

Increased *Vibrio*
parahaemolyticus Shellfish-
Borne Disease Incidence
in New England:
Climate-Related influences



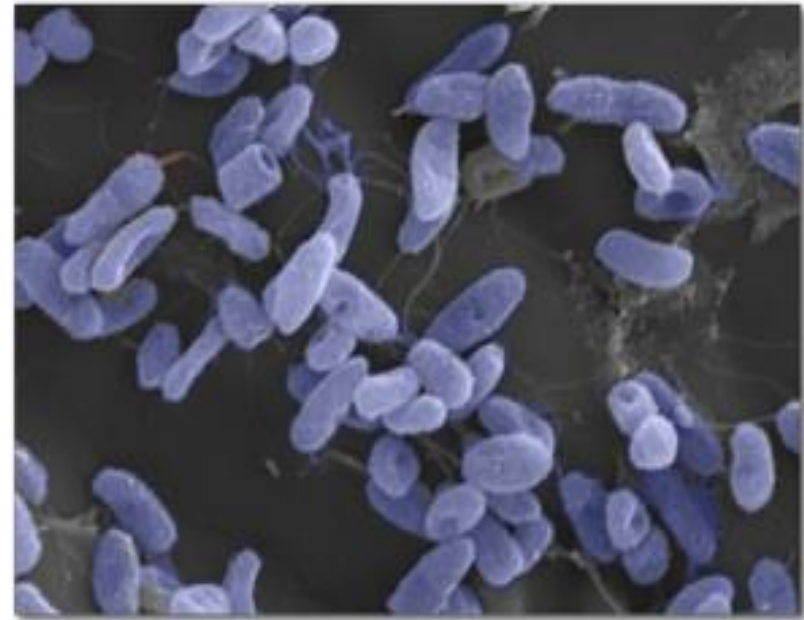
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Ashley Marcinkiewicz, Randi Foxall

**UNH Northeast Center
for *Vibrio* Disease & Ecology**

Vibrio parahaemolyticus

- Gram negative bacteria
- Halophilic- living in brackish salt water
- Warm waters
- Pili for attachment
- Potential 8-9 minute 'doubling time' (60-70 times increase per hour)

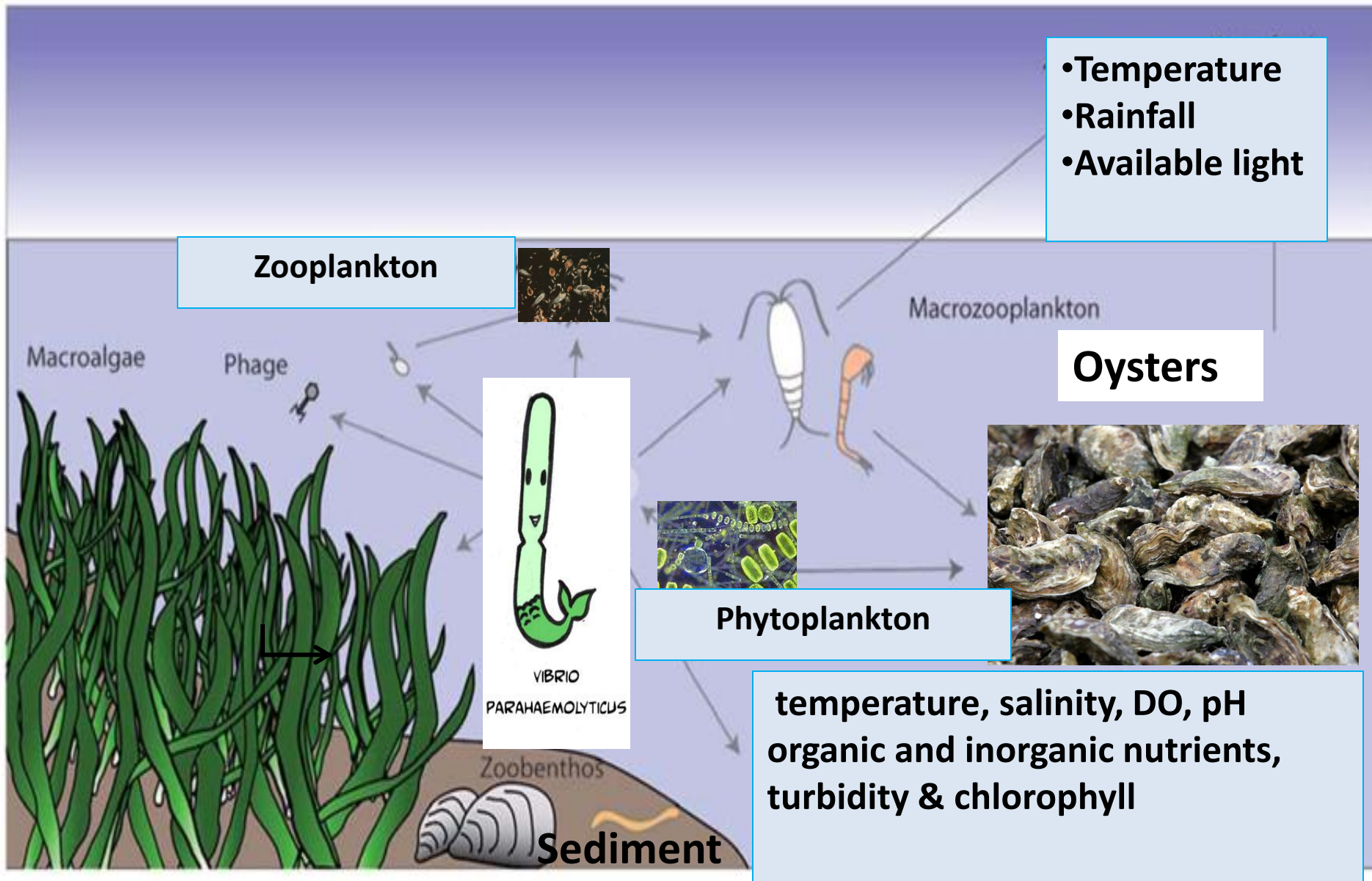


V. parahaemolyticus as a Human Pathogen

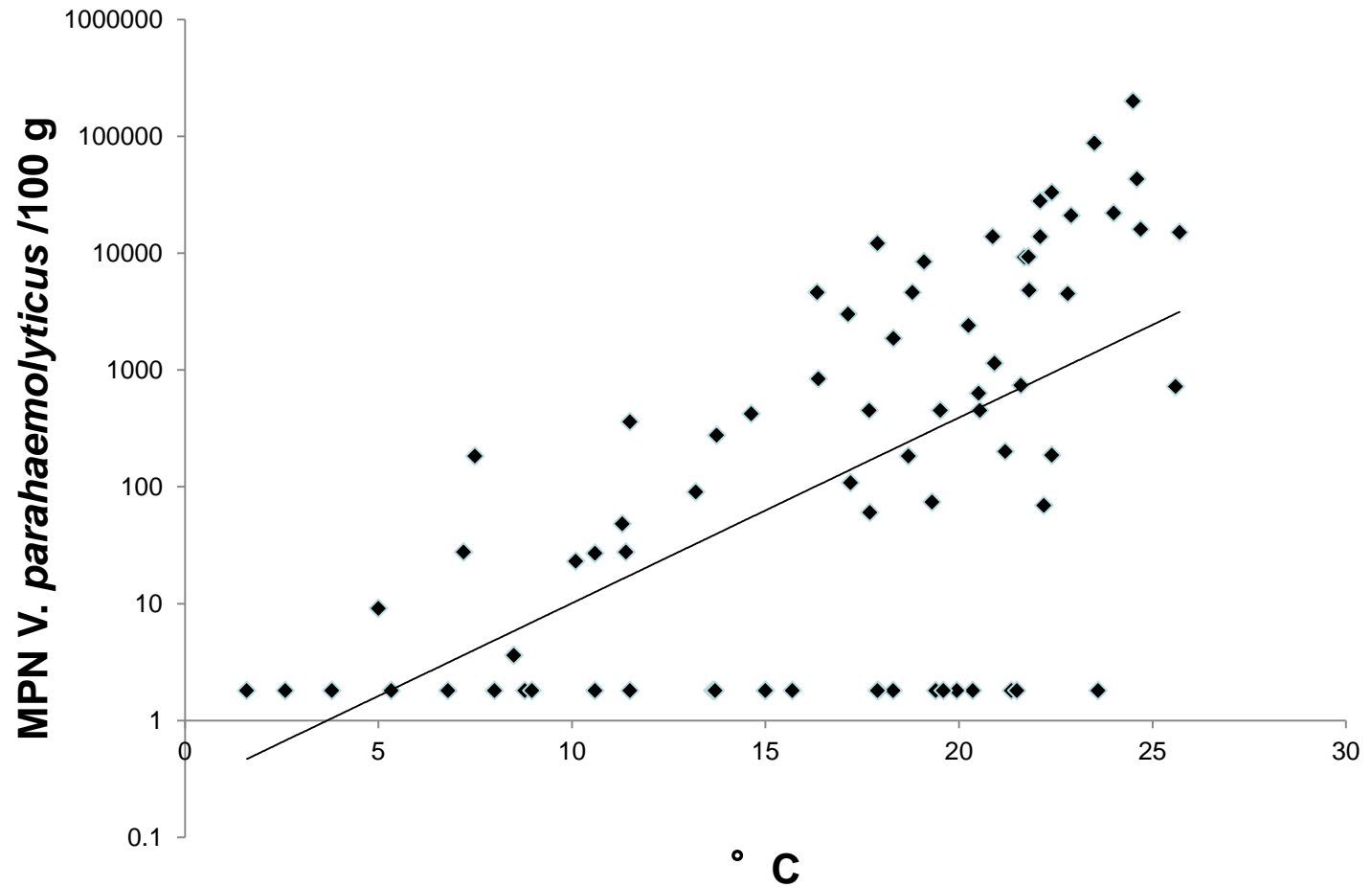
- A small minority of strains are human pathogens
- Can cause inflammatory gastroenteritis & septicemia
- Typical exposure via seafood consumption



Vibrio parahaemolyticus, the ecosystem picture



Water temperature & *V. parahaemolyticus* (Vp) concentrations in OYSTERS at Nannie Island: 2007-13

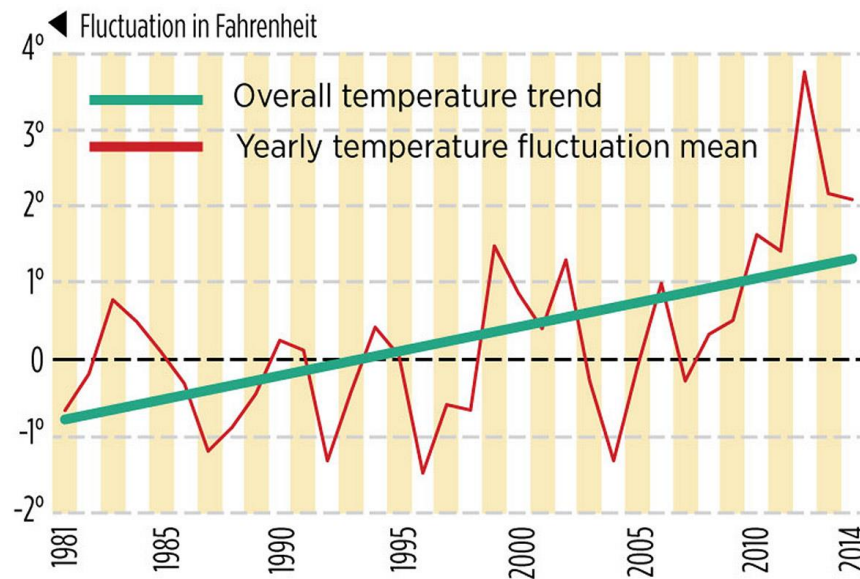


“Big changes occurring in one of the fastest warming spots on earth”

Colin Woodward, Portland Press Herald, Oct. 25, 2015

Getting warmer

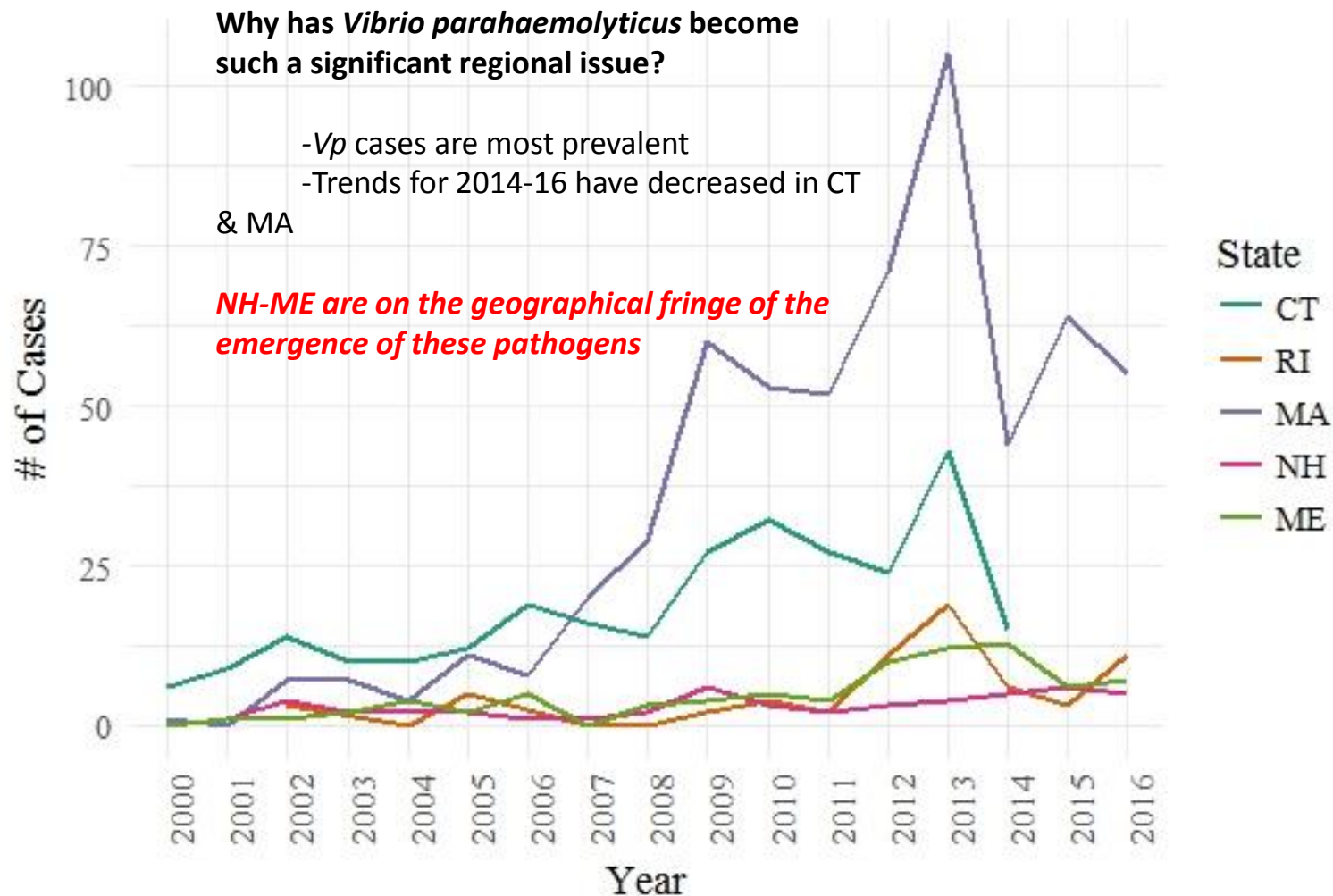
Sea surface temperatures in the Gulf of Maine have been rising over the past 35 years, and at nearly the fastest rate on the planet over the last 10. 2012 had the warmest readings in the 150 years humans have been collecting them.



