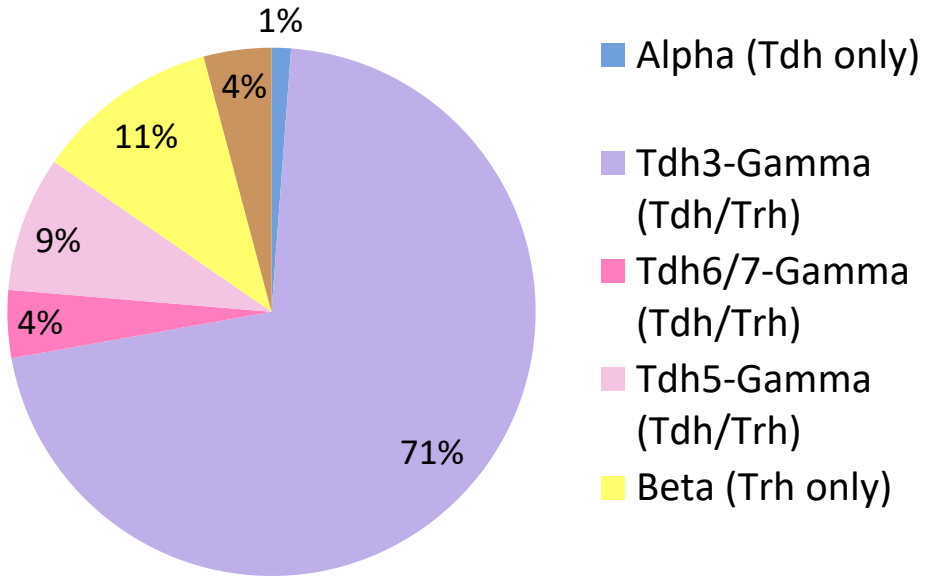
- 90% of clinical isolates carry virulence genes (diagnostic markers) in one of three VPaI elements: Vibrio Pathogenicity Island = VPaI
  - These promote disease
  - could promote environmental fitness
  - Islands copy themselves and spread through populations like a virus

The demographics of pathogenic Vp in the Northeast US

80% of “strains” we encounter **<u>in the environment</u>** are not native



The vast majority of local source **<u>infections</u>** are caused by strains (ST36 & ST631) from the Pacific Northwest that contain VPaI- γ which contains both *tdh* and *trh.*



Xu et al., 2015, 2017 and unpublished

Pre-harvest Air Exposure/Temperature Abuse

What length of re-submergence time is required for Vp levels to return to background levels?

