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Genetic and phylogenetic analysis of *Vibrio parahaemolyticus* reveals distinct differences in strains from the Pacific Northwest of the U.S.

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<https://cedar.wvu.edu/ssec/2014ssec/Day3/4>

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Genetic and phylogenetic analysis of
Vibrio parahaemolyticus reveals distinct differences in
strains from the Pacific Northwest of the U.S.

Rohinee Paranjpye, Jeffery Turner, William Nilsson,
Gladys Yanagida and Mark Strom

Vibrio parahaemolyticus

- Gram-negative, halophilic bacteria
- Natural inhabitant of marine estuarine habitats
- Wide temperature and salinity range
- Accumulate in shellfish through filter-feeding

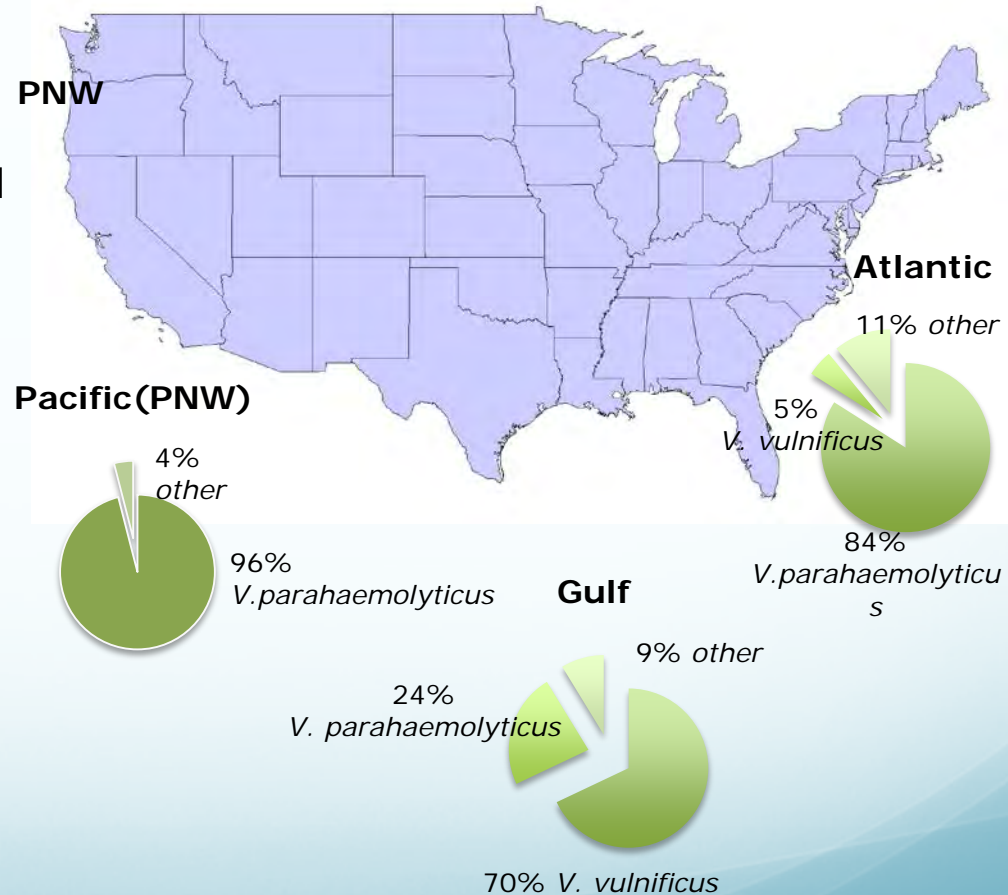


C. Stehr

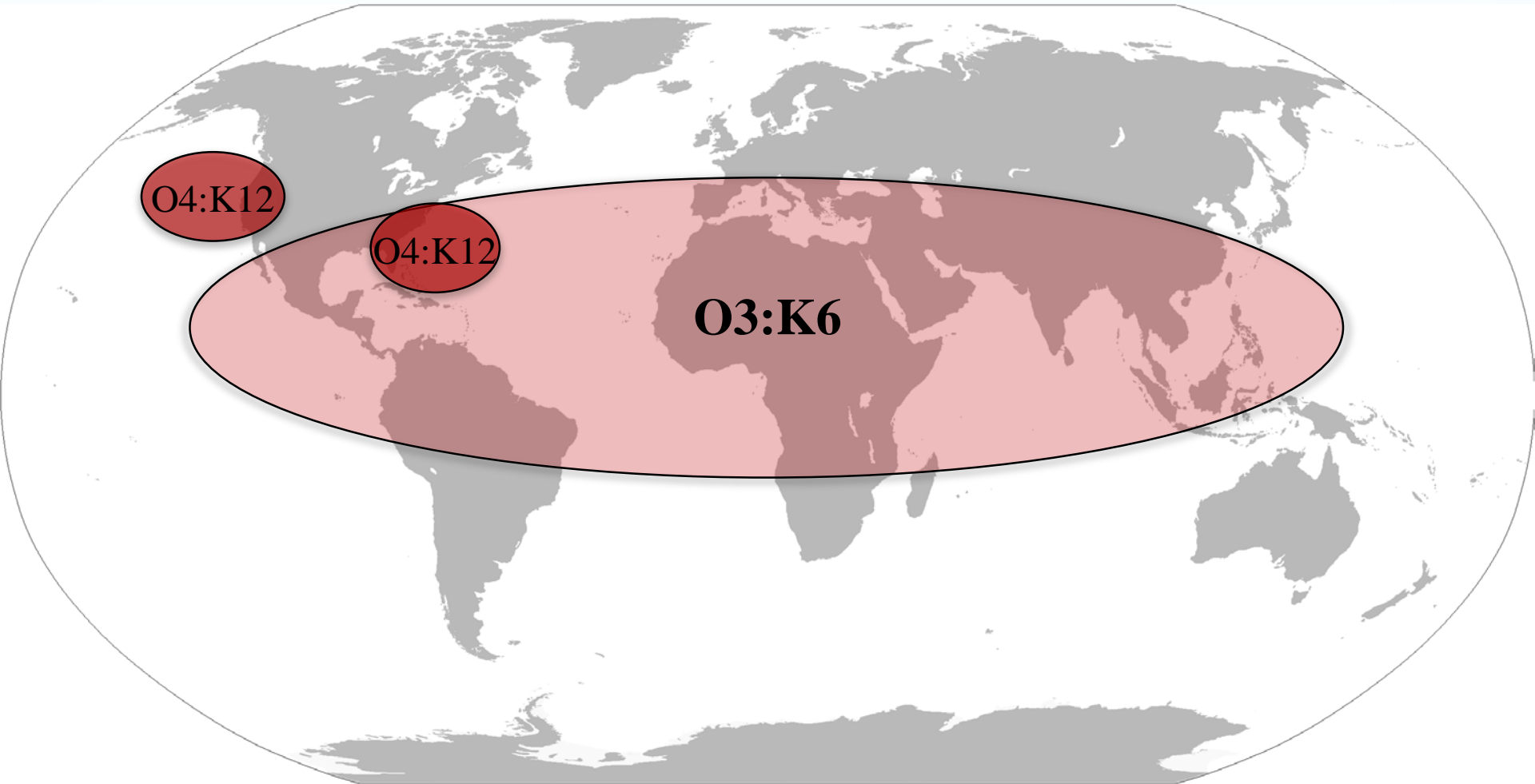
V.p on crab shell

Vibrio parahaemolyticus