SOURCE: Andrew J. Pershing/Gulf of Maine Research Institute

STAFF GRAPHIC | MICHAEL FISHER

Annual cases of vibriosis in humans in New England states

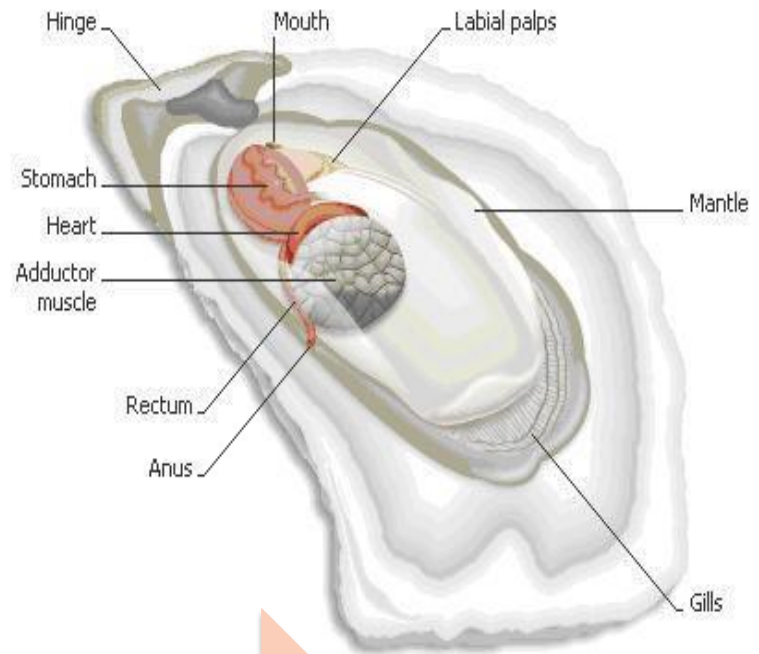


Annual cases of vibriosis in humans for Maine (ME), Massachusetts (MA), New Hampshire (NH), Rhode Island (RI) and Connecticut (CT) for 2000 through 2016. Species include *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, *V. alginolyticus*, *V. fluvialis*, and 'unknown'.

Data from CDC, MA DPH, NH DHHS, ME CDC, RI DH.

Oyster Uptake & Purging

- *Crassostrea virginica*
- Up to 50 gallons per day
- Filter Feed via gills

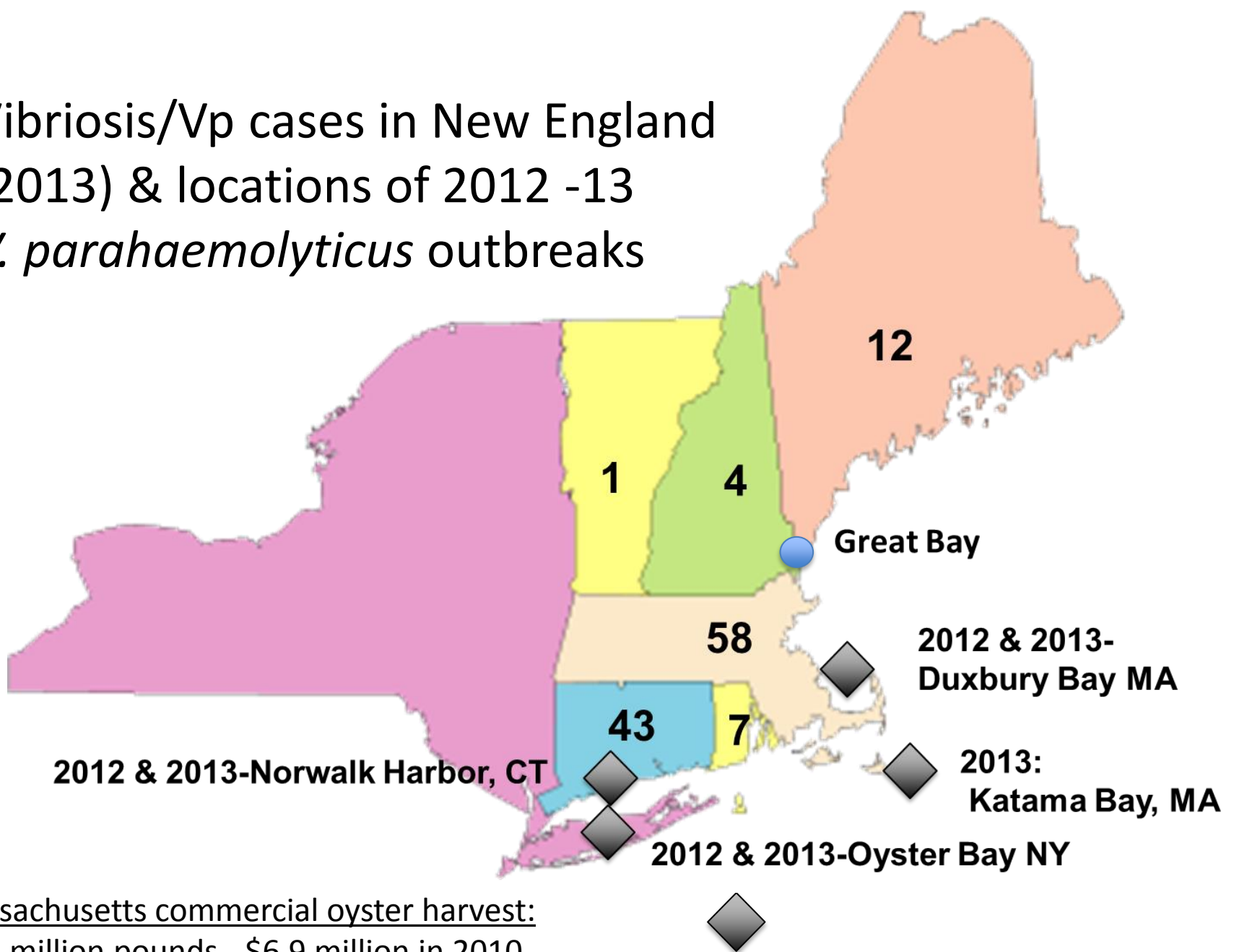


Vibrio spp. in
water / on
particles

Oyster uptakes
water / particles

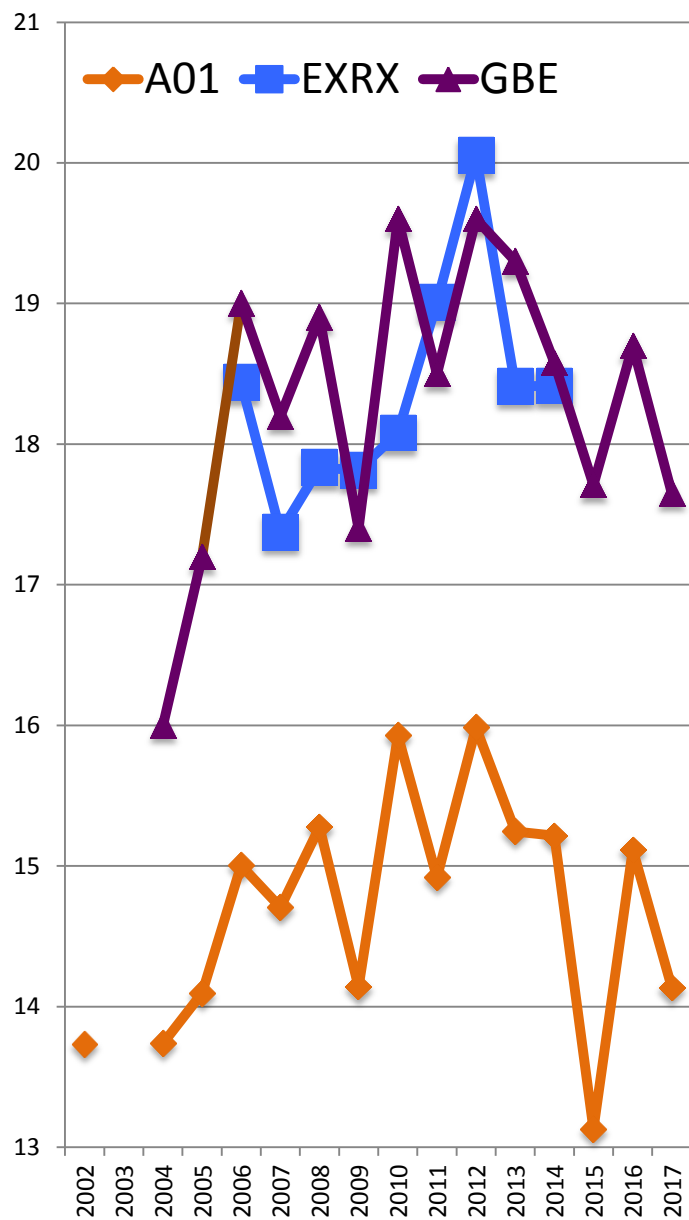
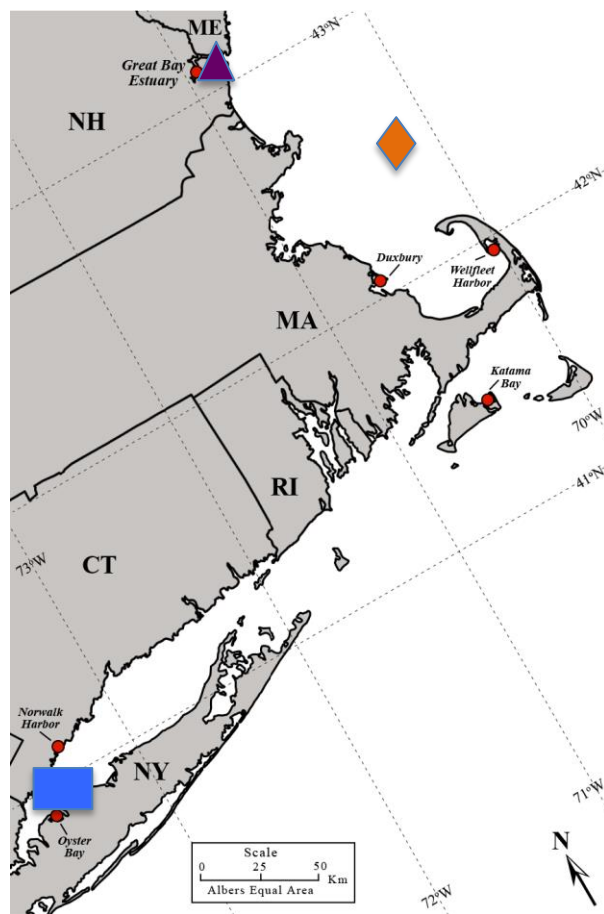
Vibrio spp.
accumulate or are
purged

Vibriosis/Vp cases in New England (2013) & locations of 2012 -13 *V. parahaemolyticus* outbreaks

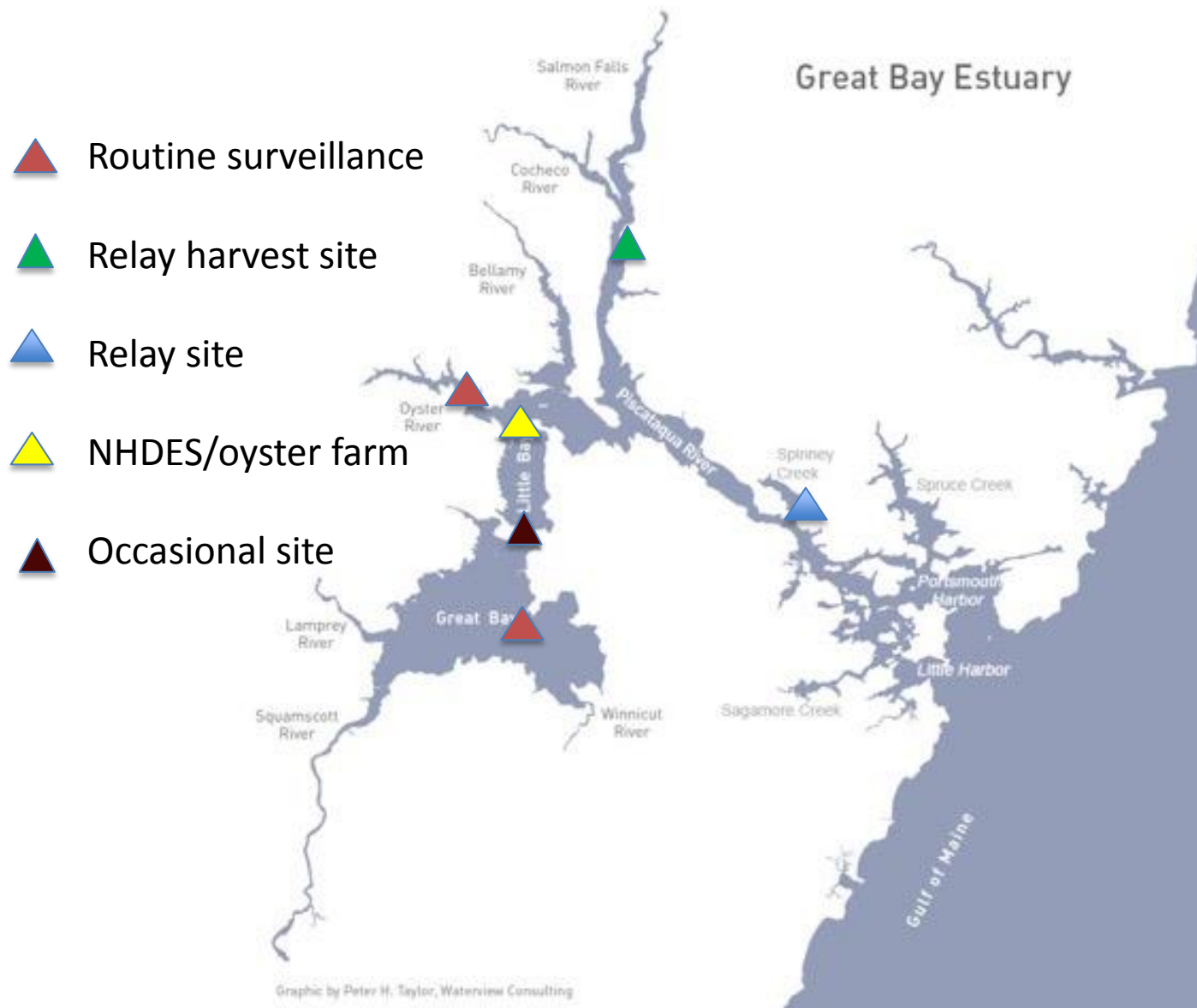


Massachusetts commercial oyster harvest:
-2.9 million pounds - \$6.9 million in 2010
-4.1 million pounds - \$11.6 million 2012

