Pathogenic *Vibrio* Species

3 *Vibrio* species are most responsible for the majority of Vibrio-borne human illness:

Vibrio parahaemolyticus (Vp)
Vibrio vulnificus (Vv)
Vibrio cholerae (Vc)

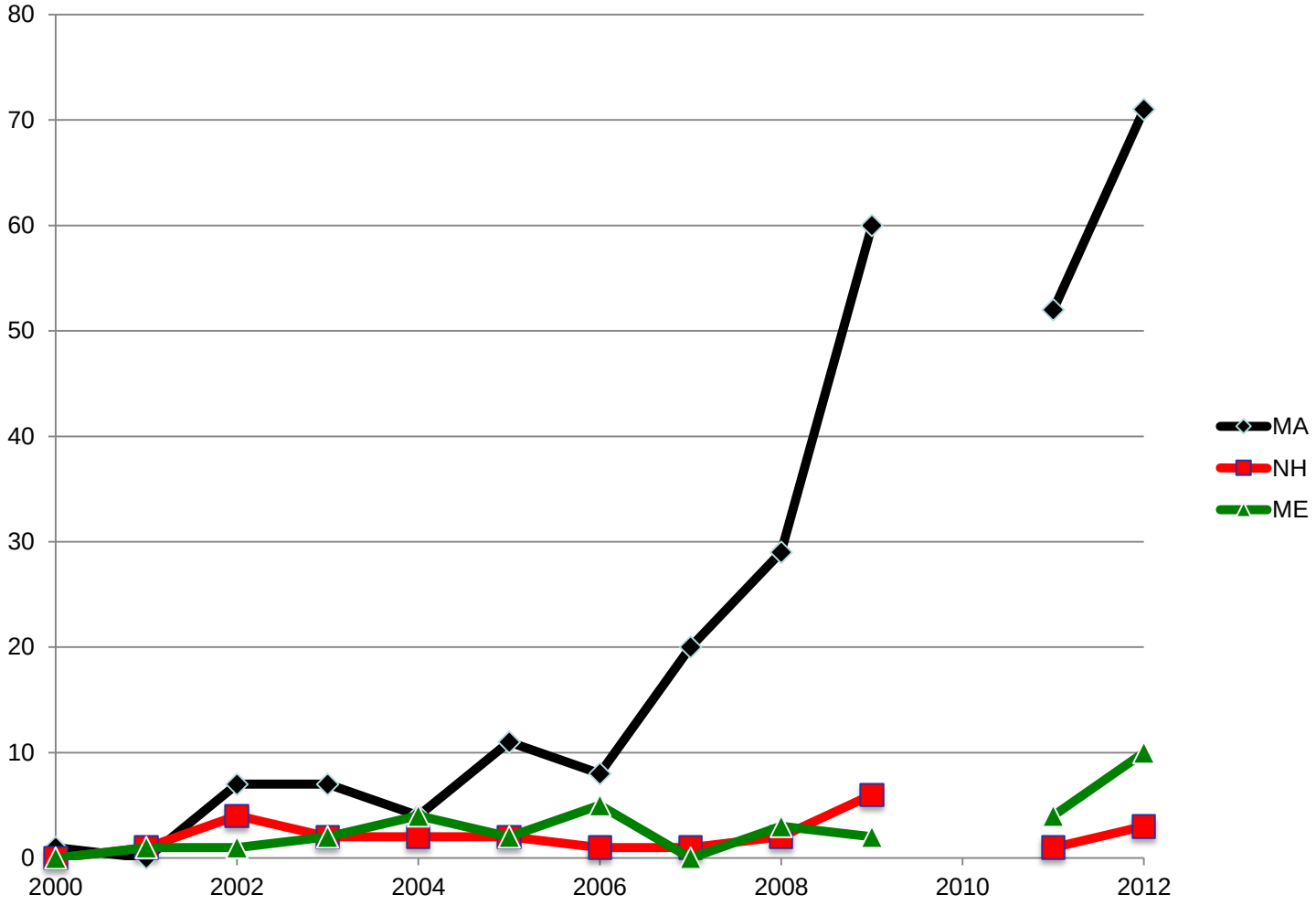
Not all strains of these species are equally capable of causing disease. Non-pathogenic strains exist; this is not well understood.

In the Northeast

-8 Vp cases from oysters harvested from Cape Cod Bay, MA in 2010-11;
->25 Vp cases from oysters harvested from Cape Cod Bay, MA in 2013; two closures with recalls in August from Duxbury & Martha's Vineyard.

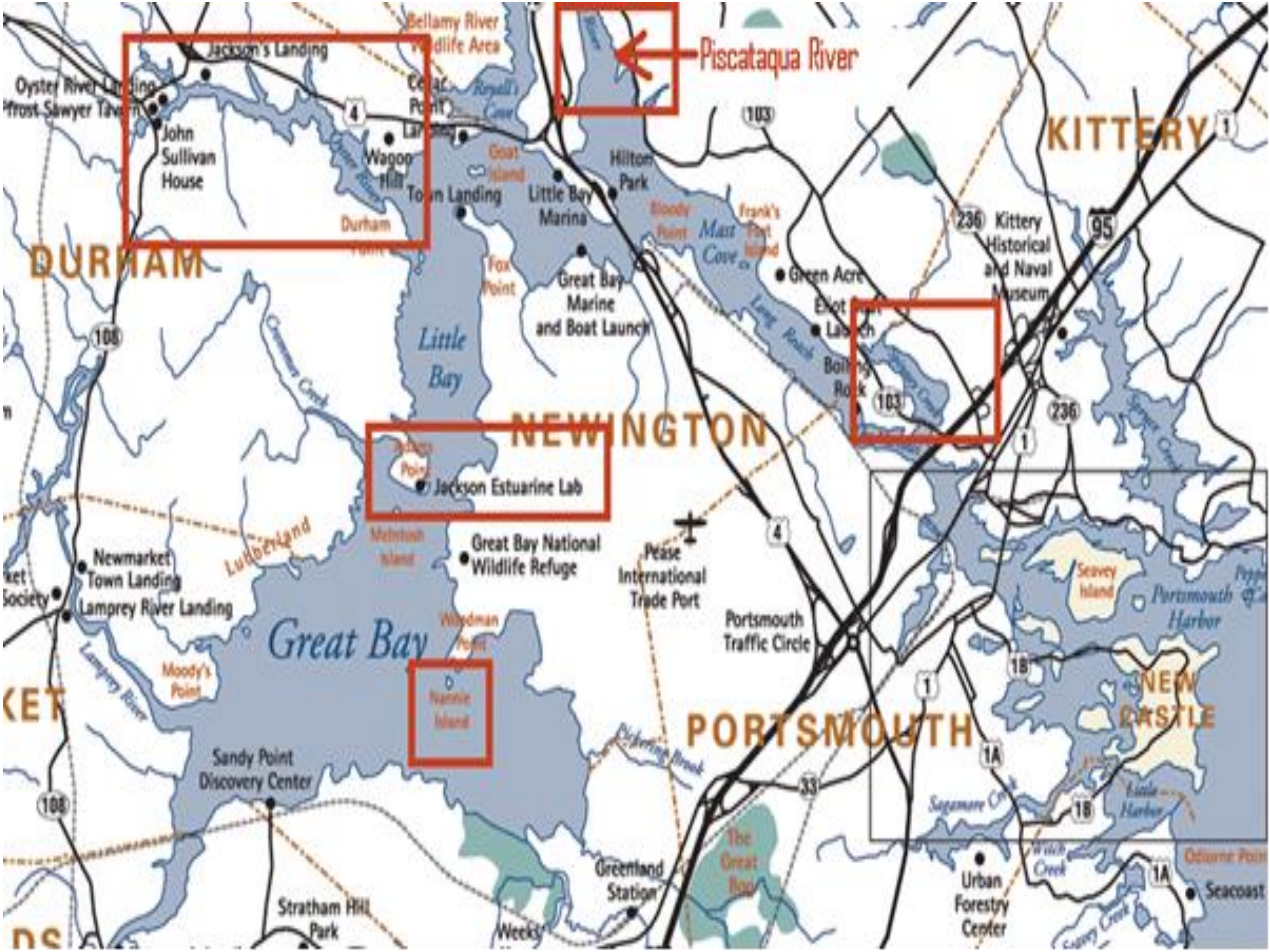
-Vp cases in Maine ~ doubled from 2011 to 2012, and ~ doubled again in 2013;
-Vc case in southern Maine associated with oysters, possibly from Maine