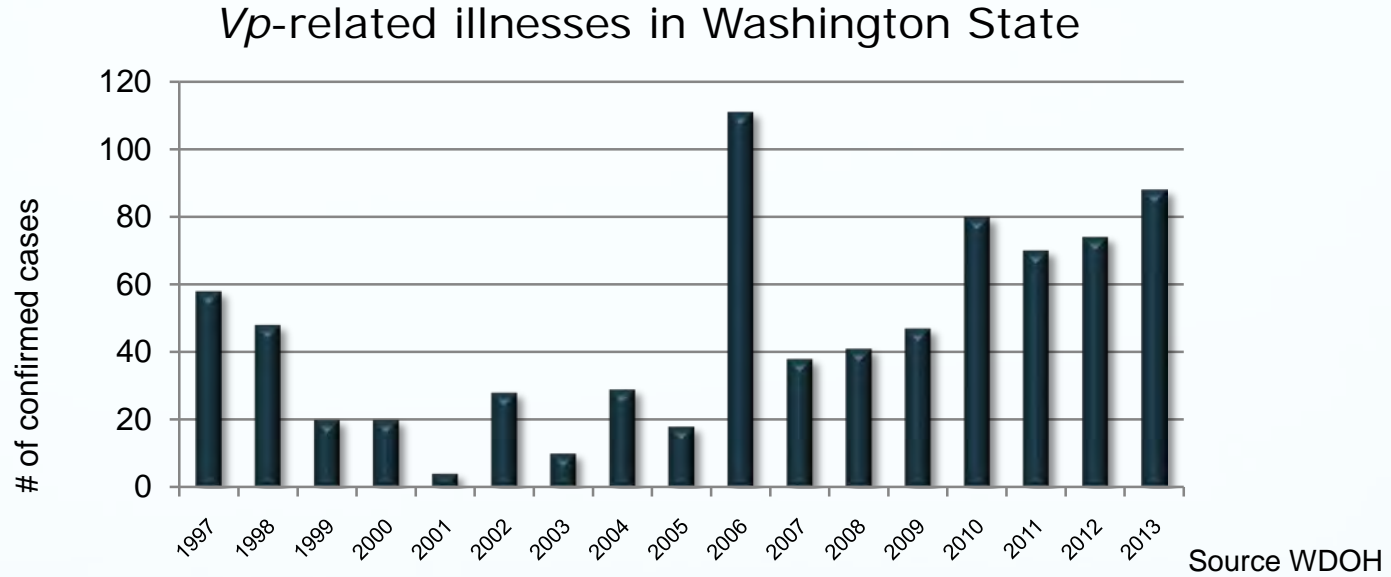
- Most common non-cholerae disease causing *Vibrio* in the U.S.
- Accounts for over 50% of reported *Vibrio* infections in the U.S.
 - Illnesses under-reported (1 in ~140 cases reported)
- Infection by consumption of raw shellfish harboring the bacterium
- Severe, self-limiting gastroenteritis, some wound infections
- Not all strains pathogenic
- Strains that are *tdh+*/*trh+* are considered potentially pathogenic