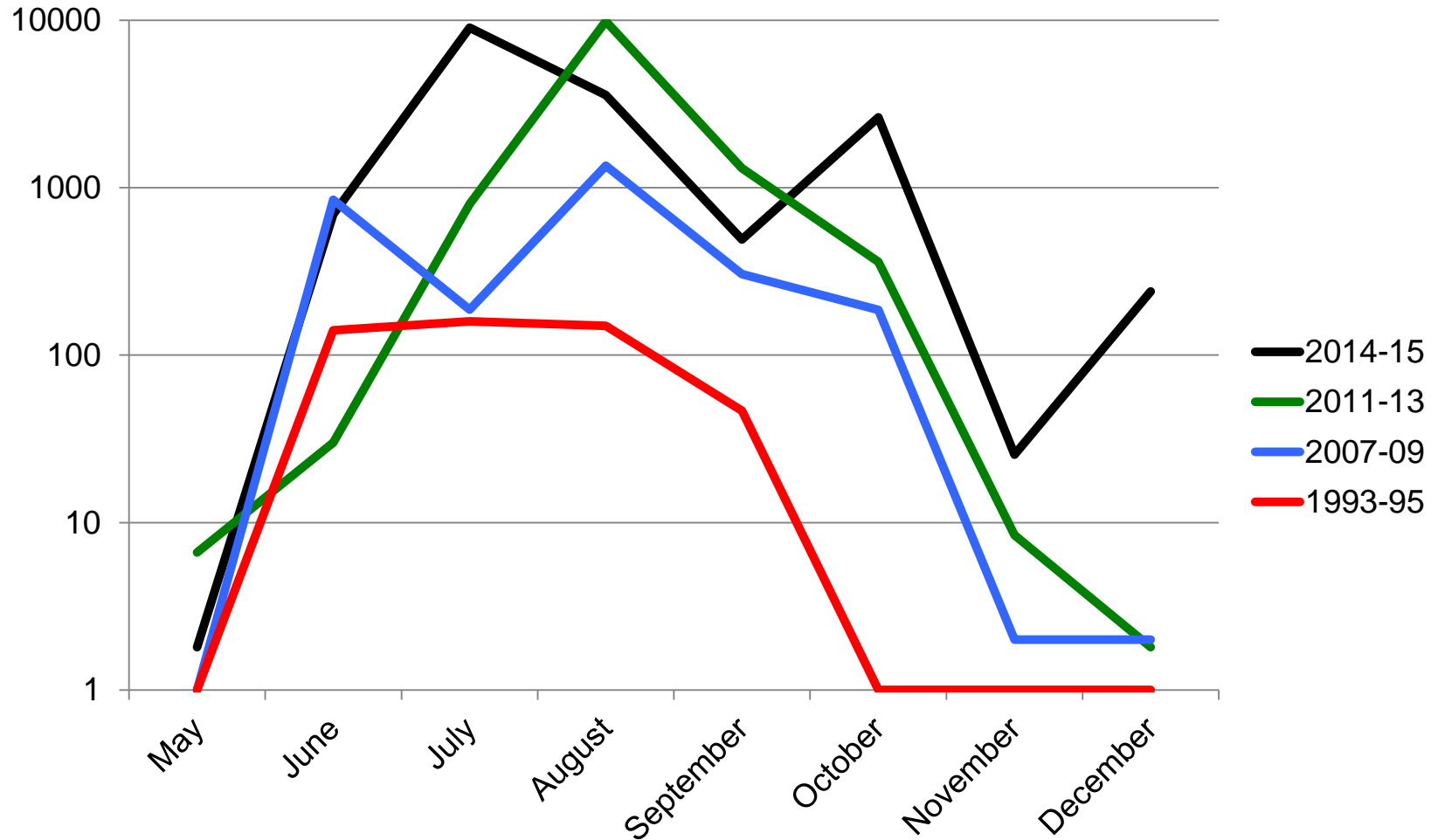
Sea surface temperature at NERACOOS buoys in Long Island Sound, Gulf of Maine and Great Bay Estuary in June: 2007-2017



Vp Sampling Sites in the GBE: 2007-17

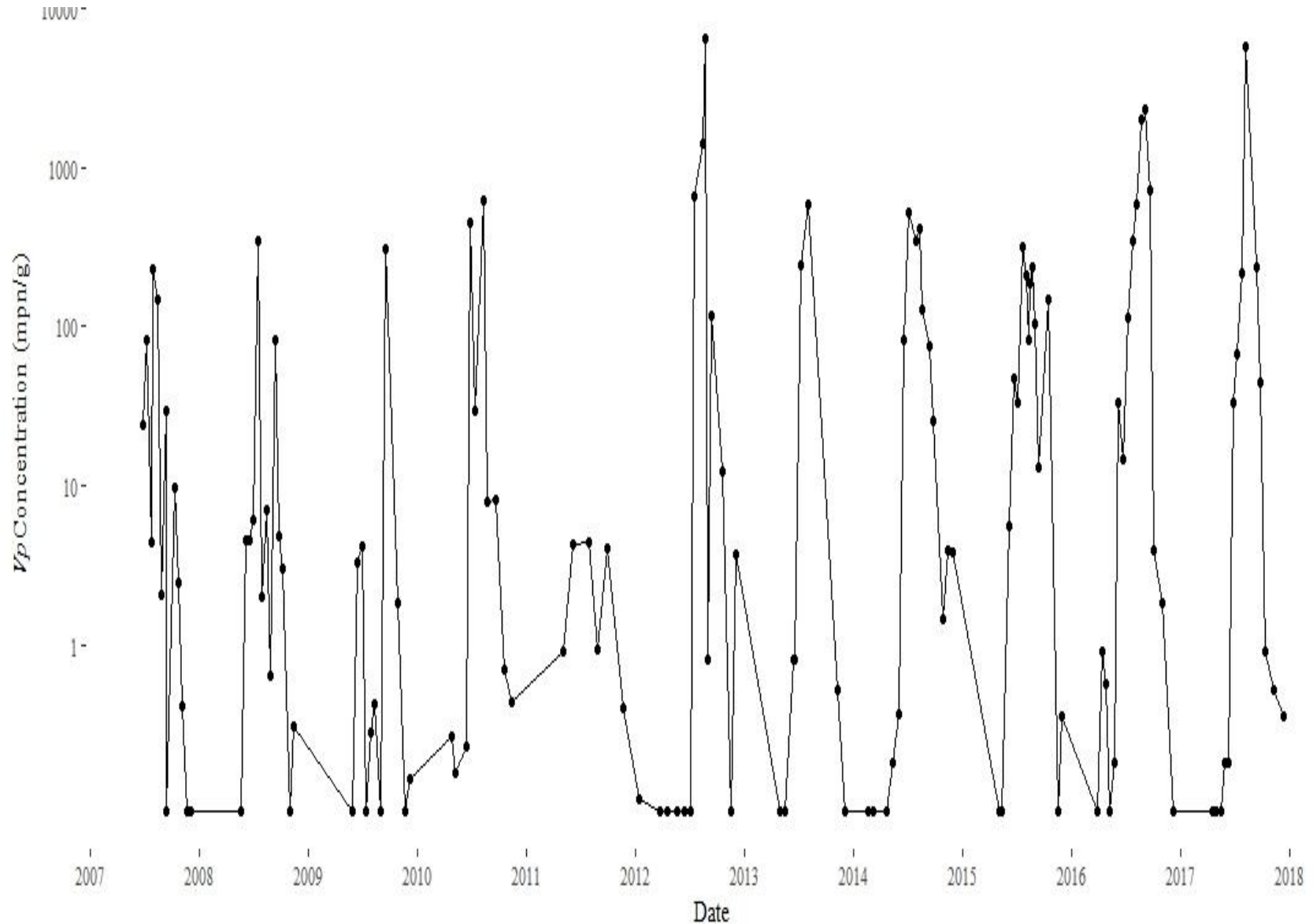


Average monthly Vp levels (Vp/100 g) in Nannie Island (NH) oysters: 1993 to 2015



Vp concentrations in oysters in the Great Bay : 2007-2017

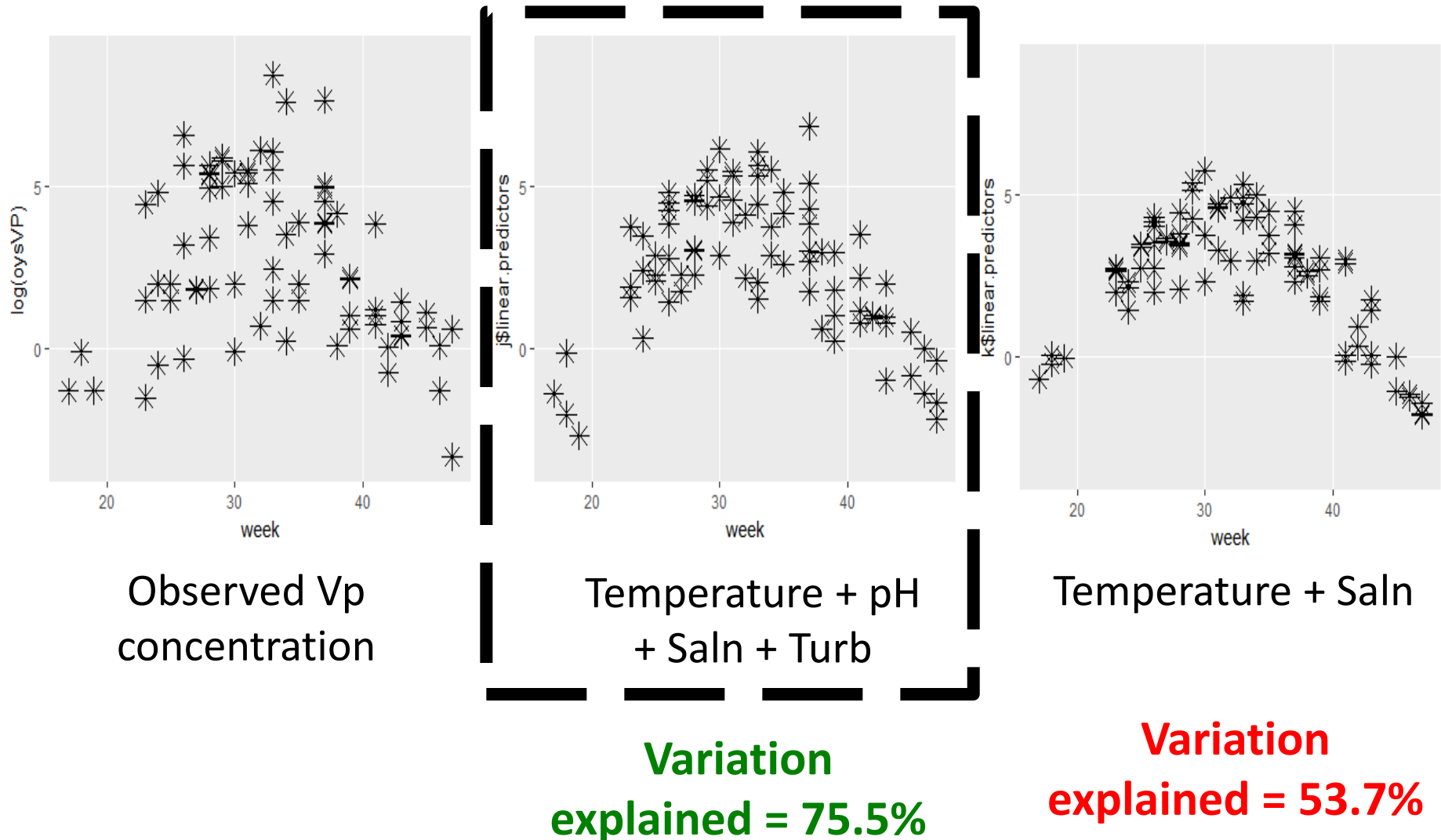
(missing August and September 2012 data)



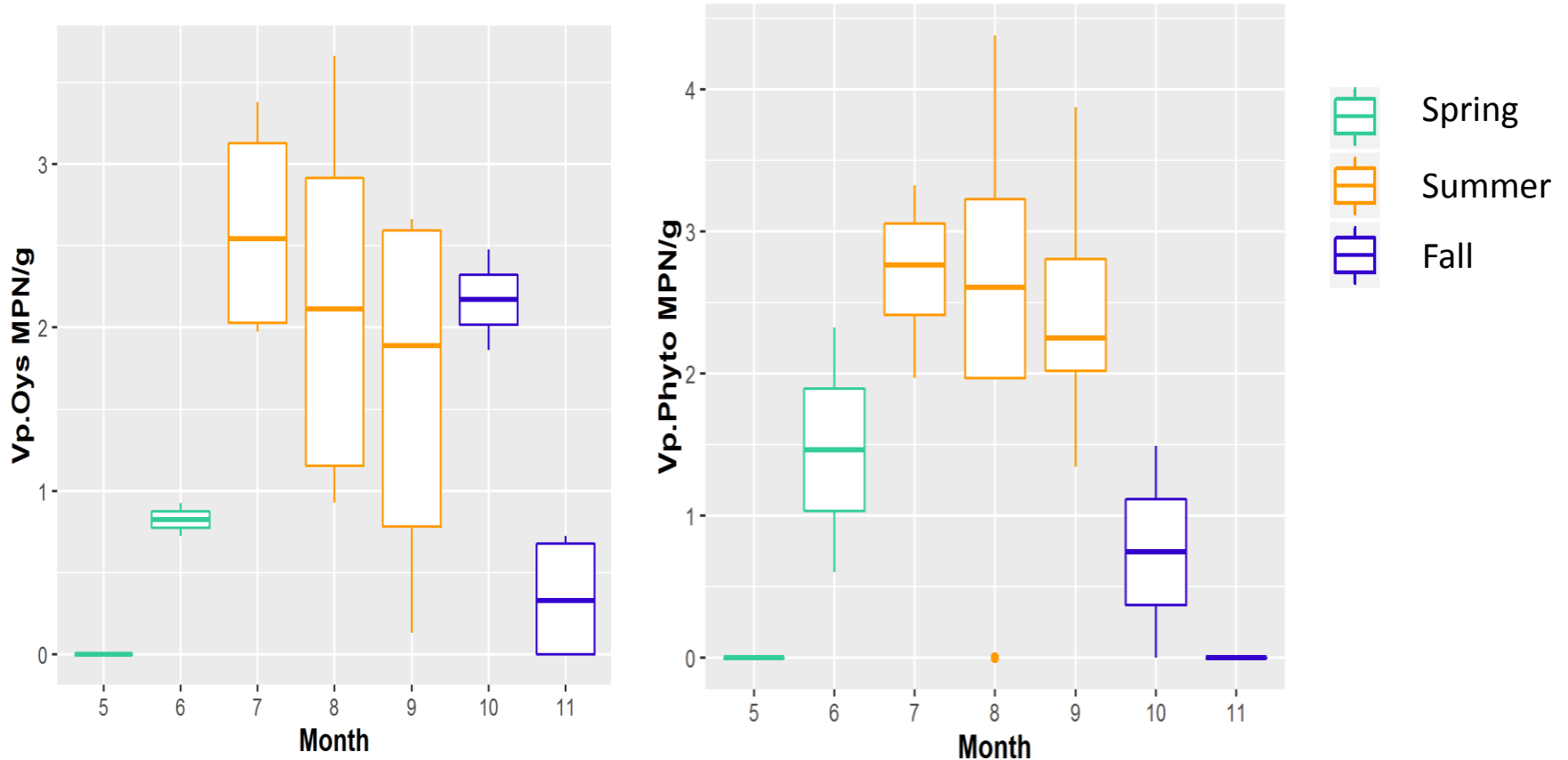
Why did Vp concentrations
not decrease
over the last three years?