Pre-exposure Air exposure Re-submergence over time

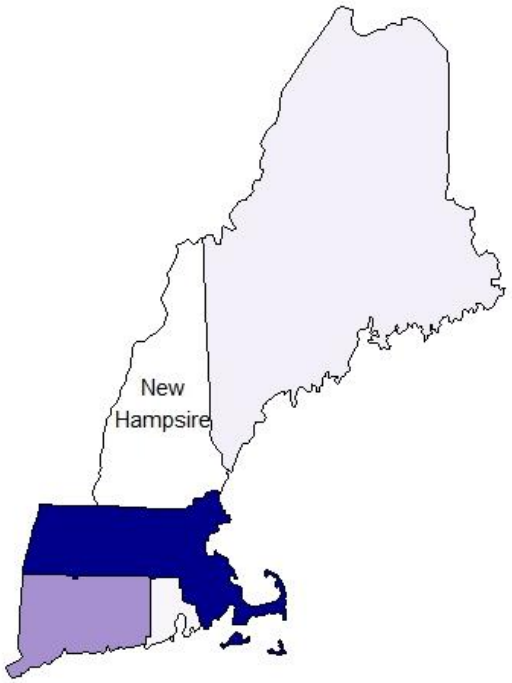
Exposed oysters

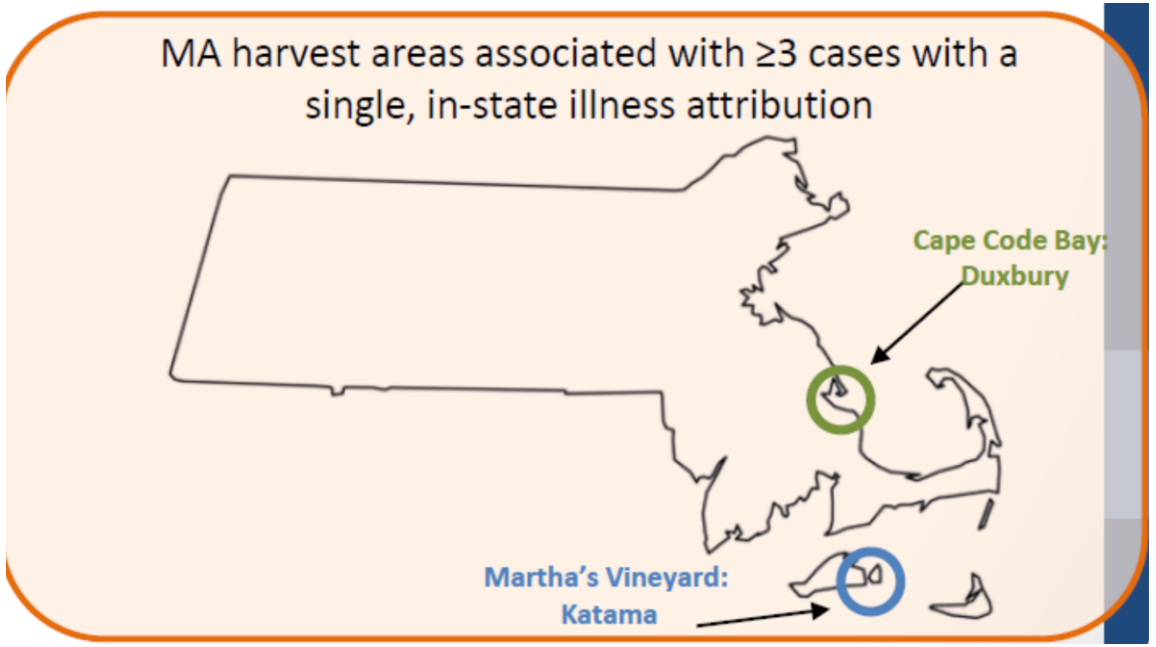
---Water level---

Control oysters (Spatially separated from exposed oysters)