Cases of “Vibriosis” in Gulf of Maine states: 2000-12



Data from CDC, MA DPH and ME CDC

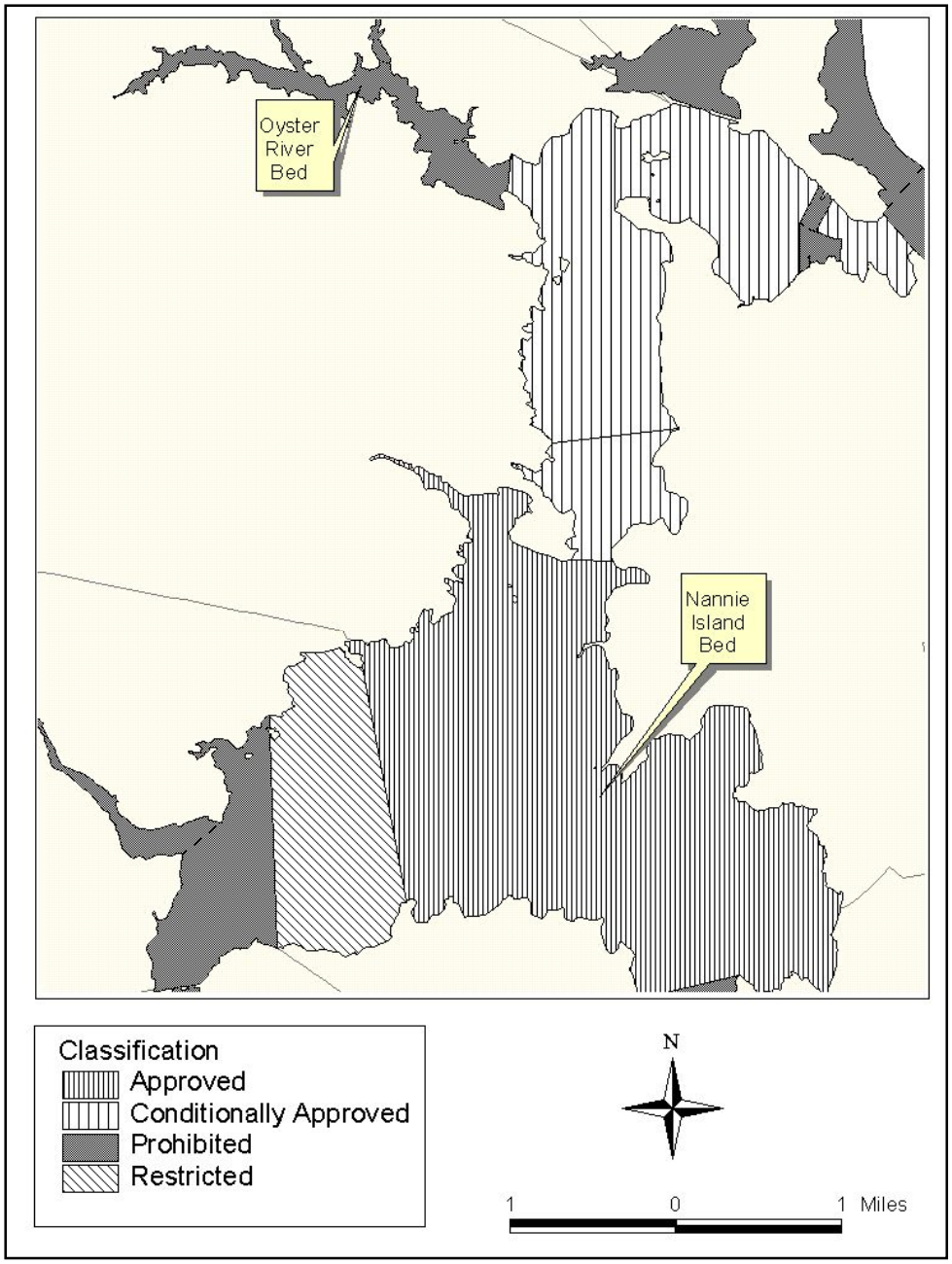
What factors could explain these trends?



Piscataqua River
Bellamy River Wildlife Area
Jackson's Landing
Oyster River Landing
Frost Sawyer Tavern
John Sullivan House
Cedar Point
Wagon Hill
Town Landing
Durham Point
DURHAM
Goat Island
Little Bay Marina
Hilton Park
Bloody Point
Mast Cove
Frank's Fort Island
Great Bay Marine and Boat Launch
Fox Point
Green Acre
Eliot Boat Launch
Long Reach
Boiling Rock
KITTERY
Kittery Historical and Naval Museum
Little Bay
NEWINGTON
Jackson Estuarine Lab
Lubberland
McIntosh Island
Great Bay National Wildlife Refuge
Pease International Trade Port
Portsmouth Traffic Circle
Newmarket Town Landing
Lamprey River Landing
Lamprey River
Great Bay
Woodman Point
Moody's Point
Nannie Island
Seavey Island
Portsmouth Harbor
NEW CASTLE
PORTSMOUTH
Pickering Brook
Sandy Point Discovery Center
Sagamore Creek
Little Harbor
Odiorne Point
Greenland Station
The Great Bog
Urban Forestry Center
Seacoast
Stratham Hill Park
Witch Creek
108
4
103
236
95
1
1A
1B
33

Oyster River:

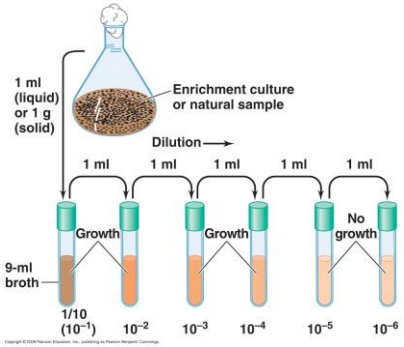
Prohibited site
warmer
reduced salinity
higher nutrients
more heterogeneous



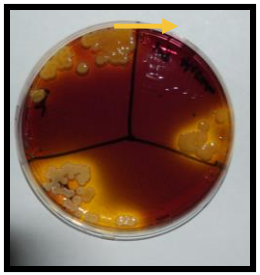
Nannie Island:

Approved site
Cooler
higher salinity
lower nutrients
more consistent

Approach:
Analysis of oysters, water & sediment



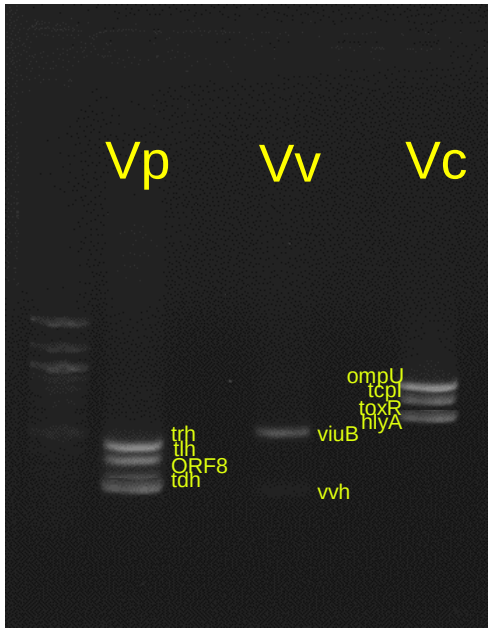
enrichment and enumeration



growth on media

(TCBS & CPC agars)