Emergence of a “pandemic” serotype

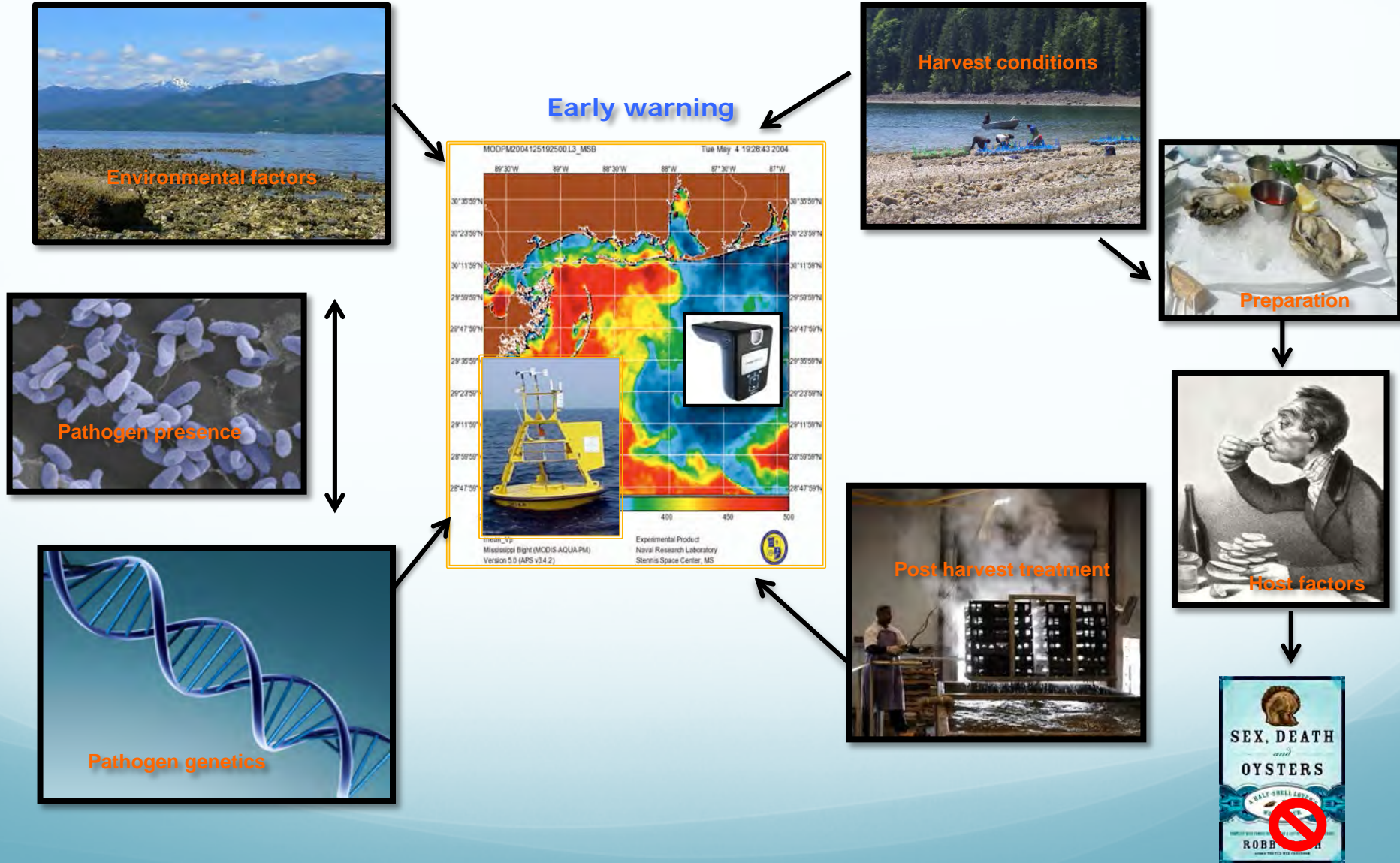


V. parahaemolyticus (*Vp*) in the Pacific Northwest of the U.S.



- Current risk models utilizing temperature and presence of total (*tl+*) or *tdh+* *Vp* have not been predictive for illness in the Pacific Northwest
- Decreasing time after harvest to refrigeration not significantly effective at reducing illness frequency
- Need a better understanding of the environmental factors
- Better markers for predicting pathogenic strains

Vibrio-risk management

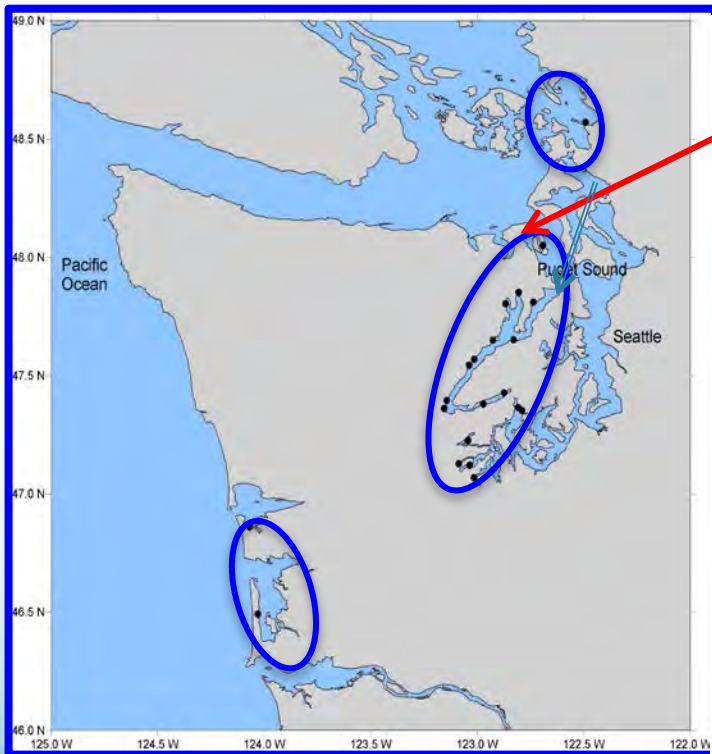


Development of early warning tools for prediction of *Vp* increase in the Pacific Northwest of the U.S.