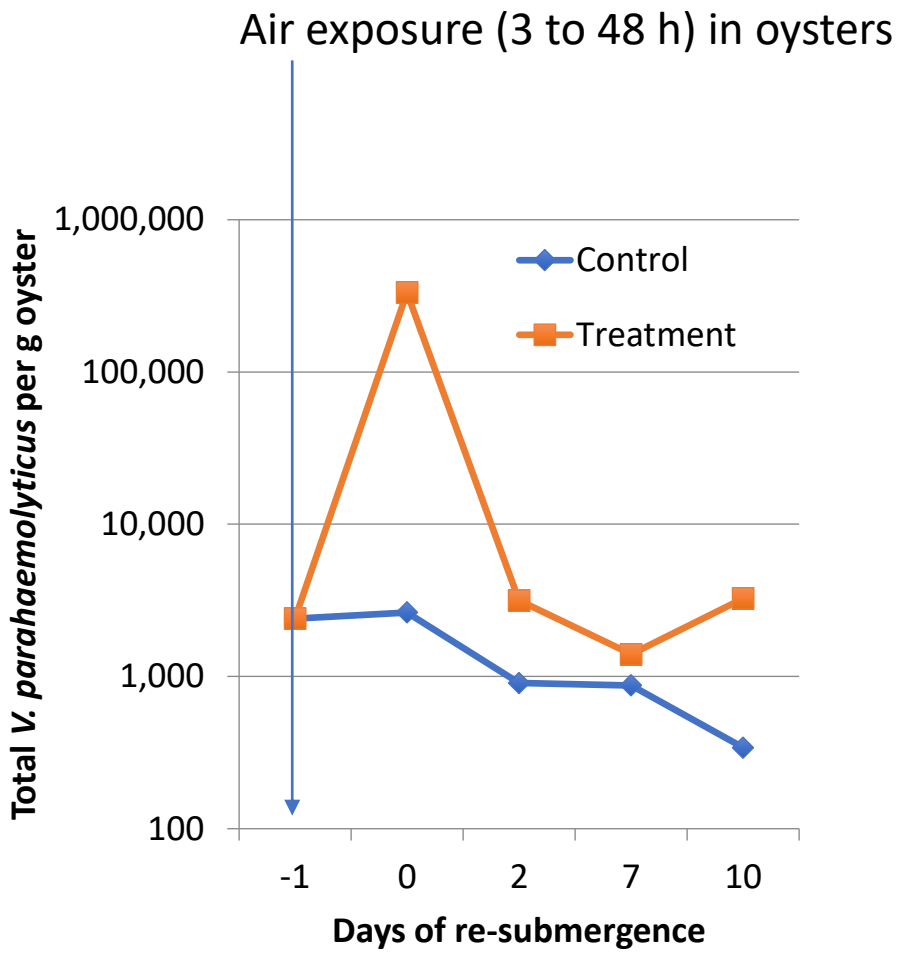
STUDY VARIABLES

Matrix of treatments:	length of exposure & recovery, gear, cultivation method, tidal exposure
Vp analysis endpoints:	total, hemolysins (*trh*, *tdh*) *PLUS* virulence markers linked to highly virulent strains
Regional study sites:	NH-Great Bay, ME- Spinney Creek, & MA-several sites (see below)
Environment conditions:	water & air temperatures, salinity (rainfall & drought considerations), dissolved oxygen, turbidity





Re-submergence effects on *total *Vp* concentrations in pre-harvest oysters



*Potential pathogenic strains only detected mostly in MA oysters

Summary Results for 4-Years of Re-submergence Field Trials (20) in Massachusetts, New Hampshire & Maine

Location			Abuse effect*	Recovery time†	
Trial conditions	Year	Trial	Log10 Vp conc inc	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	