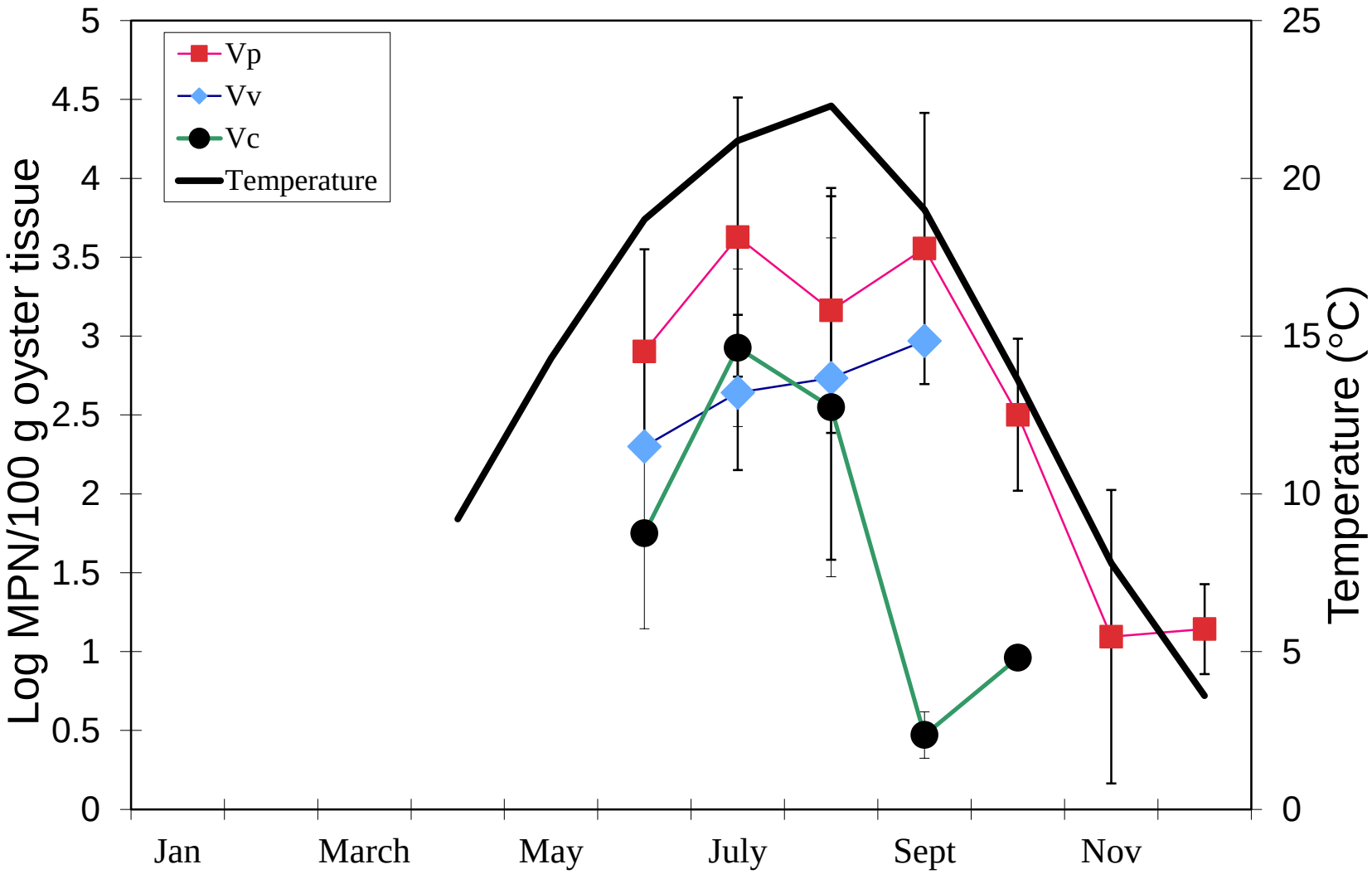
CHROMagar



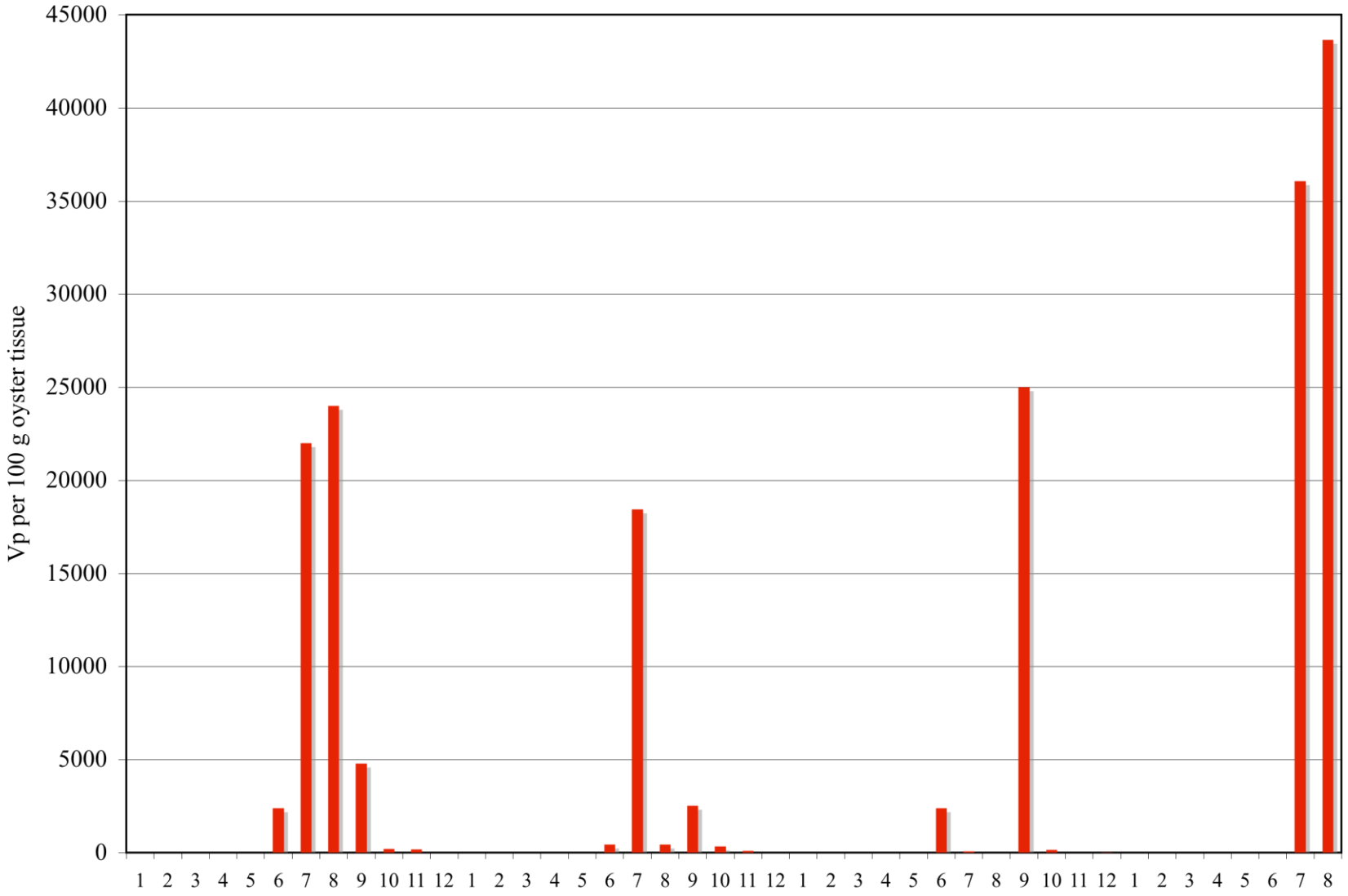
multiplex PCR

→ or use q-PCR

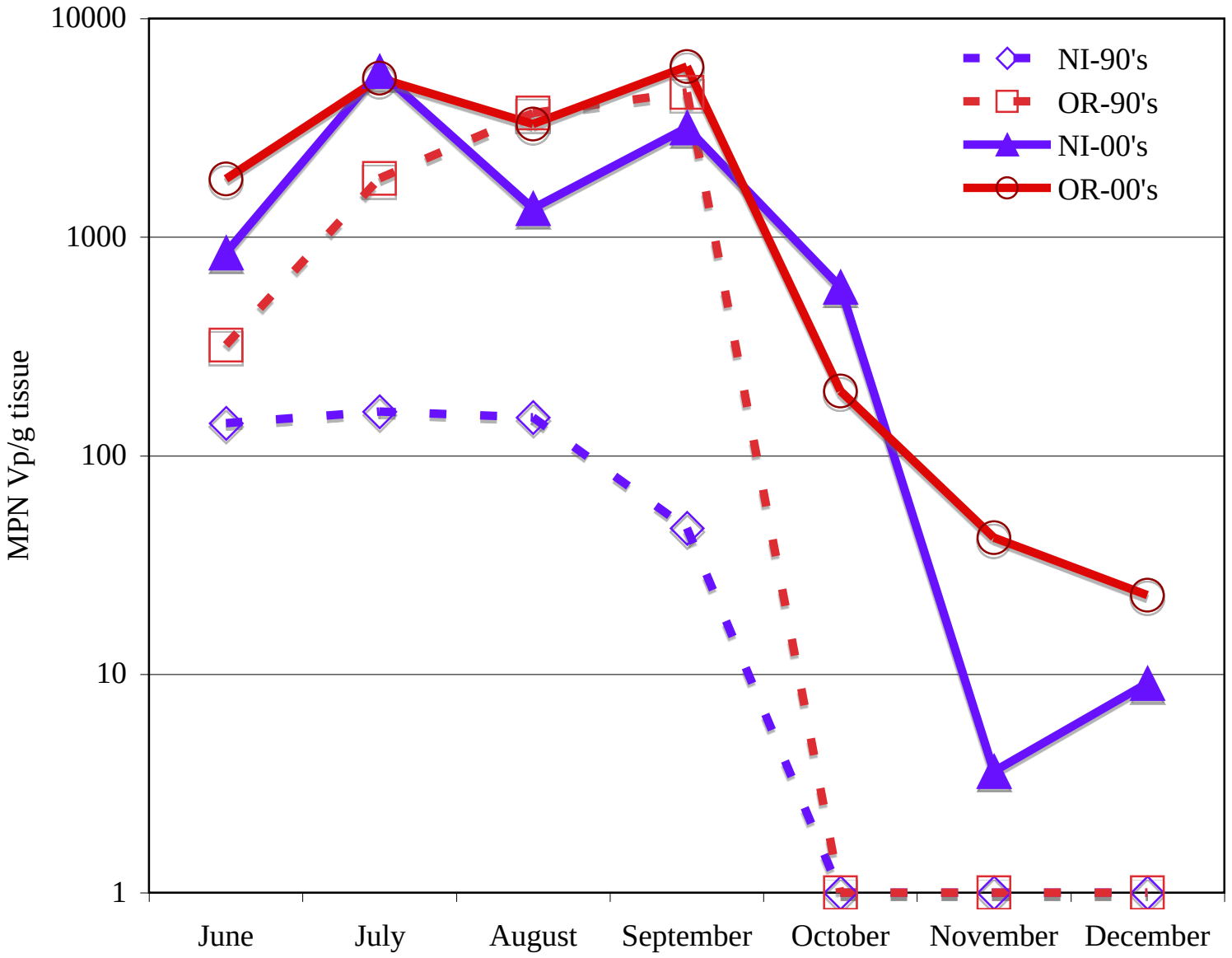
Vibrio abundance is seasonal in the GBE



Inter-annual *Vibrio parahaemolyticus* concentrations in oysters from the Oyster River*: 2007-10*



V. parahaemolyticus levels at Nannie Island (NI) and Oyster River (OR) were different in 1993-95. They were not different in 2007-09.