Temperature doesn't tell the whole story for Vp concentration variation

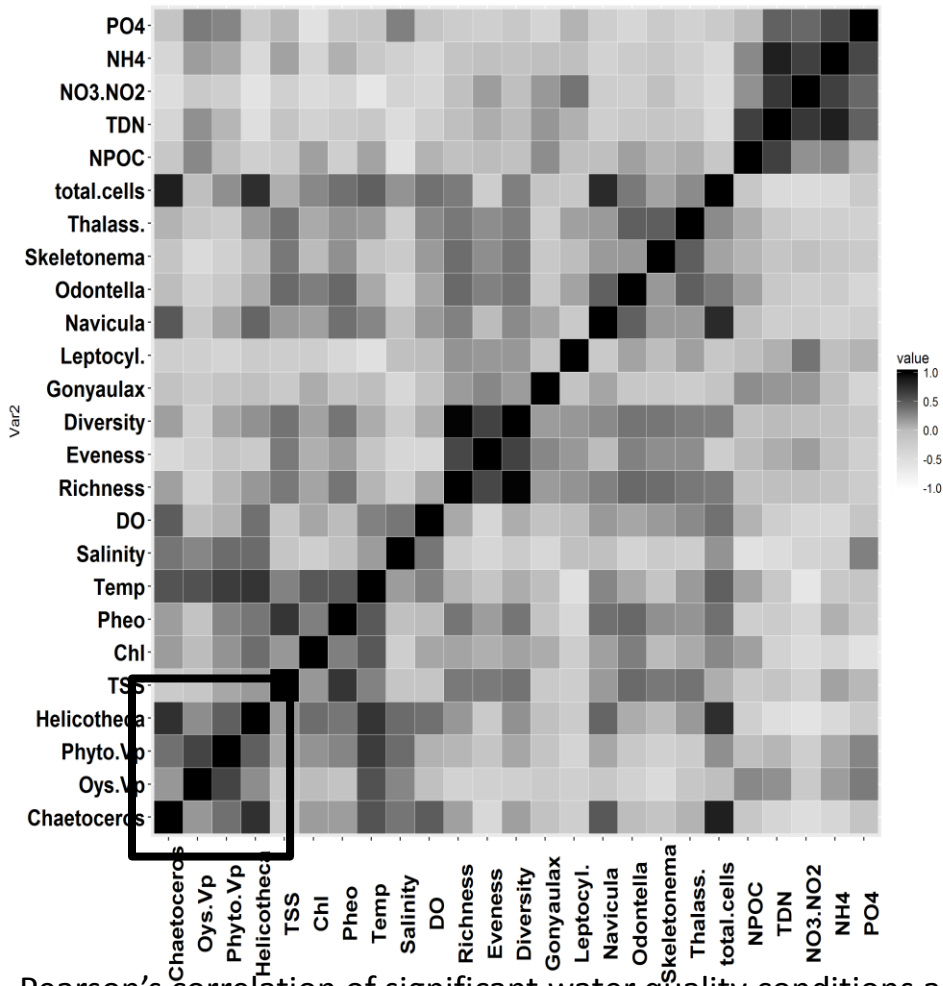
Multiple Regression Estimation Models for Seasonal Variation



Seasonal *V. parahaemolyticus* Concentrations in Oysters and Phytoplankton



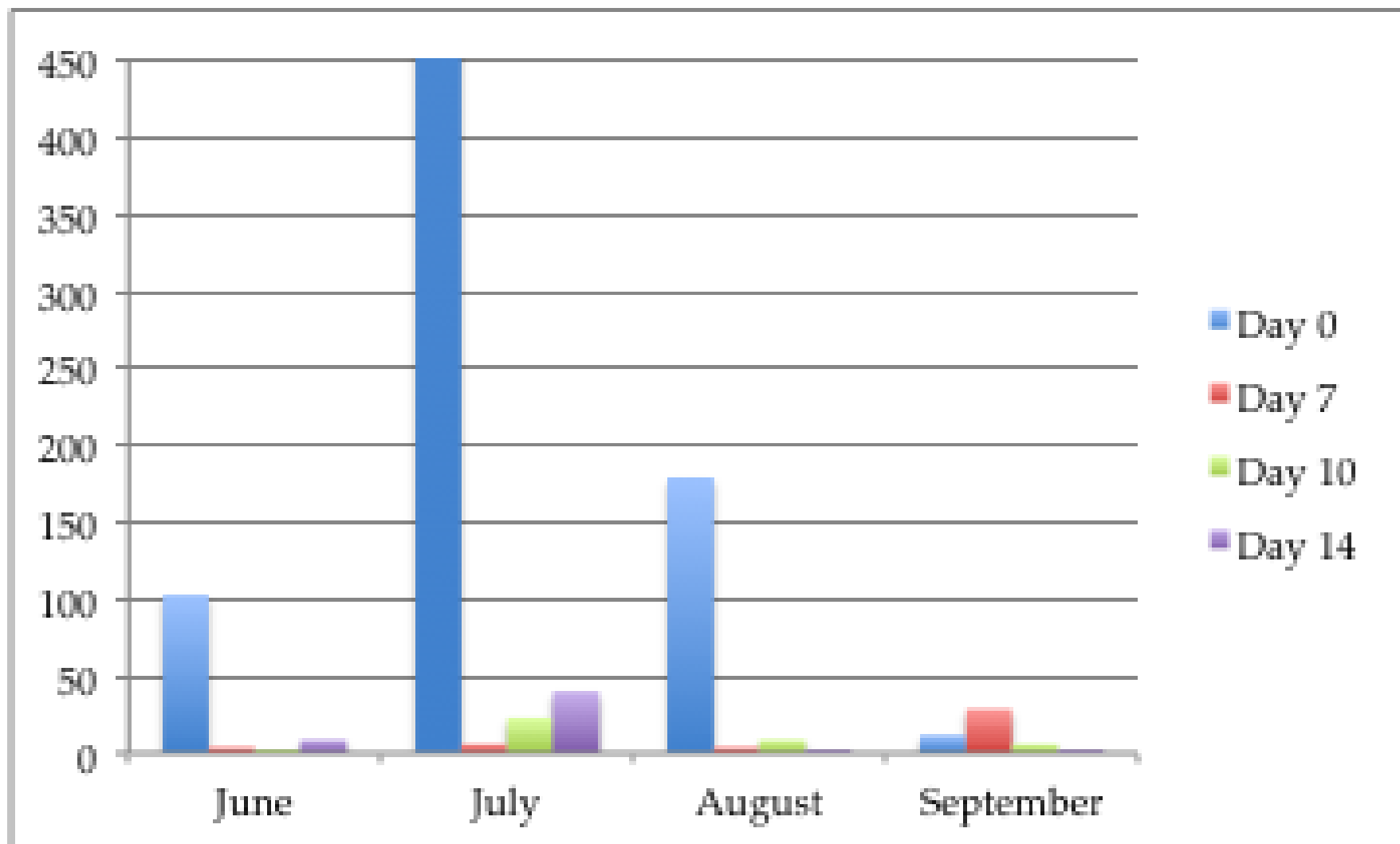
Nutrients, Chlorophyll, Phytoplankton and *V. parahaemolyticus* Dynamics



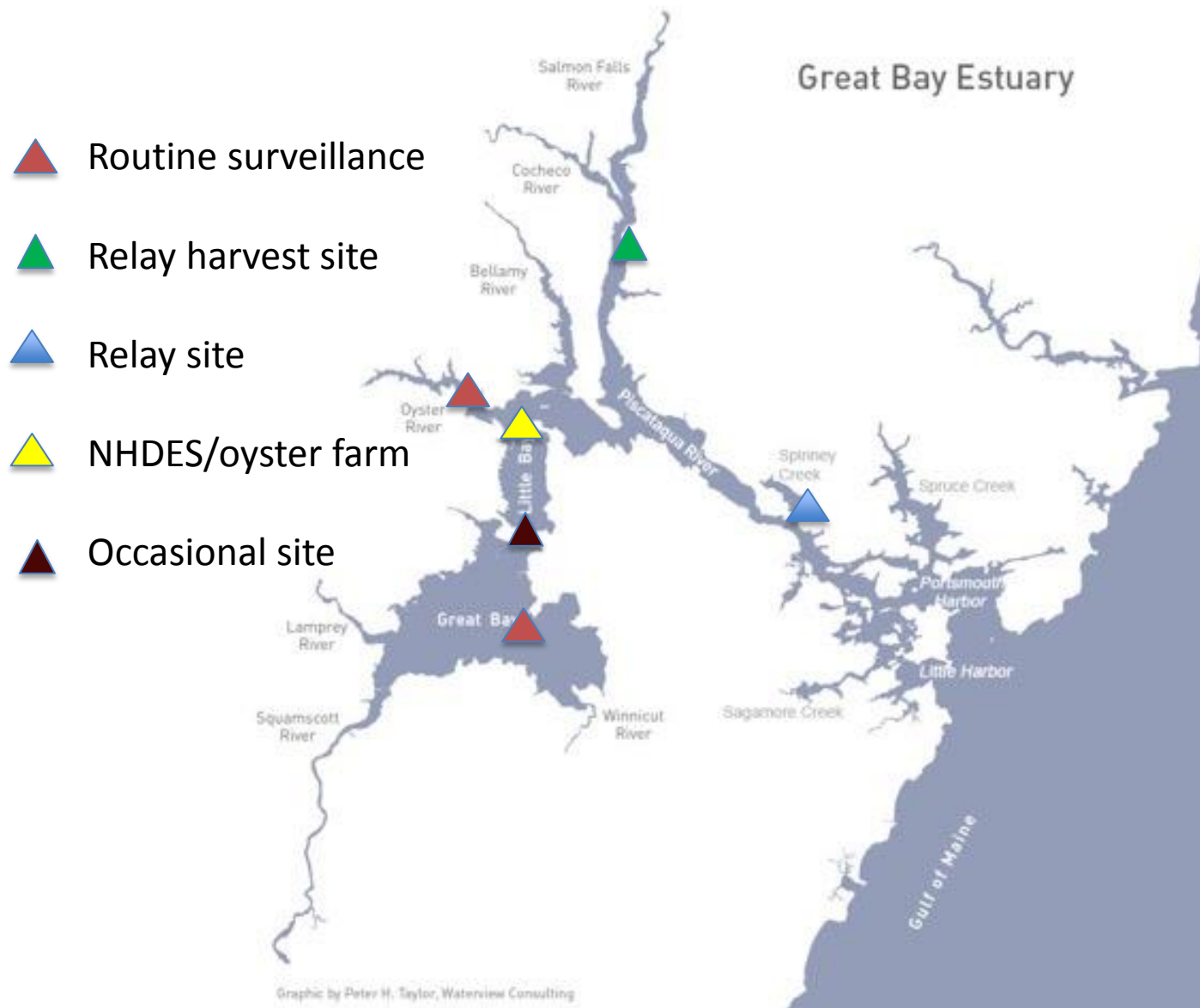
V. parahaemolyticus
is strongly associated
with blooms of the
phytoplankton diatom
Chaetoceros

Pearson's correlation of significant water quality conditions and phytoplankton communities. *Chaetoceros* sp. and *Helicotheca* sp. blooms co- occur and often coincide with increases in *V. parahaemolyticus* associated with phytoplankton.

Reduction in Vp concentrations following “Relay”: 2012



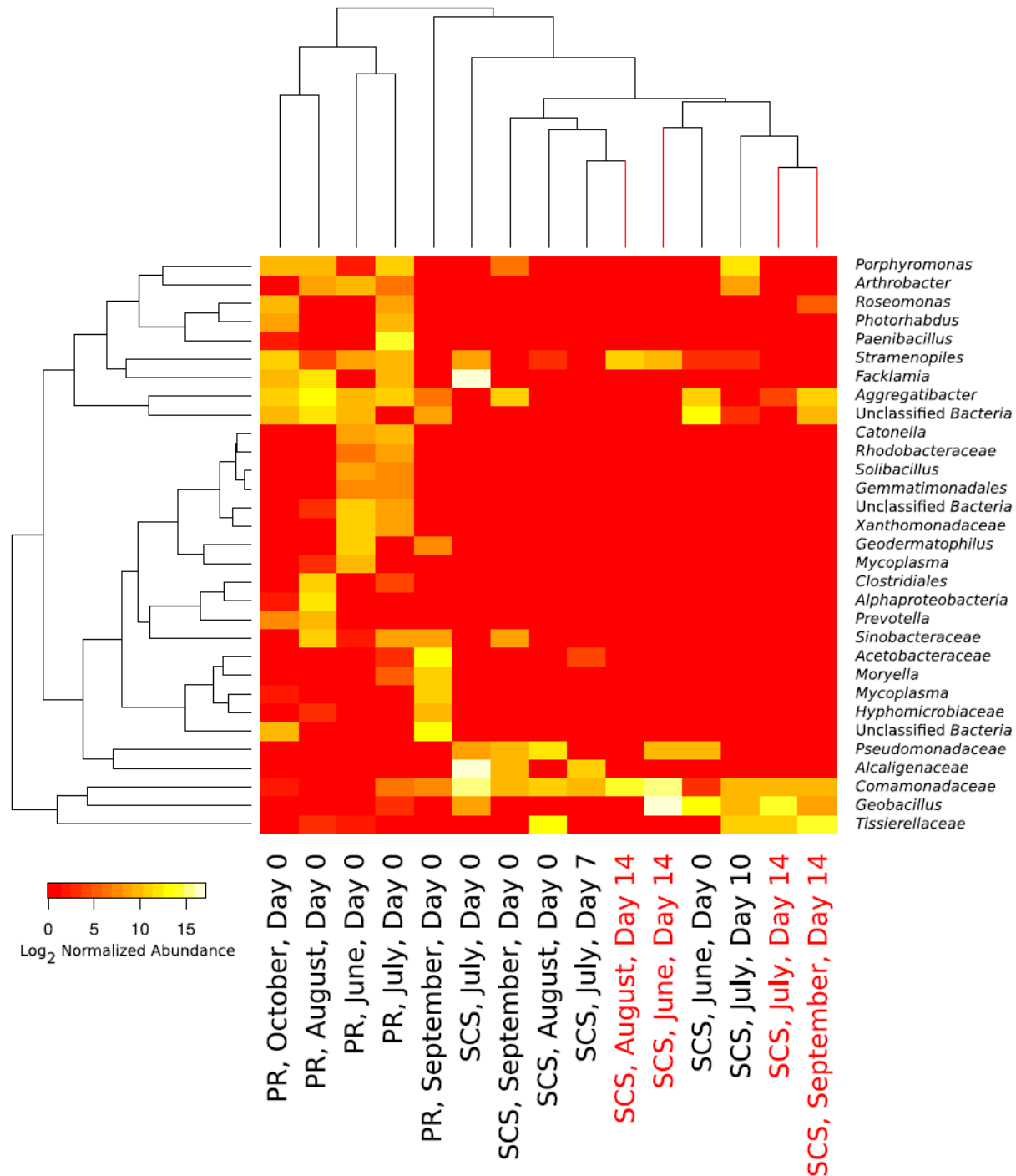
Vp Sampling Sites in the GBE: 2007-17



The relative abundance of significant taxa in water and their relatedness: 2011-12

Water microbiome community profiles are different between the two sites.