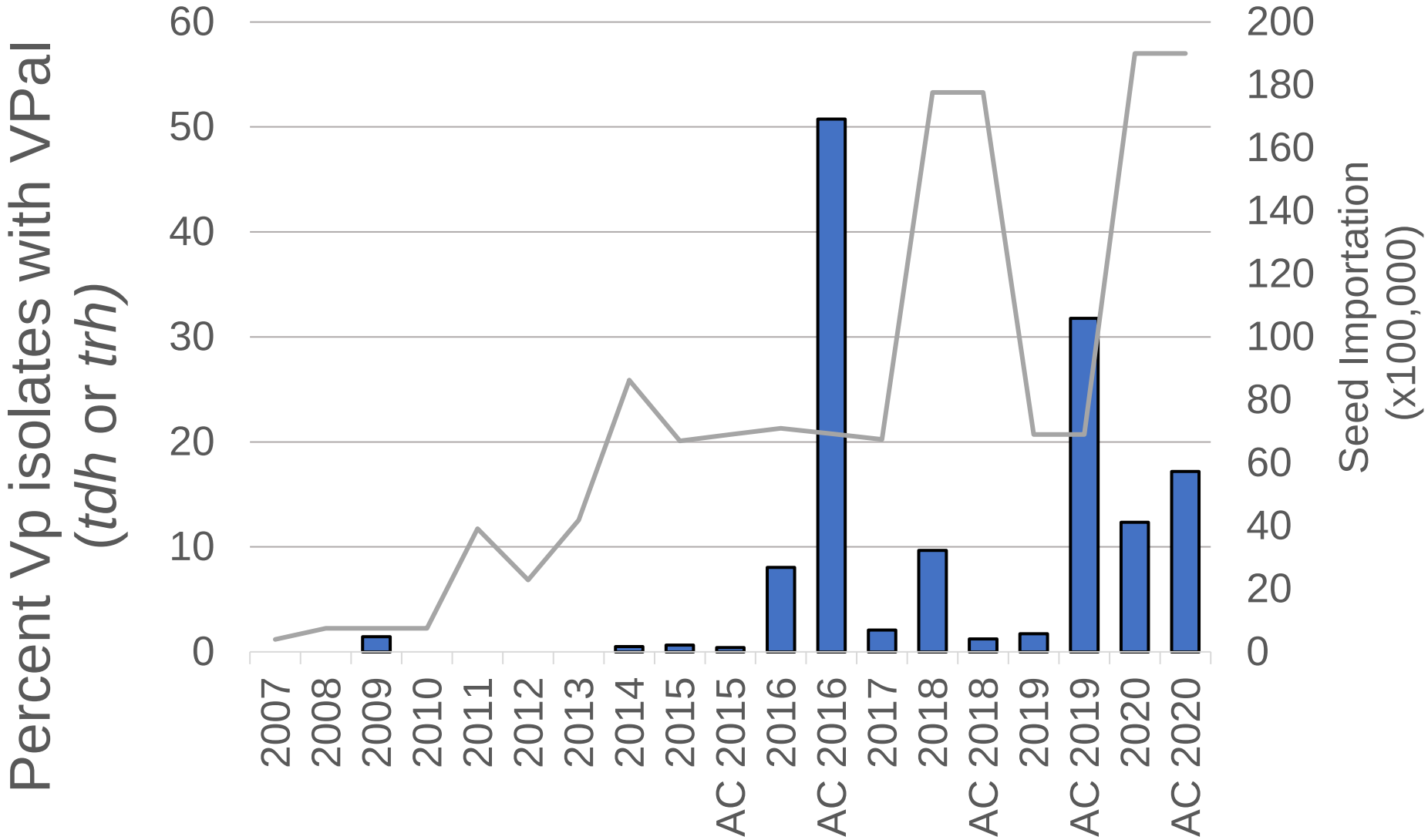
*the increase in Vp concentration in oysters following temperature abuse and before re-submergence

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

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.

The recovery times for *trh*, *tdh* & *tdh* 3/6 were ≤ total Vp except
2020 MA subtidal trial
6d for *trh* versus 4 d for total Vp

The prevalence of VPaI-harboring strains increased as aquaculture and seed importation increased