Environmental conditions and *Vibrio* spp. concentrations in oysters

1993-1995: Multiple Stepwise Regression (14 variables)

Log *V. vulnificus* = 0.03 Temp + 0.16PO4 + **0.13 DOC**

Log *V. parahaemolyticus* = 0.09Temp + 0.05 TSS +0.25 PO4 - 0.09 TSSorg

2008-2009: Multiple Stepwise Regression (4 variables)

V. cholerae (non-O1 serotypes): temperature, **rainfall***, salinity (DO)

Vp & Vv: many different significant relationships, depending on site, year

Vibrio cholerae in the Oyster River-Schuster et al. 2011

Abundance and environmental factor correlations

	Total Maximum MPN		Oyster Maximum MPN		Water Maximum MPN	
	Pearson Correlation [b]	Stepwise Regression [a]	Pearson Correlation	Stepwise Regression	Pearson Correlation	Stepwise Regression
Temperature						
12hr	0.355*	NC	0.230	NC	0.271	NC
72hr	0.389*	NC	0.270	NC	0.266	NC
Salinity						
12hr	-0.369*	NC	-0.355*	NC	-0.236	NC
72hr	-0.461*	0.1768	-0.375*	0.378	-0.367	0.450
Rain						
72hr	0.472**	0.049*	0.112	0.4753	0.623**	< 0.001**
96 hr	0.566**	0.006*	0.555**	0.001**	0.228	0.976
Dissolved O_2						
Percent	-0.333*	NC	-0.261	NC	-0.223	NC
mg/L	-0.355*	NC	-0.247	NC	-0.255	NC
Turbidity	0.112	NC	0.134	NC	0.112	NC

[a] *p* statistic is given otherwise the environmental condition was excluded from the stepwise model and no correlation (NC) is reported

[b] *r* statistic is given

* $p < 0.05$

** $p < 0.001$

NOTE: *Vp* & *Vv* also correlate with, and may be predicted by, these environmental conditions

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

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

NOTE: Similar reductions have been shown with relaying for *V. parahemolyticus*

GBE Vibrios are culturable under unique conditions and have rare clinical markers

Extreme temperatures:

Detection of Vp in oysters at -0.5^{o}C (January 2011)

Detection of Vc in water at 13.6^{o}C (October 2008)

Wide salinity tolerance range:

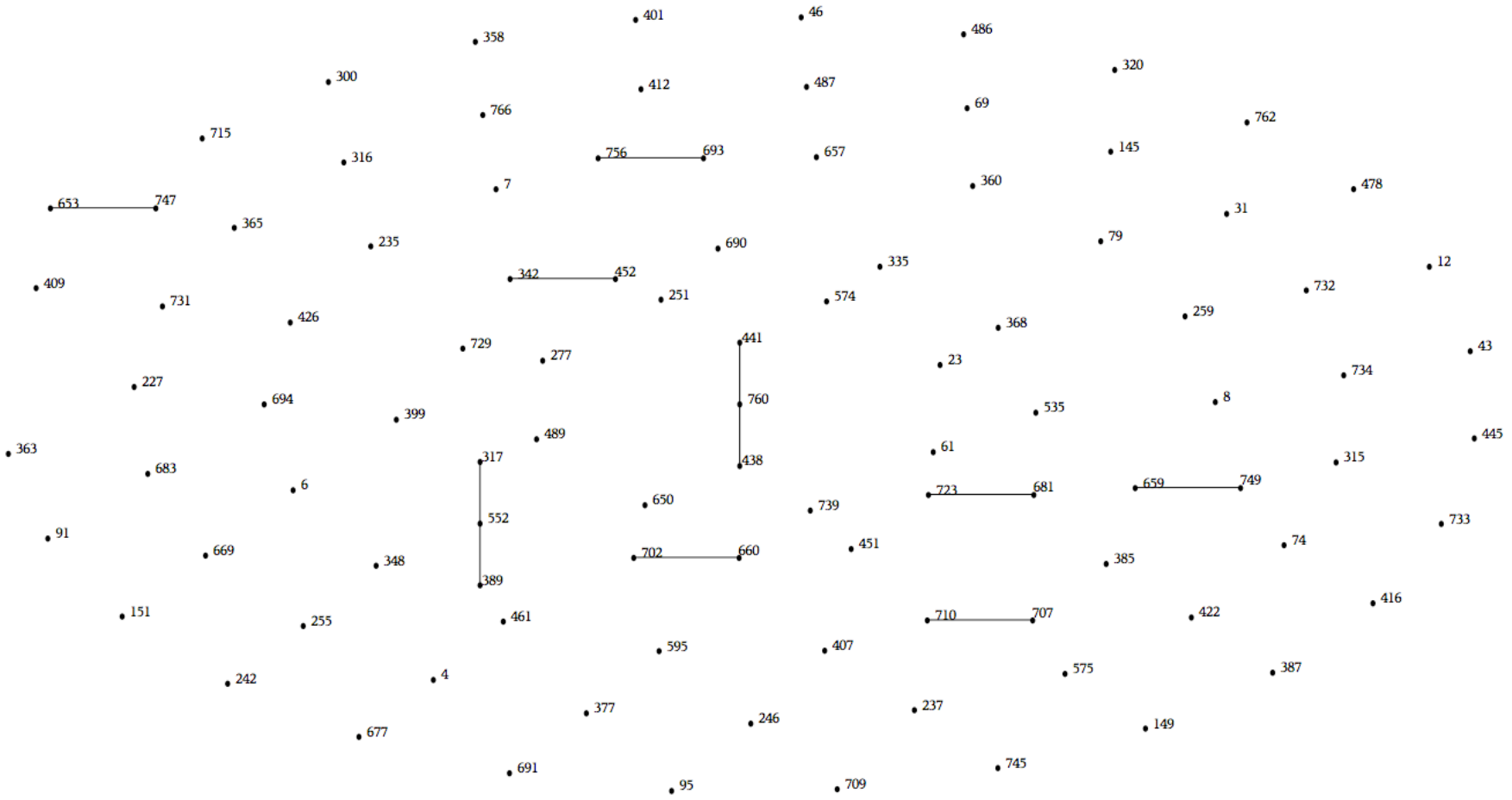
Growth of Vc at 8% NaCl

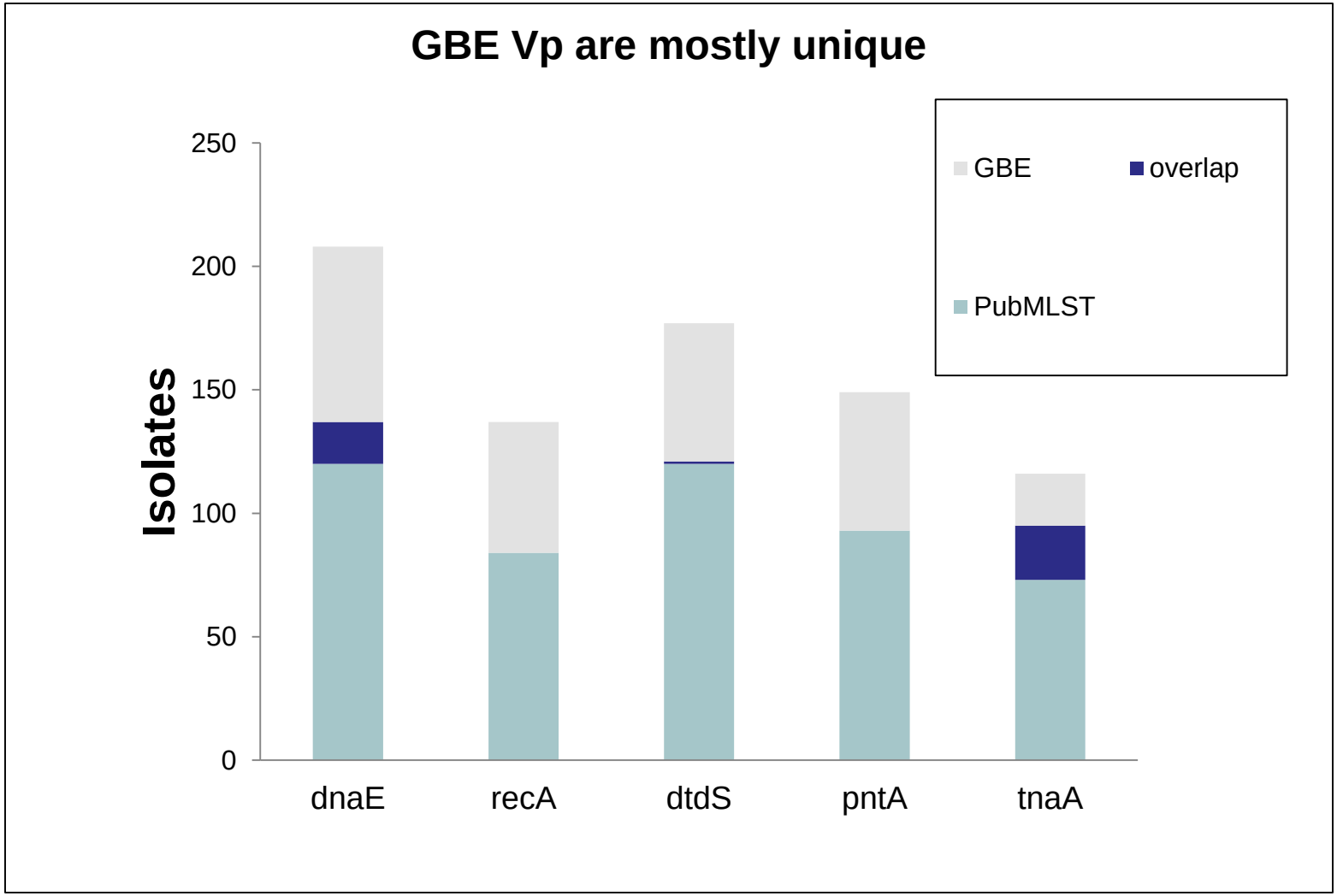
Clinical marker detection:

Not detected in 4 years *EXCEPT: tdh/trh* in Vp by qPCR in temperature abused oysters (28^{o}C for 18h) collected during August, 2009, more frequent during 2012, infrequent in 2013

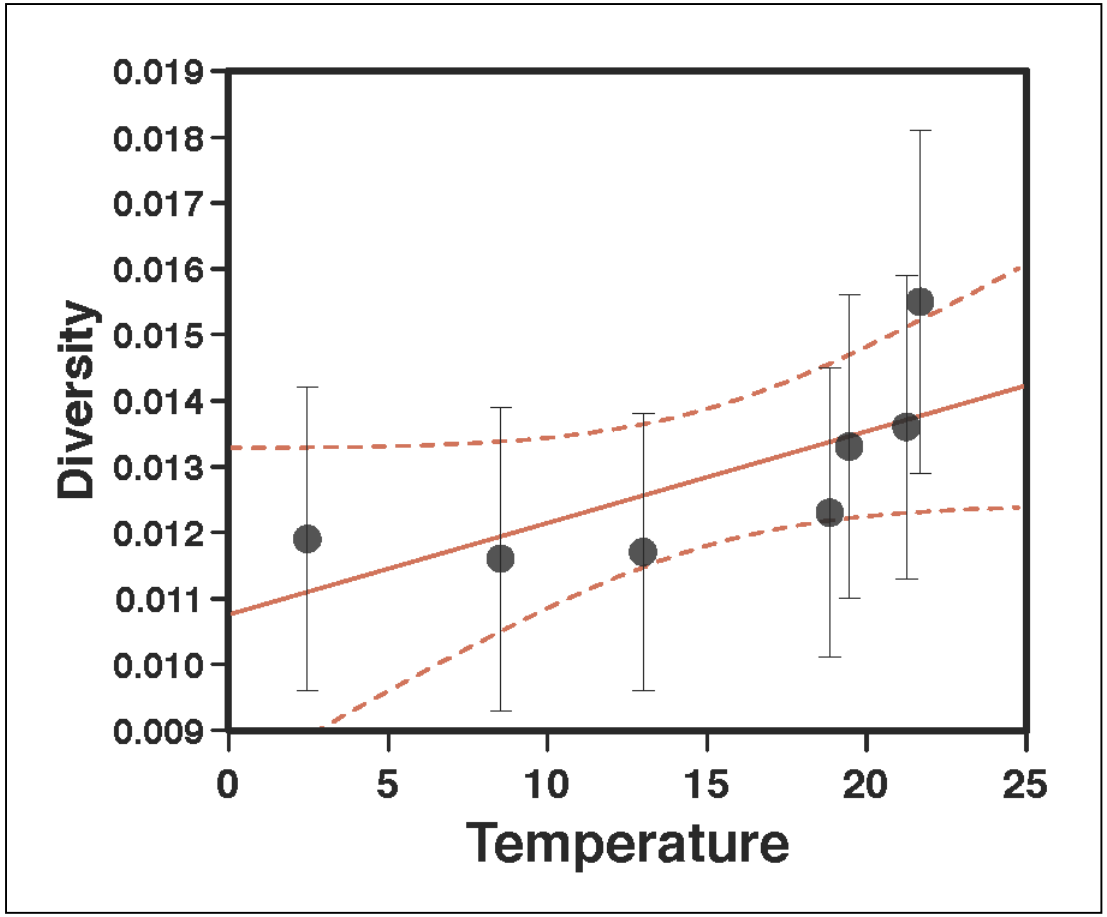
The *V. parahaemolyticus* population is extremely diverse.

Multilocus sequence typing (MLST) with 7 housekeeping genes:
-154 Sequence Types of 188 isolates: only 29% in clonal complexes




GBE Vp are mostly unique
Isolates
250
200
150
100
50
0
GBE
overlap
PubMLST
dnaE
recA
dtdS
pntA
tnaA

The Vp population diversity appears to vary with season/water temperature.