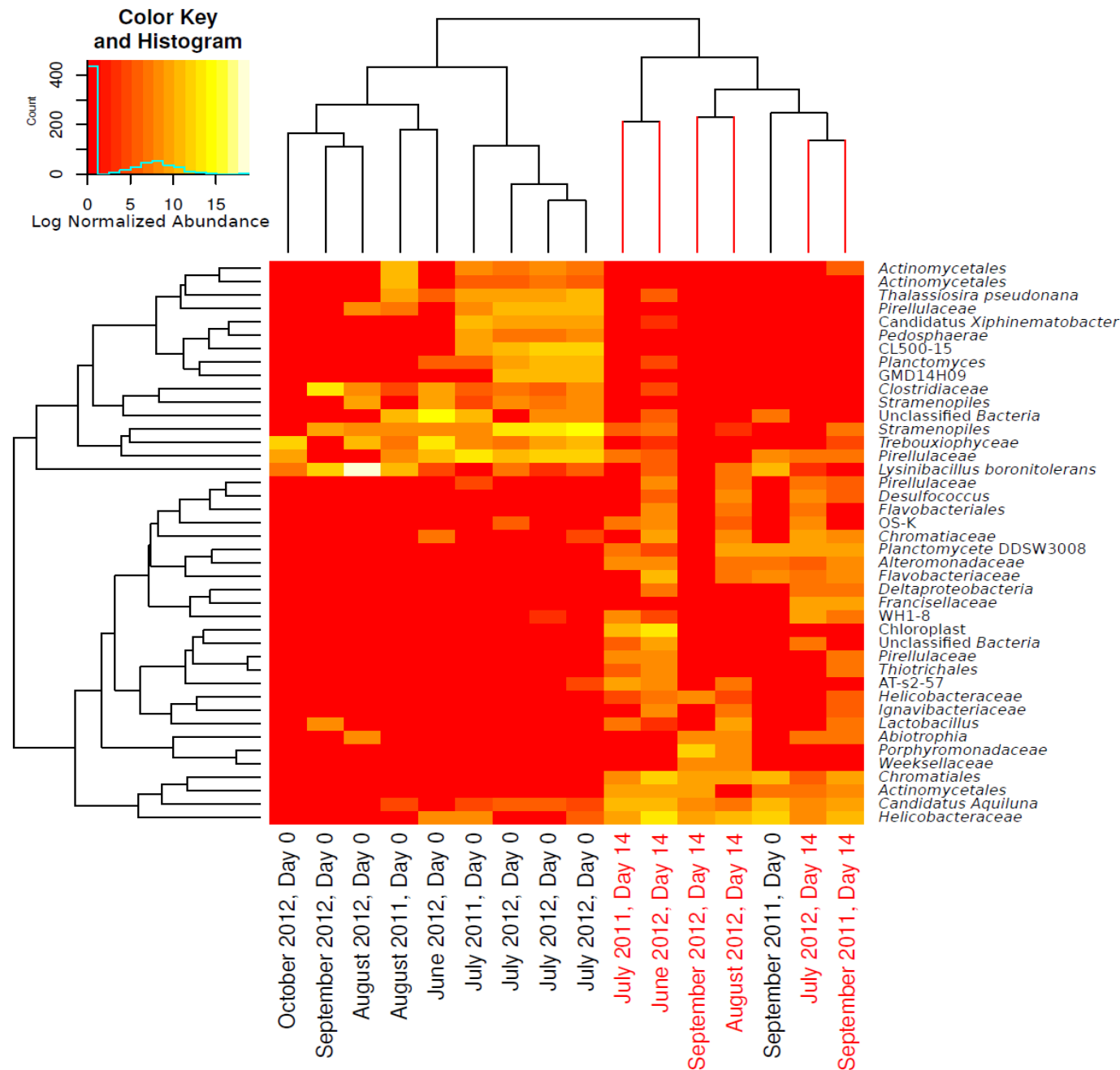
This is a probable mechanism for why Vp levels change when oysters are relayed.



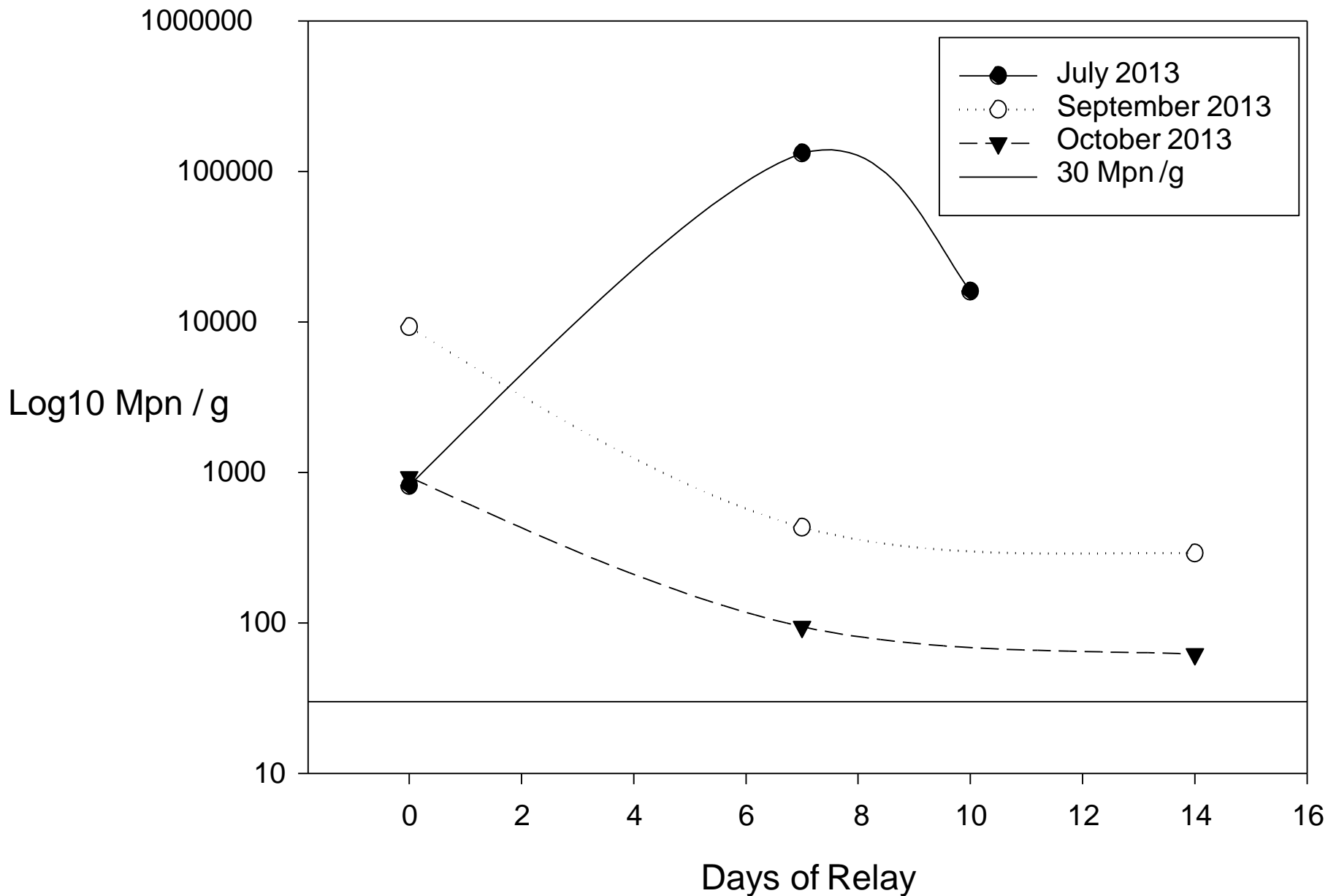
The relative abundance of significant taxa in oysters and their relatedness: 2011-12

Oyster microbiome community profiles change when oysters are exposed to different environmental conditions.

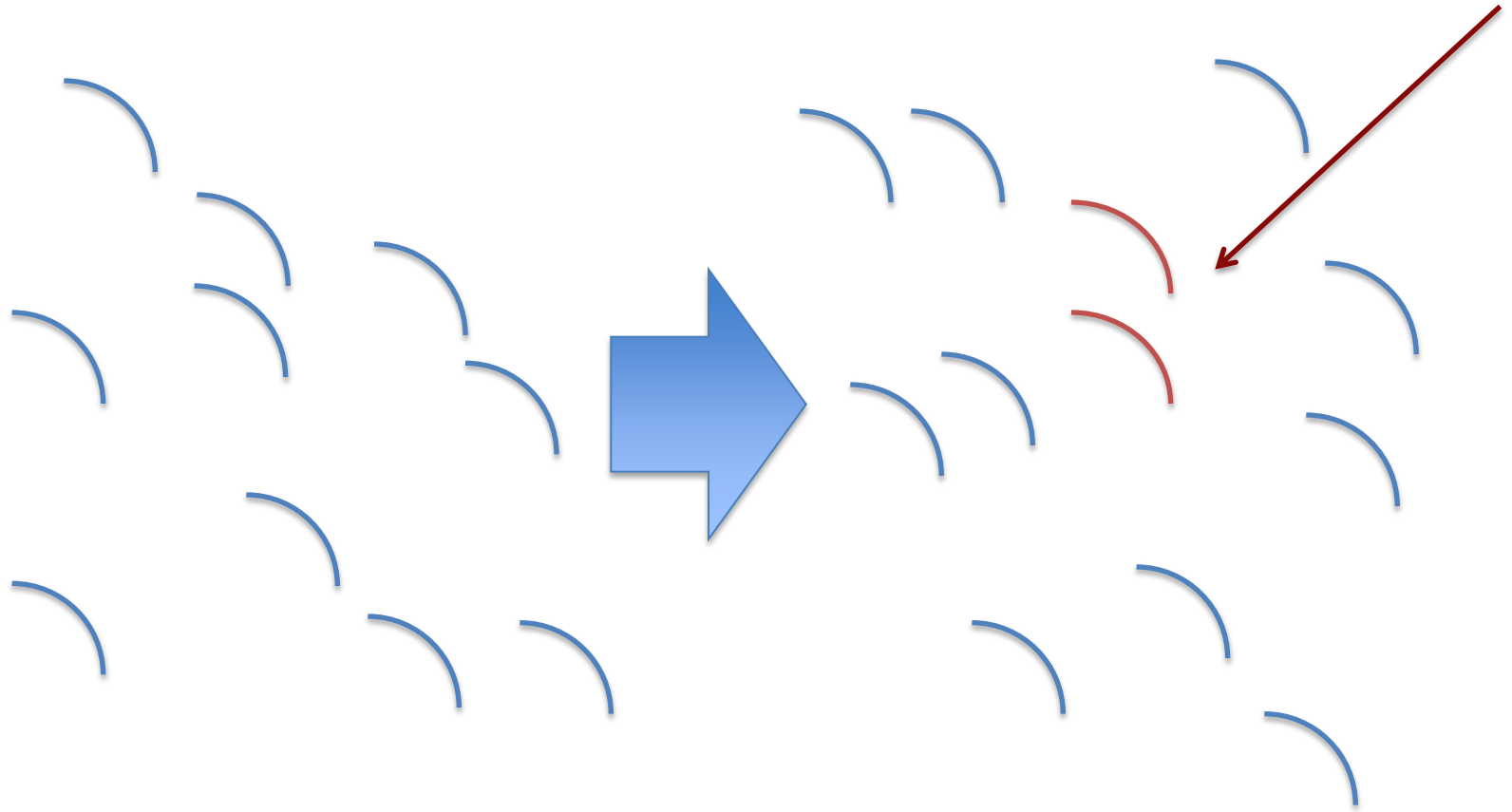
This may help to explain why Vp levels declined in relayed oysters.



Relay 2013

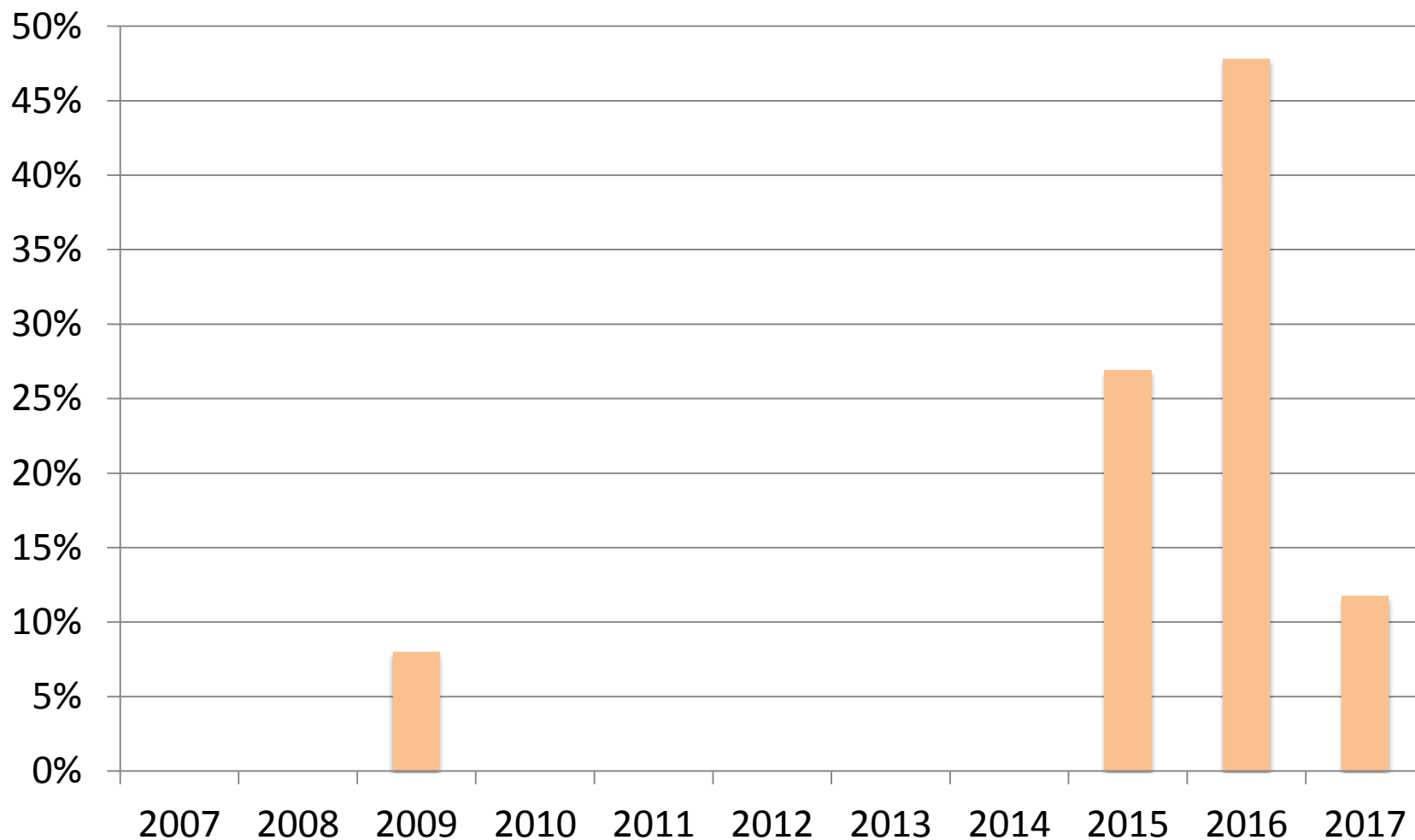


Vp strains in regional populations: Emergence of pathogenic strain indicator markers (*tdh*, *trh*)

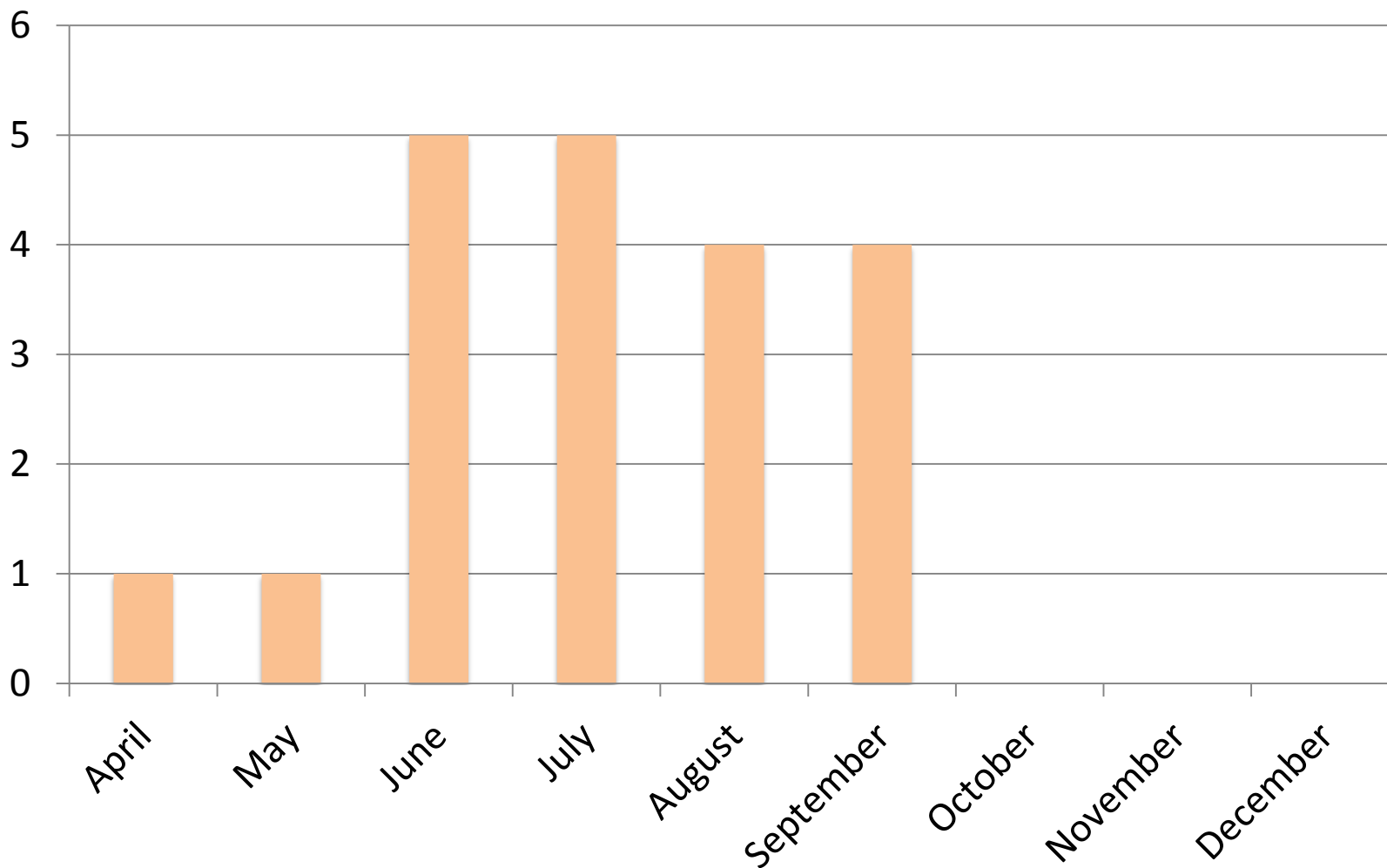


Do the two population-types track one another,
or do they respond to environmental conditions differently?