- Ecological factors
 - abiotic (T, Sal, nutrients etc)
 - biotic (e.g. plankton)
 - hydrodynamic models??
- Pathogen variation
 - strain differences
 - virulence markers

Comparison of environmental variables PNW, GC, MD

Shellfish growing areas in WA

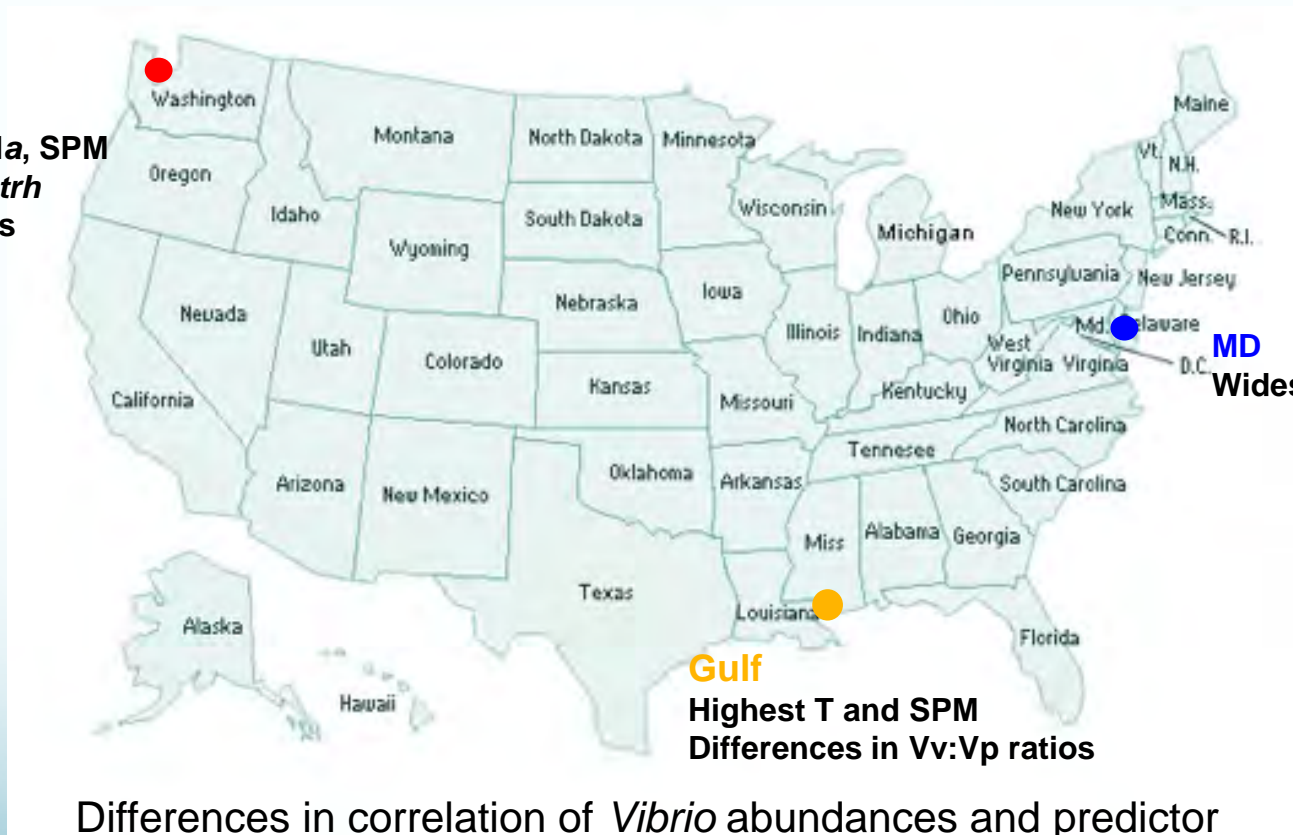


Effect of environmental variables on *Vp* populations

All locations: Temp correlated with *tlh*, but not *tdh* or *trh*

PNW

Higher Sal
Lower T, chl_a, SPM
Higher *tdh*, *trh*
in sediments



MD
Widest T range

Gulf
Highest T and SPM
Differences in *Vv*:*Vp* ratios

Differences in correlation of *Vibrio* abundances and predictor variables depending on geographic location