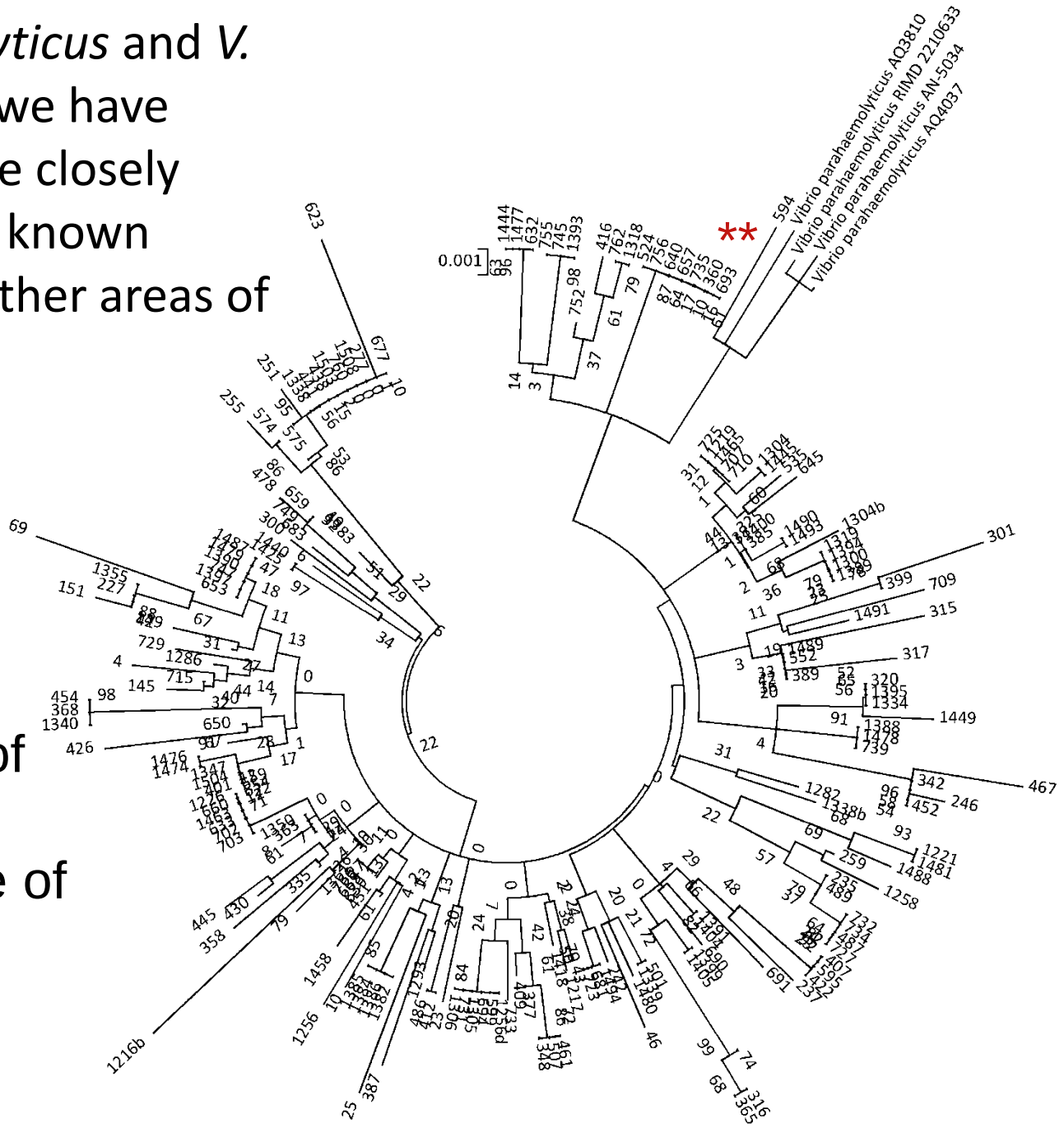


Diversity increases as water temperatures rise above 15˚ C

Are there pathogenic *Vibrio* strains in New Hampshire?

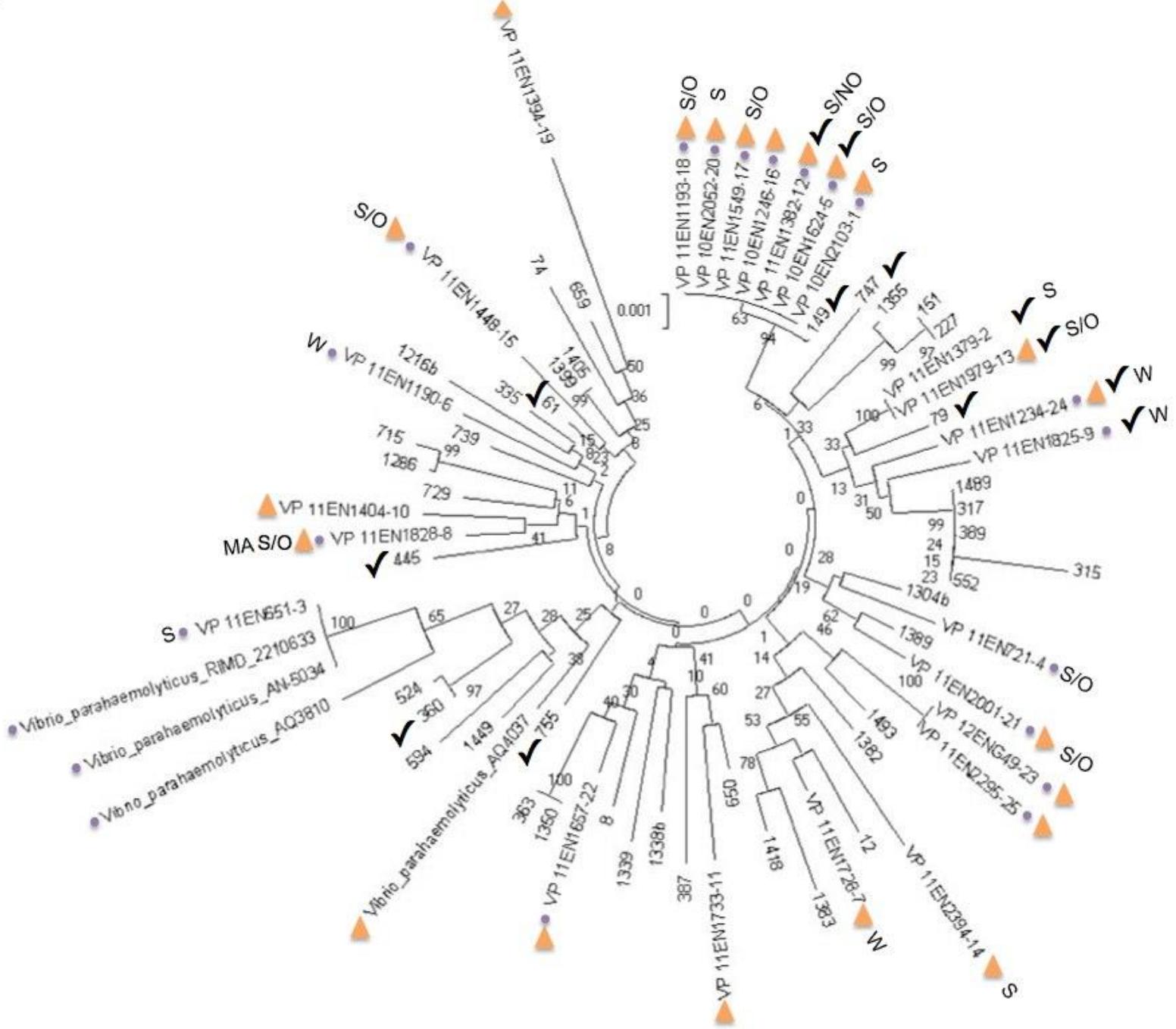
Some *V. parahaemolyticus* and *V. cholerae* strains that we have found in Great Bay are closely related genetically to known clinical strains from other areas of the world.