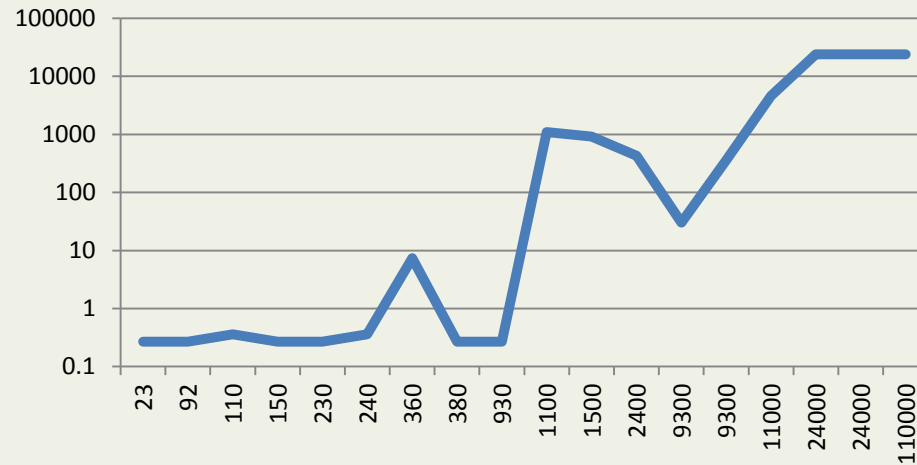
% Marker Detection in Oyster Samples with Detectable Vp from the 2 Routine Surveillance Sites: 2007-17



Monthly Incidence of Markers in Oyster Samples with Detectable Vp from the Routine Surveillance Sites: 2009, 2015-17

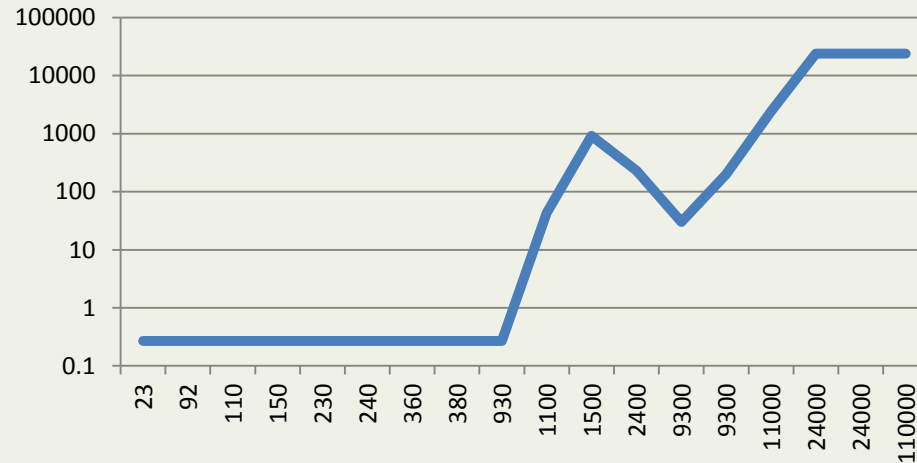


trh



tdh and *trh* markers were detected at levels that roughly track total Vp levels.

tdh



There is an approximate threshold of incidence corresponding to a total Vp level of ~900 MPN/g oyster tissue.

Why are we seeing a higher incidence of pathogenic type strains in the region?

Have they been here the whole time but conditions just didn't favor them?

Have new strains invaded?

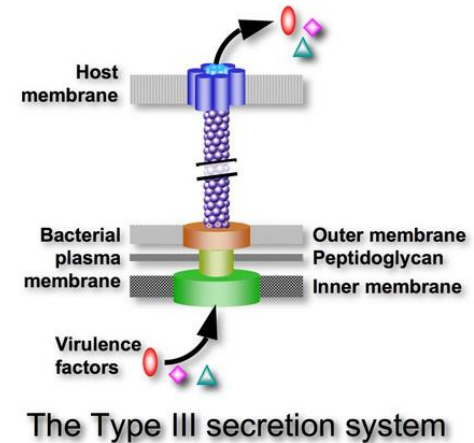
Have new pathogens evolved (how and why)?

Genomics comparisons can help us understand pathogen evolution and inform better surveillance



Tdh and Trh hemolysins

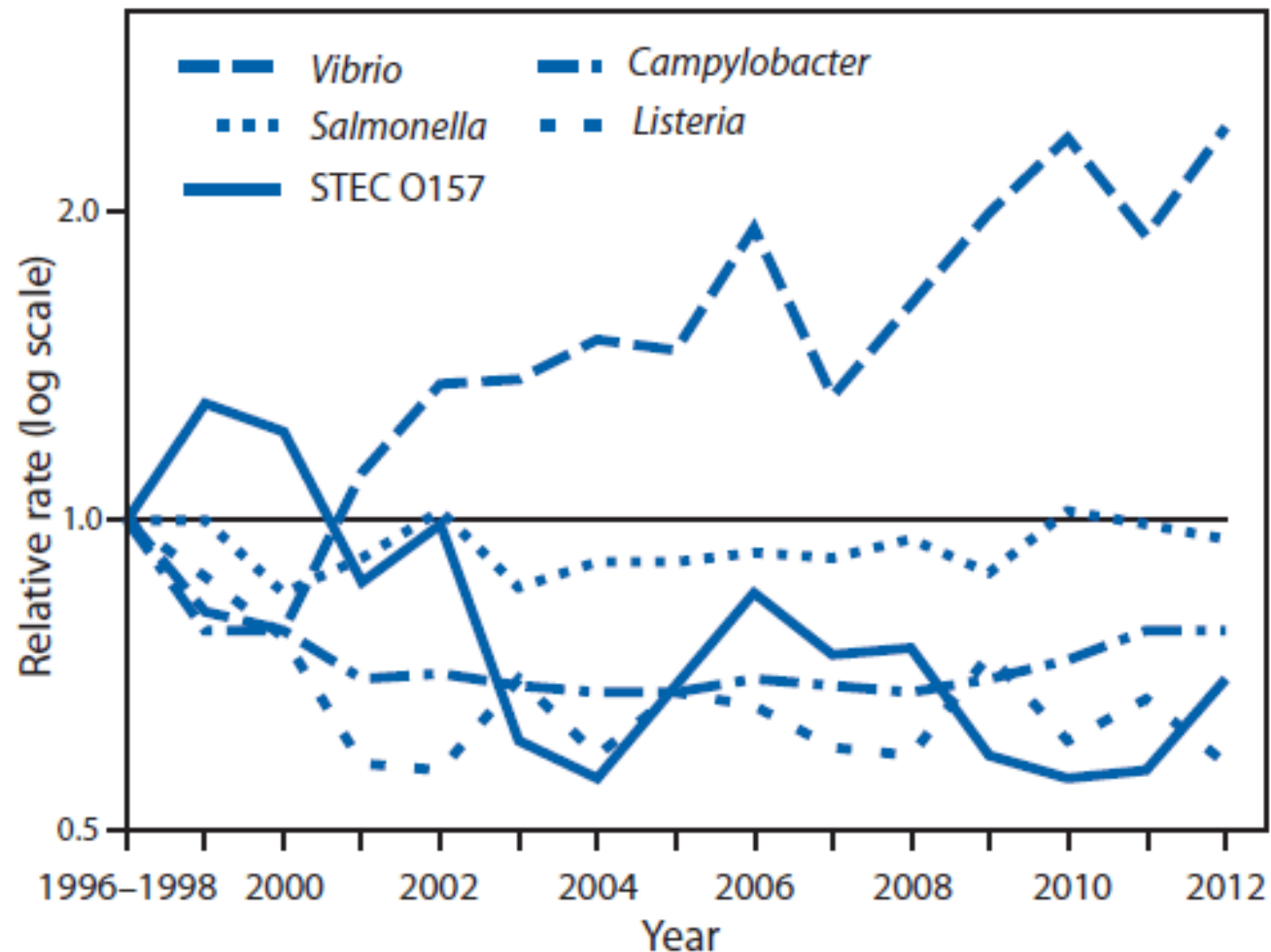
M. Nishibuchi



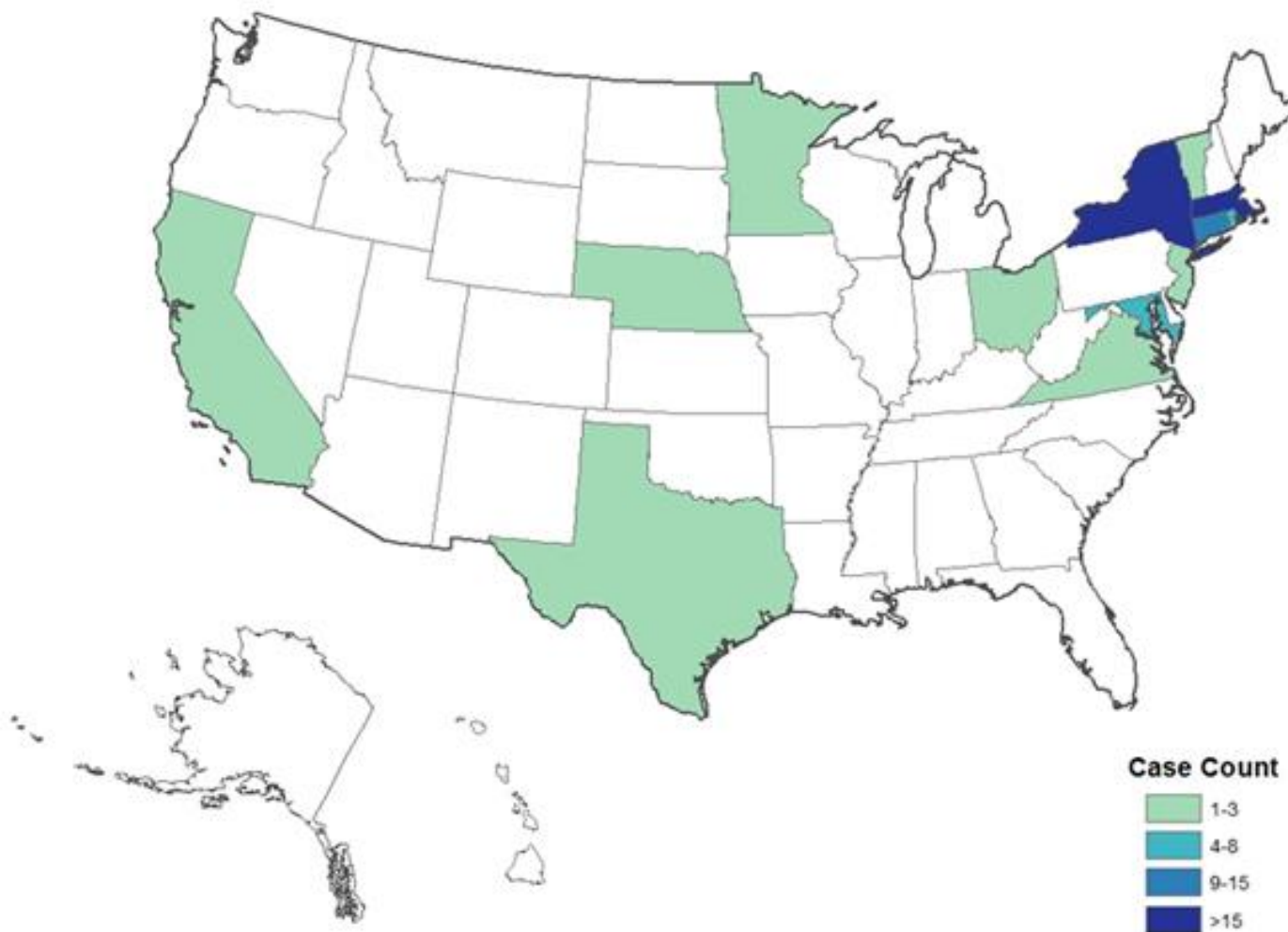
<http://carbon.bio.ku.edu/research.html>

- Not all Vp are human pathogens and it remains challenging to tell a pathogen and non-pathogen apart
- Pathogens typically carry virulence genes in a DNA element called a Vibrio Pathogenicity Island (VPaI)
 - Mosaic and assembled from pieces of DNA that can cut and paste themselves together
 - Carry genes that promote infection and disease
 - Entire VPaI can be copied and donated to unrelated bacteria: they spread through populations like a virus
- Unrelated pathogens can carry similar or different VPaI depending on where or from whom they were acquired

Relative rates of laboratory-confirmed infections with *Campylobacter*, STEC* O157, *Listeria*, *Salmonella*, and *Vibrio* compared with 1996–1998 rates, by year — Foodborne Diseases Active Surveillance Network, United States, 1996–2012



Gillis D. et al. 2013. Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996–2012. *MMWR* 62(15);283–287.



One hundred and four *Vibrio parahaemolyticus* isolates with the same DNA “fingerprint” were reported to PulseNet from persons in 13 states who became ill from May 12, 2013 through August 19, 2013. Of the 104 *Vibrio parahaemolyticus* isolates, 76 have been serotyped and all 76 were found to be serotype O4:K12.