Precise environmental variables that trigger
increases in pathogenic *V. parahaemolyticus* still
unclear

Risk assessment models will likely need to take into
account differences in geographic locations

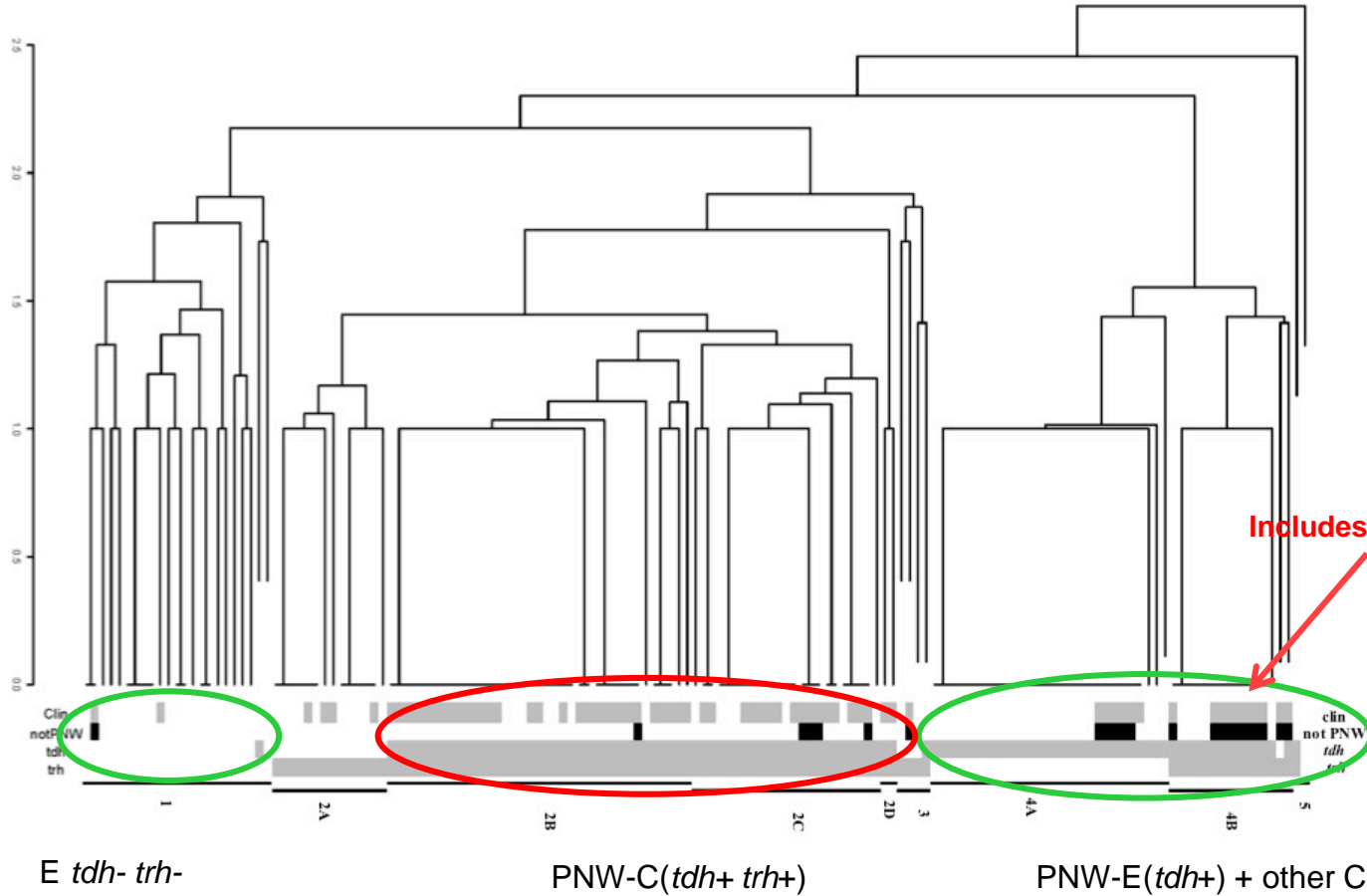
Genetic diversity of *V. parahaemolyticus* strains
from the Pacific Northwest and identification of
biomarkers that can be used for detection of
potentially pathogenic isolates