Again, most strains of these species are probably not capable of causing disease, especially in colder climates.



1-1 Genotyping of Vp outbreak strains

Strains	tlh[a]	tdh[b]	trh[c]	ORF8[d]	vscC2[e] (T3SS2)	*vop*P[f] (T3SS2)	MTase[g]	*vop*C[h]	VPA1376[i]
3	+	+	-	+	+	+	+	+	+
4, 6	+	+	-	-	+	+	+	+	+
9	+	+	-	-	+	-	+	-	+
7	+	-	+	-	+	-	-	-	+
19	+	-	+	-	-	-	-	-	+
10, 14	+	-	+	-	+	-	+	-	+
11	+	-	+	-	-	-	+	+	+
13	+	-	+	-	-	-	-	-	-
1,20,21,22 ,23,25	+	+	+	-	-	-	-	-	+
5, 16	+	+	+	-	+	+	+	+	+
8	+	+	+	-	-	-	+	-	-
12, 17, 18	+	+	+	-	+	-	+	-	+
24	+	+	+	-	+	-	-	-	+
15	+	+	+	-	-	-	+	-	-
NH-A	+	+	+	-	-	-	-	-	-
ME	+	+	+	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-	-


VP 11EN1394-19
S/O
VP 11EN1193-18
S
VP 10EN2052-20
S/O
VP 11EN1549-17
S/NO
VP 10EN1246-16
S/O
VP 11EN1382-12
S
VP 10EN1624-5
VP 10EN2103-1
S/O
VP 11EN1448-15
W
VP 11EN1190-6
VP 11EN1379-2
S
S/O
VP 11EN1979-13
W
VP 11EN1234-24
W
VP 11EN1825-9
VP 11EN1404-10
MA S/O
VP 11EN1828-8
445
S
VP 11EN651-3
Vibrio_parahaemolyticus_RIMD_2210633
Vibrio_parahaemolyticus_AN-5034
Vibrio_parahaemolyticus_AQ3810
Vibrio_parahaemolyticus_AQ4037
VP 11EN1657-22
VP 11EN1733-11
VP 11EN1728-7
W
VP 11EN2394-14
S
VP 11EN721-4
S/O
VP 11EN2001-21
S/O
VP 12ENG49-23
VP 11EN2295-25
0.001

Virulence of strains in an alternative invertebrate infection model

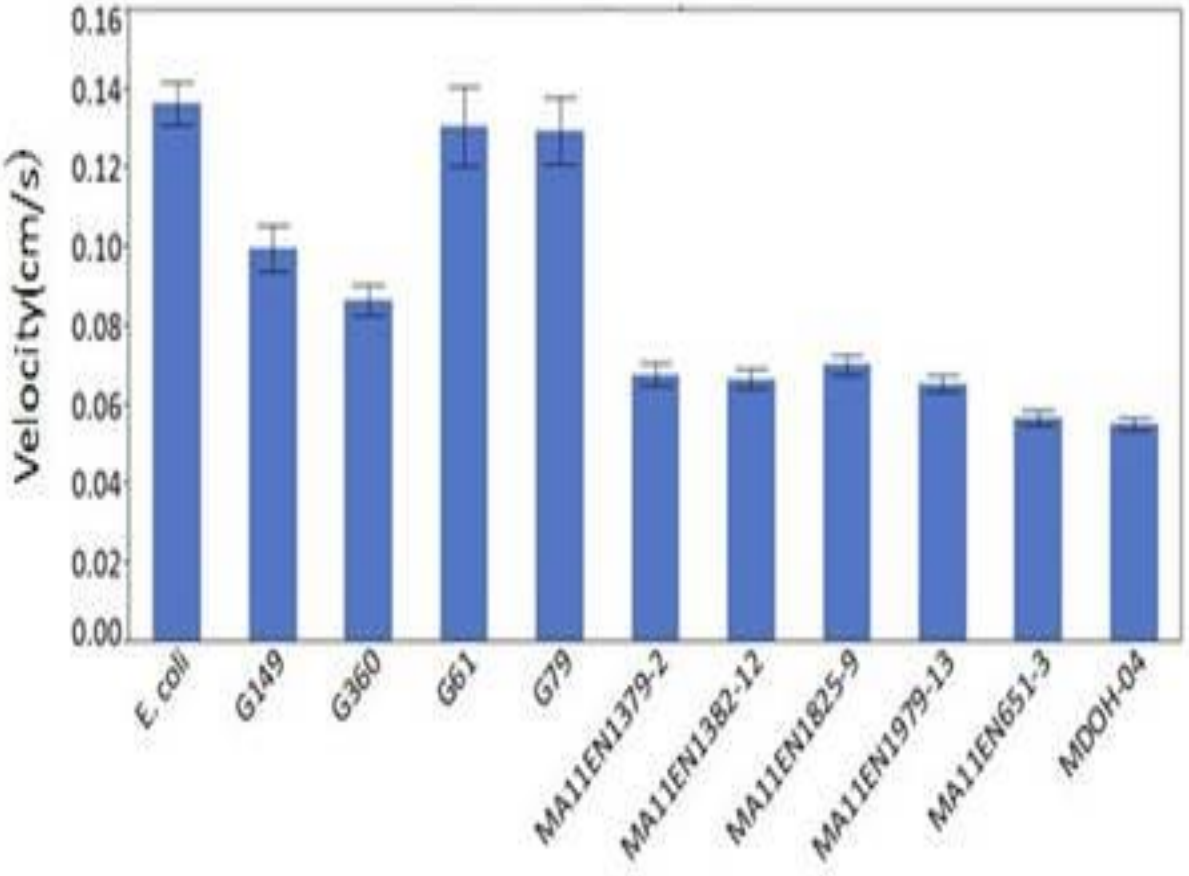


Figure 4. Quantification of virulence using a nematode gastric model. Four clinical strains form MA, including a member of the dominant endemic pathogenic lineage (11EN1382-12), one harboring no known virulence genes (MA11EN1379-2) and four environmental strains from the GBE, including non-pathogenic reference strain (G61) were compared to a highly virulence VpPCC strain (MDOH-04-5M732) and a non-pathogenic E.coli (food). All clinical strains reduced the motility of nematodes. Only environmental strains 149 and 360 showed significant albeit less virulence compared to the clinical strains based on ANOVA. Error bars represent SD from two replicates of 15 worms per treatment.