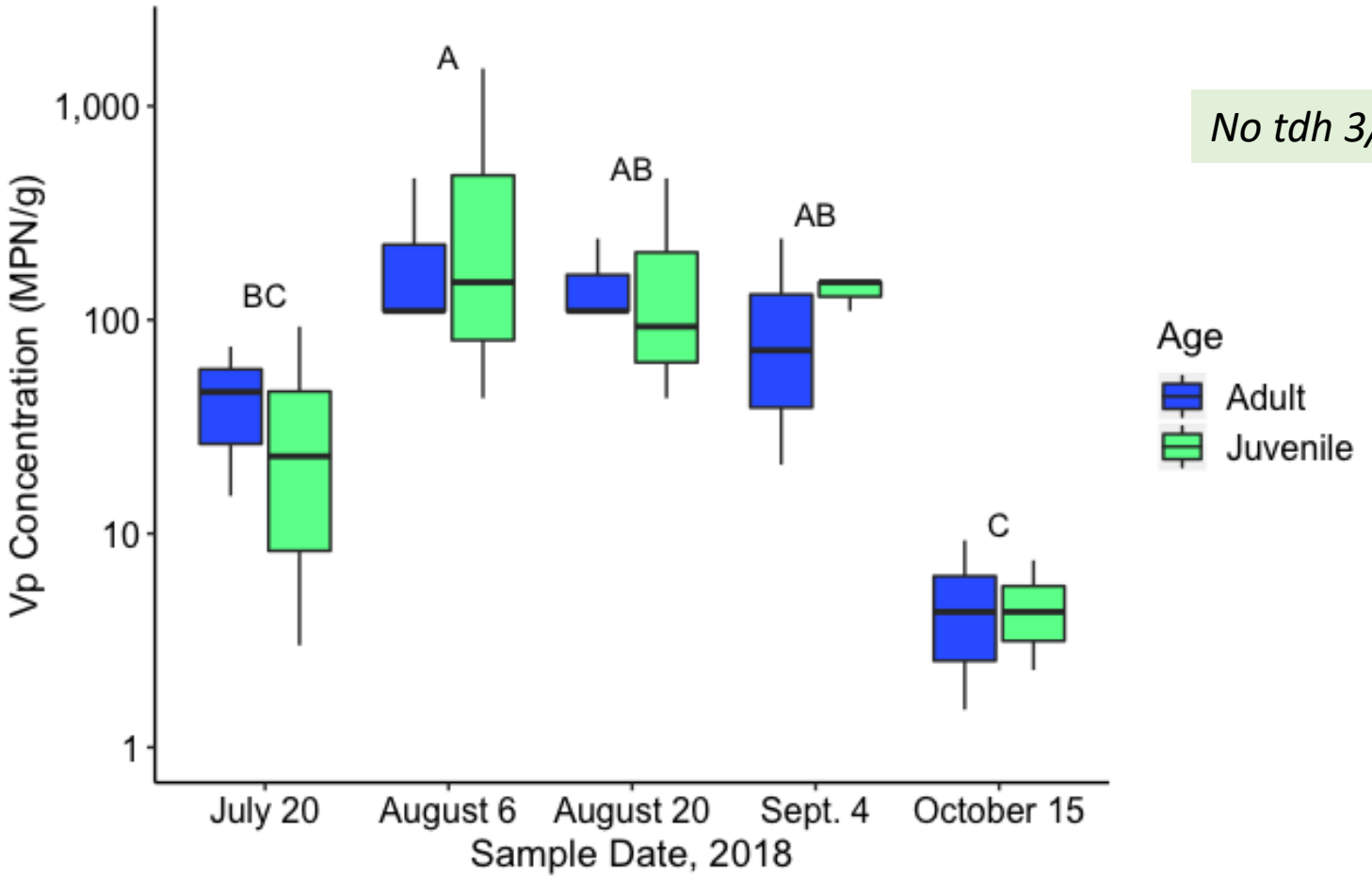


New Hampshire Shellfish Importation Public Health Policy

Fis 802.05 states NHFG will deny issuance of a wildlife/fish importation permit

"**if there is any significant disease, genetic, ecological, environmental, health, safety or welfare risks to the public** or other wildlife species."

"No import of these species will be from a location that has had illness traced to *Vibrio parahaemolyticus* sequence types (ST) 36 and/or clade II 631 or where a harvest closure(s) due to multiple *V. parahaemolyticus* related human illnesses has occurred. "



No tdh 3/6 strains were detected

V. parahaemolyticus concentrations in adult and juvenile oyster samples* on 5 sampling dates. Dates not connected by the same letter are significantly different.

**Oyster seed from hatcheries that are "allowed" exporters and routinely used by NH oyster farmers*

ONGOING & FUTURE Work

-Test juvenile seed oysters from areas where importation is prohibited (overcome resistance at source…)

-Testing pathogenic Vp strains (ST 36 & 631) in laboratory experiments to help define management-related risk reduction strategies

Acknowledgements and Support

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Thank you!
Questions?