Genetic diversity of clinical and environmental *Vp* isolates

Clinical and environmental isolates (150) PNW, and other geographic regions

- Genotyping 22 genes (e.g. hemolysins {*tdh*, *trh*}, pandemic markers, T3SS, *gbpA*)
- REP-PCR-Finger print analysis
- Multilocus Sequence Typing (MLST)
- Genome sequencing and comparison-23 strains

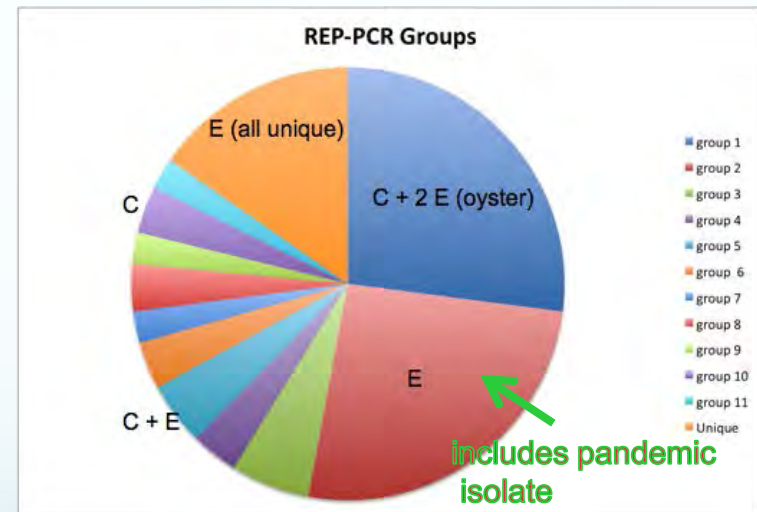
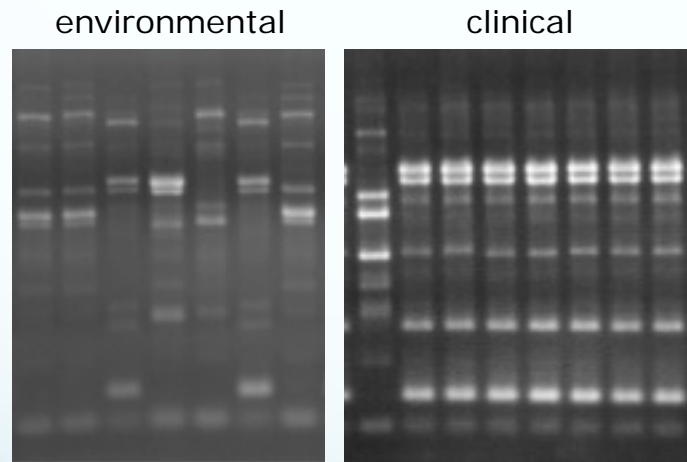
Cluster analysis of *V. parahaemolyticus* isolates



Differences in pathogenic *Vp* strains in the PNW from the pandemic strain-Rep-PCR

Genotypic comparison of selected isolates with clinical isolates from WA state and world-wide

- Rep-PCR (Repetitive extragenic palindromic PCR)