Increase in *Vibrio parahaemolyticus* illnesses associated with consumption of shellfish from several Atlantic coast harvest areas, United States, 2013

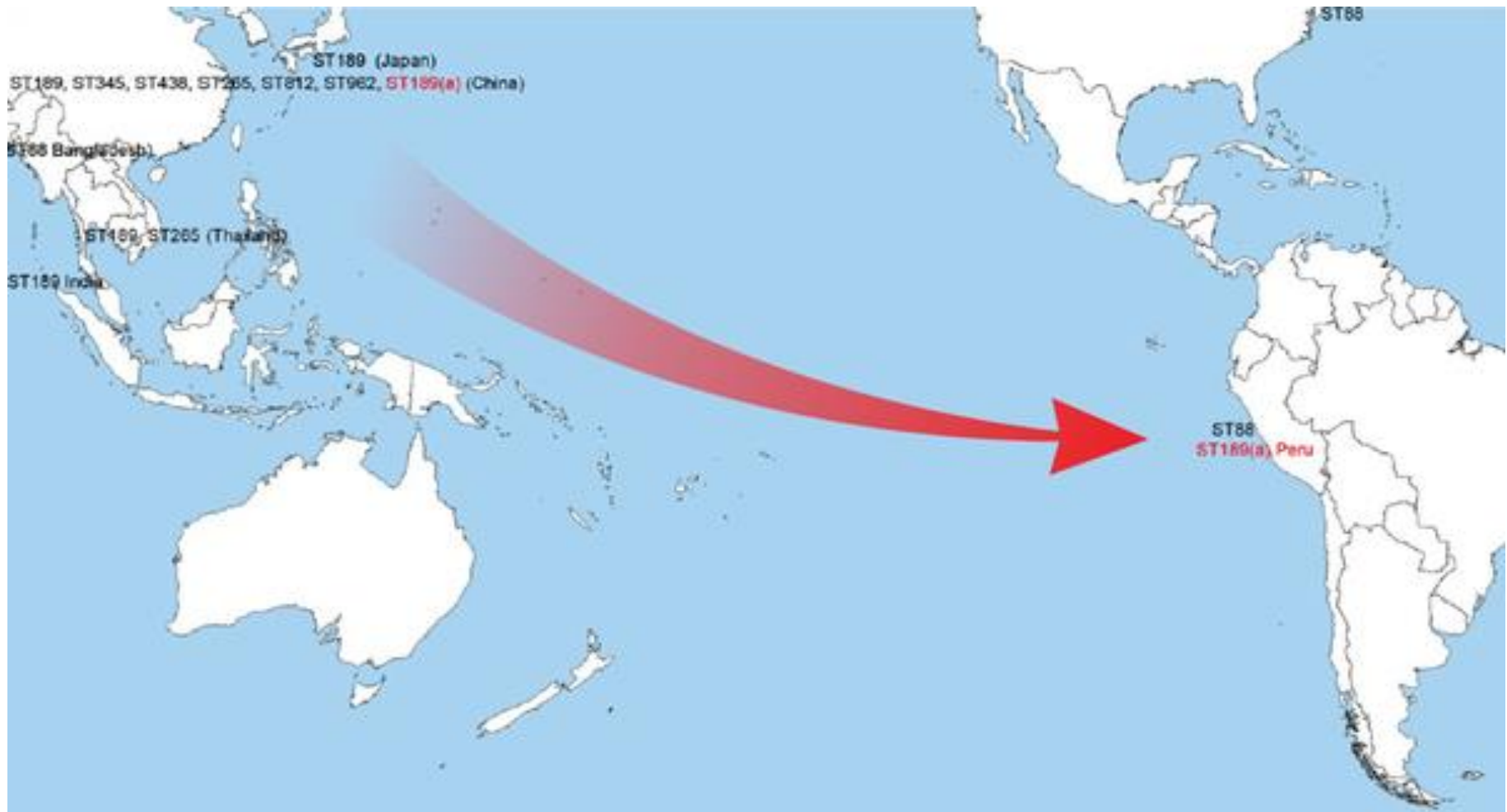
Posted October 21, 2013 11:45 AM ET. <https://www.cdc.gov/vibrio/investigations/vibriop-09-13/map.html>

Table 1. *Vibrio parahaemolyticus* Strains from Outbreaks in 2012 in the United States and Spain and Closely Matching Pacific Northwest Isolates.*

Strain	Year of Isolation and Characterization	Source	Location	Serotype
F11–3A	1988	Environmental	Washington State	O4:K12
48057	1990	Clinical	Washington State	O4:K12
10296	1997	Clinical	Washington State	O4:K12
029–1(b)	1997	Environmental	Oregon	O4:K12
10329	1998	Clinical	Washington State	O4:K12
CDC_2012V-1109	2012	Clinical	Oyster Bay Harbor, New York	O4:K12
CDC_2012V-1108	2012	Clinical	Oyster Bay Harbor, New York	O4:K12
CDC_M12–108 G	2012	Clinical	Oyster Bay Harbor, New York	O4:KUT
CDC_2012V-1131	2012	Clinical	California	O4:KUT
CDC_2012V-1132	2012	Clinical	California	O4:K12
CDC_2012V-1134	2012	Clinical	Oyster Bay Harbor, New York	O4:KUT
G35	2012	Clinical	Spain	O4:K12
G36	2012	Clinical	Spain	O4:K12
G37	2012	Clinical	Spain	O4:K12

* All isolates, which were positive for thermostable direct hemolysin and thermostable direct-related hemolysin, were multilocus sequence type 36.

Sequence types (STs) identified at both side of the Pacific Ocean and route for the movement of waters associated with El Niño event (red arrow).

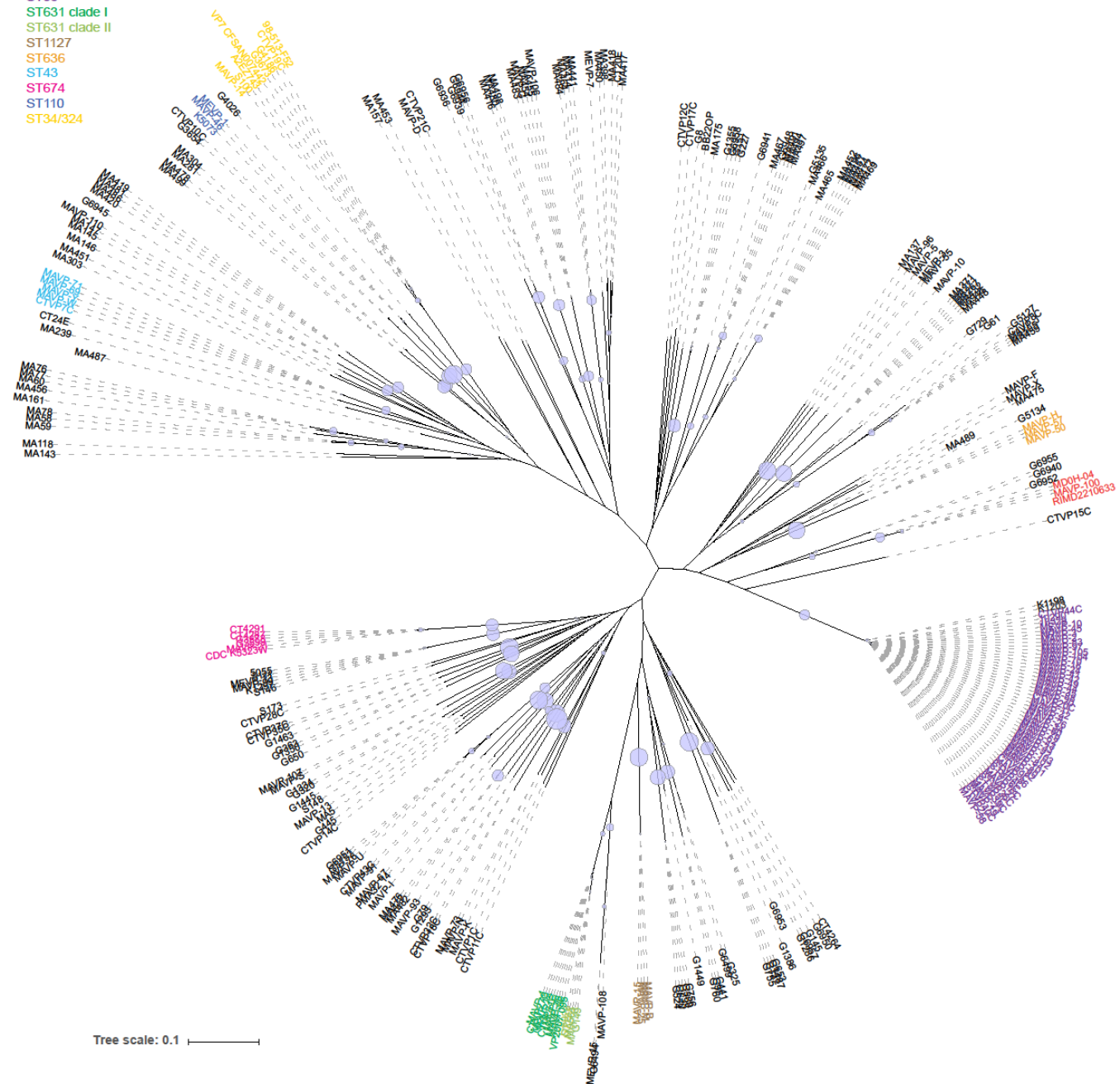


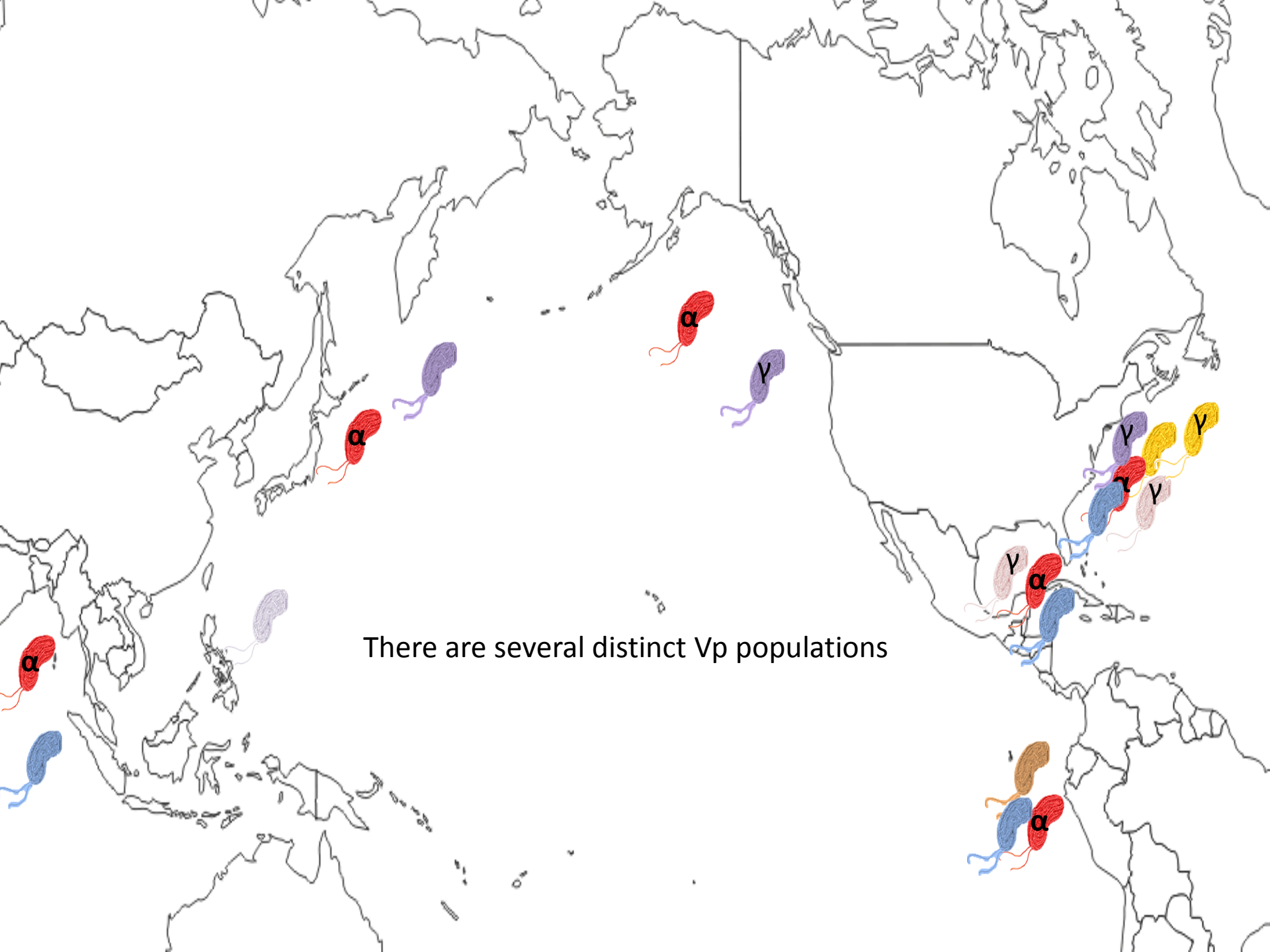
González-Escalona N, Gavilan RG, Brown EW, Martinez-Urtaza J (2015) Transoceanic Spreading of Pathogenic Strains of *Vibrio parahaemolyticus* with Distinctive Genetic Signatures in the *recA* Gene. PLOS ONE 10(2): e0117485. <https://doi.org/10.1371/journal.pone.0117485>
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0117485>

What strains of Vp cause people in the Northeast to get sick and how can we detect them?

Northeast Vp Population

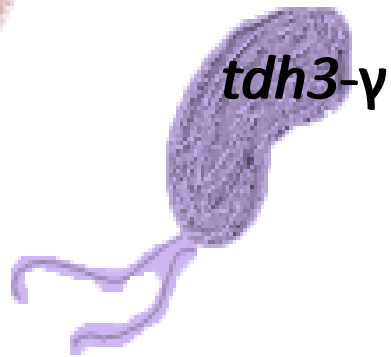
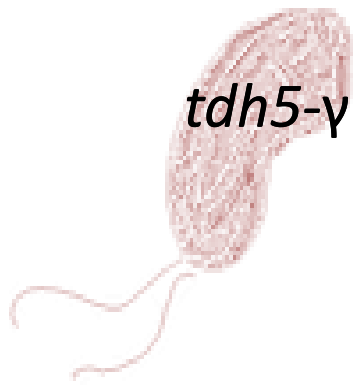
- ST3
- ST36
- ST631 clade I
- ST631 clade II
- ST1127
- ST636
- ST43
- ST674
- ST110
- ST34/324



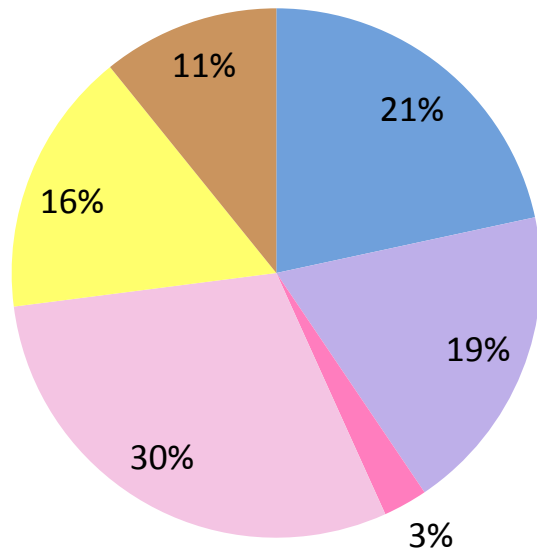


There are several distinct Vp populations

Non-native strains have donated VPal to local strains leading to evolution of new pathogens



The demographics of Northeast US pathogenic Vp

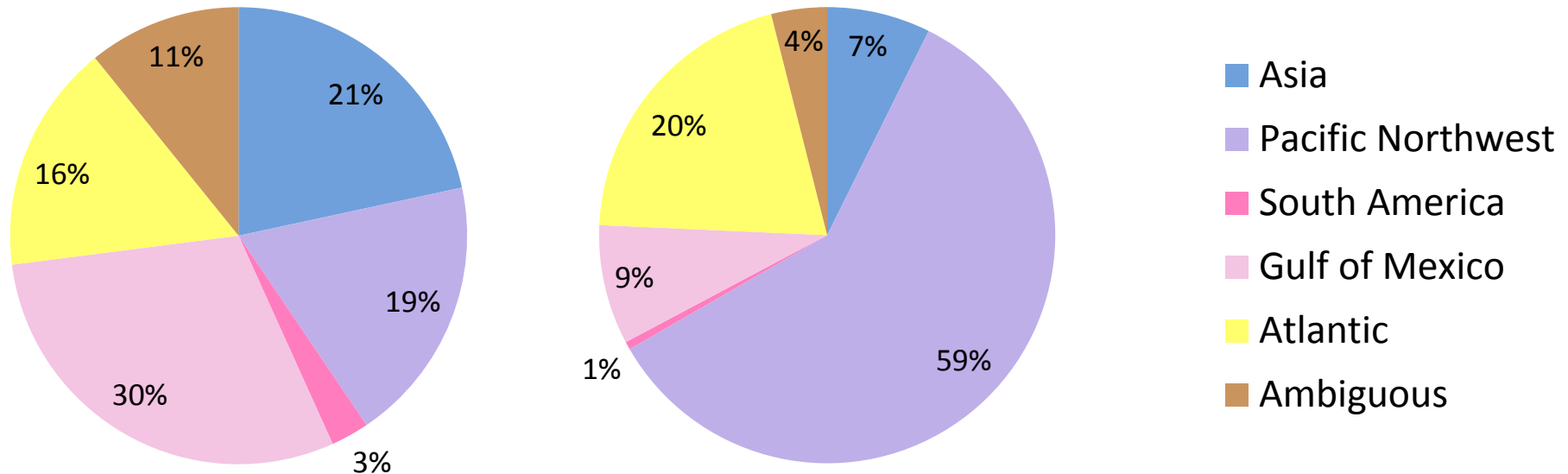


- Based on 177 clinical isolates, from 4 NE states, 2010-2016
- 38 unique “strains” or lineages (known as Sequence Types)
- 84% of “strains” in the Northeast are not native to the Atlantic
- Most introduced pathogenic strains are from the Gulf of Mexico population

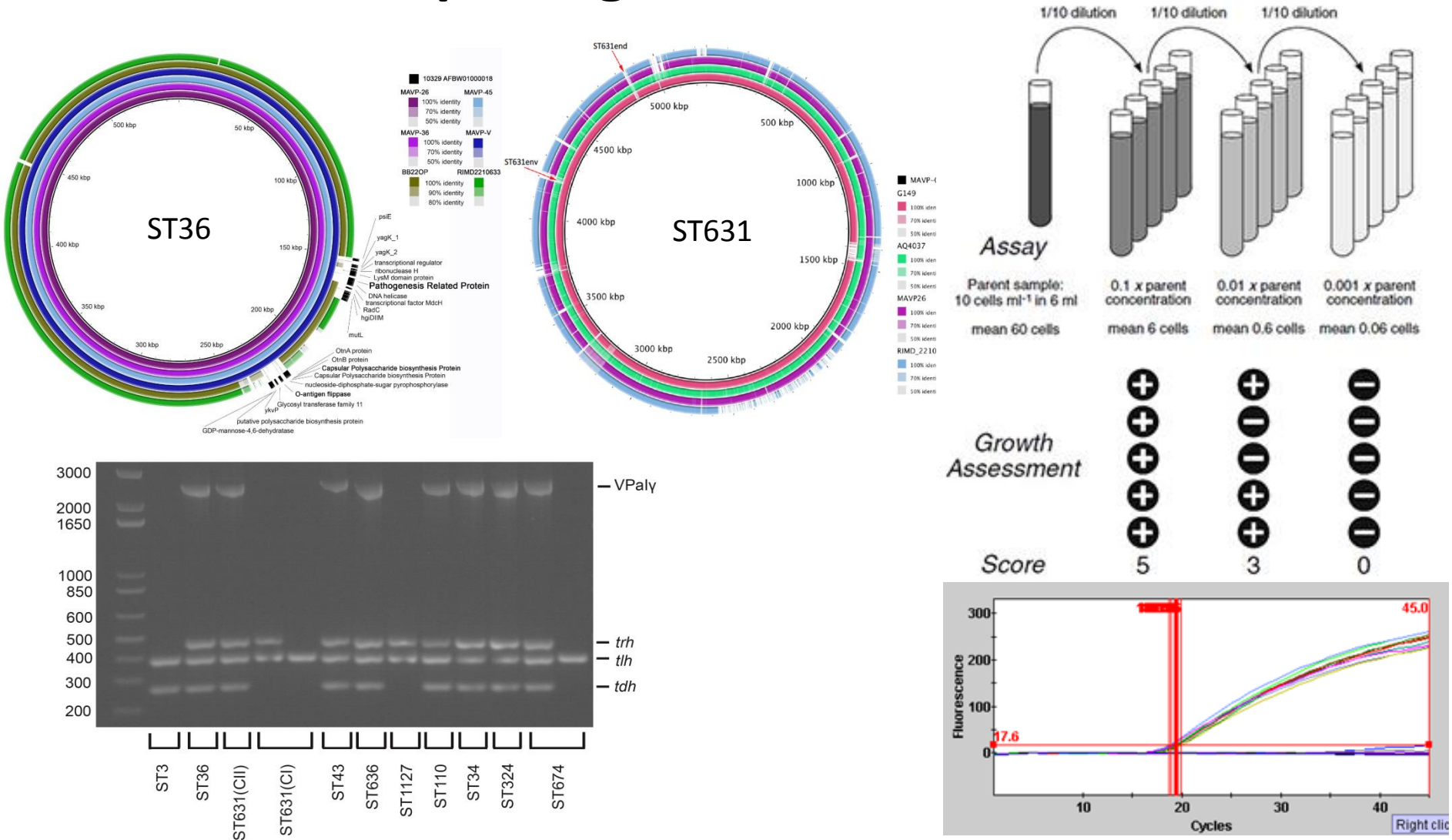


The demographics of Northeast US pathogenic Vp

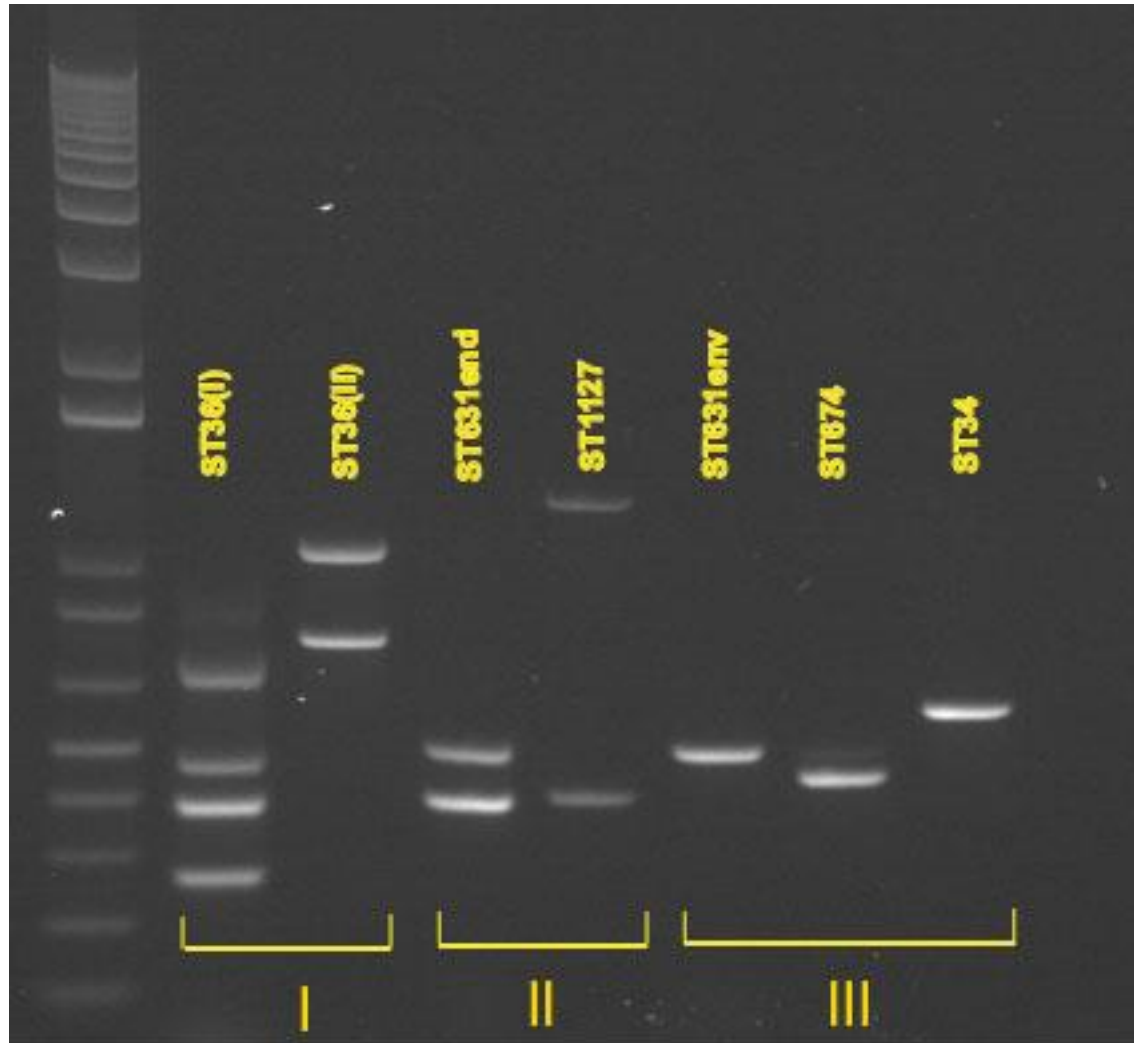
- But most infections are caused by strains from the Pacific Northwest population of North America (ST36) or a single lineage from the Atlantic (ST631) suggesting these are more virulent
- Gulf of Mexico strains, while relatively abundant in the environment, infrequently cause infections



Use of genome comparisons to develop strain- and VPal-specific detection assays for improved pathogen surveillance



Sequence-type based identification



What can we do to manage Vp?

- Improve forecast modeling for risk conditions related to ecosystem/climate conditions
- State shellfish program Vp management plans
- Evolving aquaculture practices

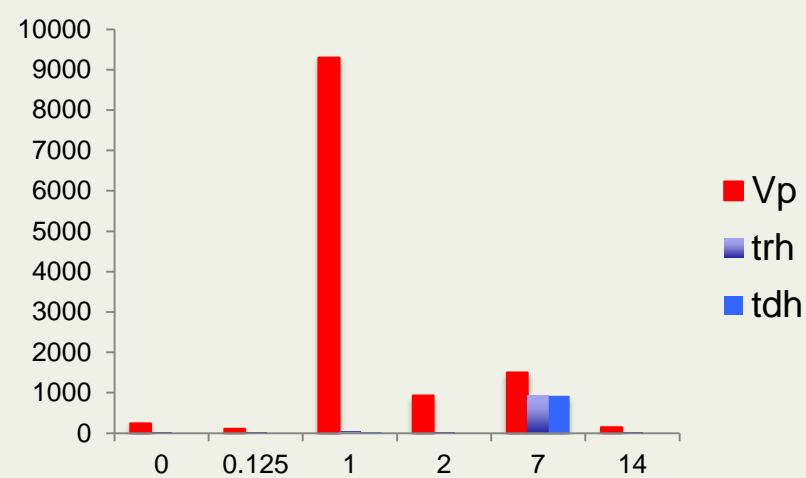
***V. vulnificus* concentrations (MPN/100 g)
in freshly harvested and relayed oysters
at Spinney Creek Shellfish Inc.**

Sample date	Fresh oysters	Relayed oysters
8/21/93	930	24
8/28/93	4600	4.3
9/4/93	1500	<3
9/19/93	150	<3
10/3/93	4.3	<3
10/16/93	<3	<3
11/7/93	<3	<3

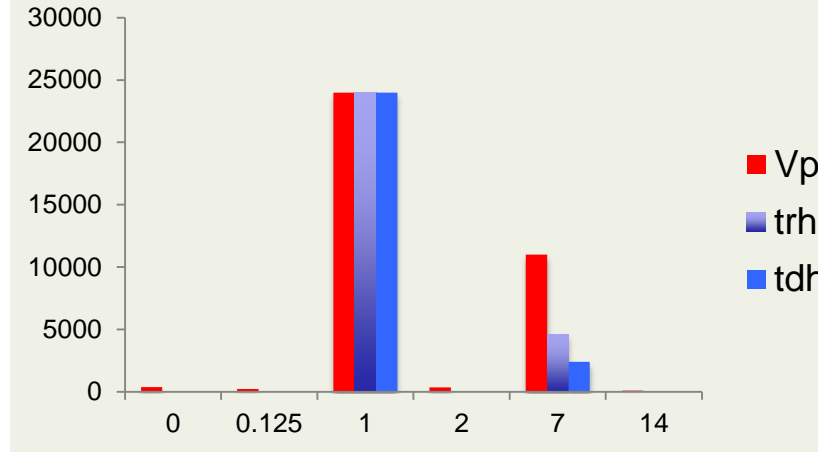
MANAGING SHELLFISH AQUACUTURE pre-harvest handling

Levels of *V. parahaemolyticus* *tdh* & *trh* markers in oysters during three 14-day re-submergence experiments in NH

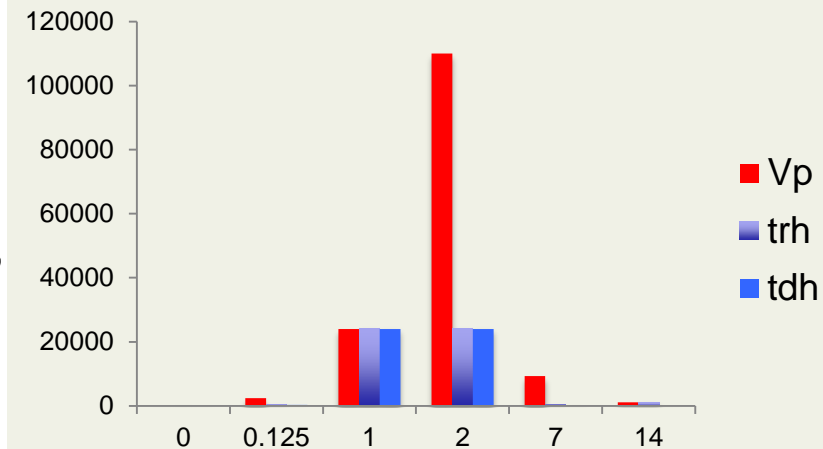
Expt #1



Expt #2



Expt #3



***trh* & *tdh* genetic marker were detected at elevated levels following re-submergence between Days 1-7, then disappeared after 7-14 days, roughly following total *V. parahaemolyticus* levels.**

Findings to Date

- The ecology of *V. parahaemolyticus* (and other pathogenic *Vibrio* species) is complex yet potentially predictable at some temporal/spatial scale(s).
- *Vibrio* populations are extremely diverse, yet there appears to be a limited number of strains that cause most of the problems-why they persist 'out there' is not understood, and their geography is complex.
- Time-temperature-condition control management strategies appear to work well in reducing illnesses, though we do not know what will happen if temperatures continue to rise and the ecosystem changes...

Future & Ongoing Research

- Are predictive models needed for every growing area, or, can we find common and useful predictive measures and 'indicators'?
- Refine Vibrio reduction strategies with changing aquaculture practices
- Application of newly developed detection methods for pathogenic strains
- Microbiome & vibriophage interactions
- What are the mechanisms of ecosystem resilience of virulent strains?

Acknowledgements

- All of our undergraduate students!!
 - MA Department of Public Health
 - MA Division of Marine Fisheries
C. Schillaci
 - NH/DHHS/ Division of Public Health Services
Debra Scoville, Jen Mahoney
 - ME CDC
 - ME Department of Marine Resources:
Kohl Kanwit
 - CT Department of Agriculture
 - Bureau of Aquaculture
K. DeRosia-Banick
- CT Department of Public Health
J. A. DeCrescenzo



**NH Agricultural
Experiment
Station**



**NEW HAMPSHIRE
DEPARTMENT OF
HEALTH AND
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