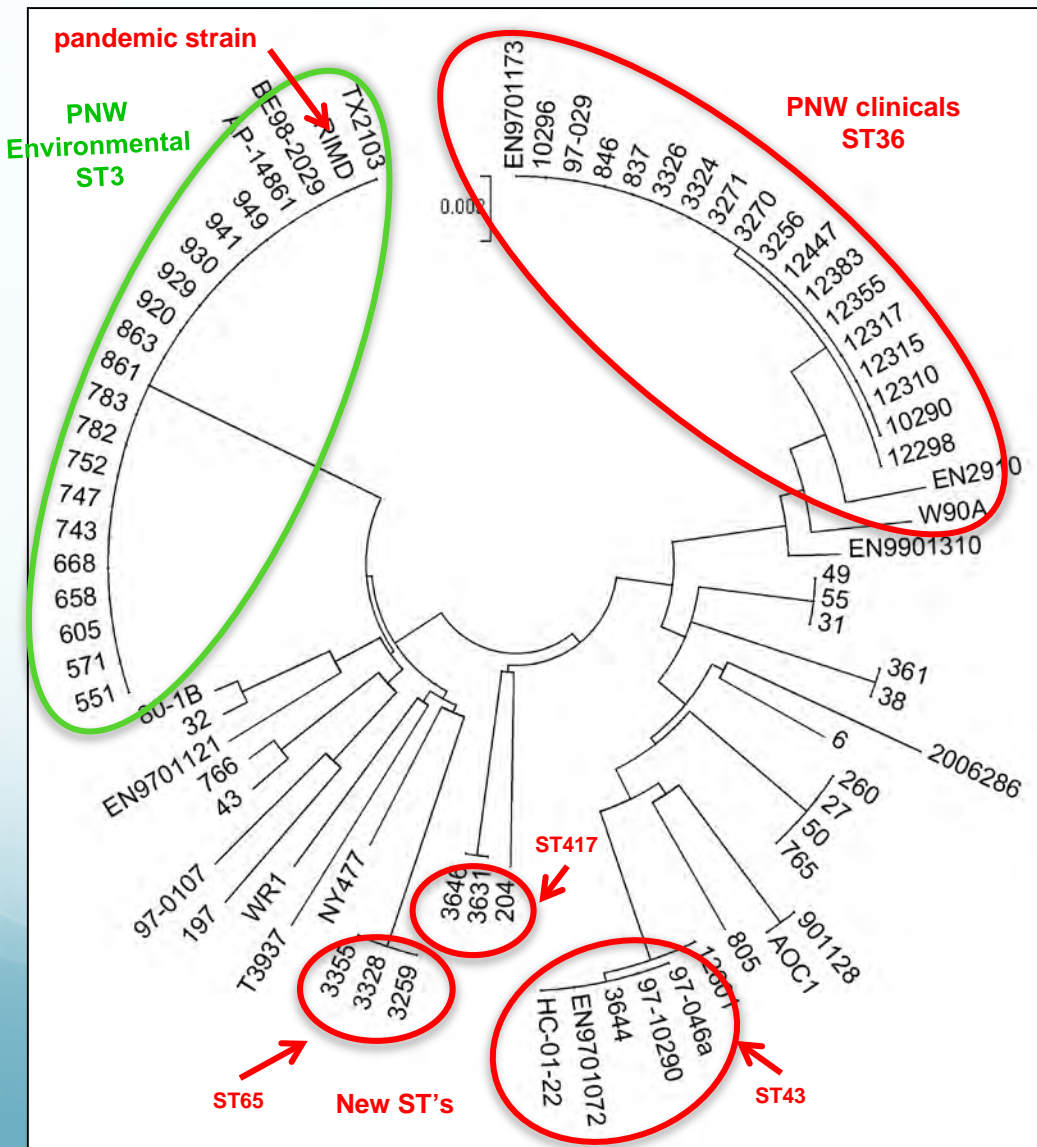


The majority of clinical isolates from the PNW have a similar profile that is distinct from that of the pandemic strain

Multilocus sequence typing

- 78 clinical and environmental isolates
- Typing based on DNA sequences of 7 housekeeping genes (*dnaA*, *dtdS*, *gyrB*, *pntA*, *pyrC*, *recA*, *tnaA*)
- Different sequences assigned to distinct alleles-alleles at 7 loci define ST

Population structure of *V. parahaemolyticus* from the Pacific Northwest



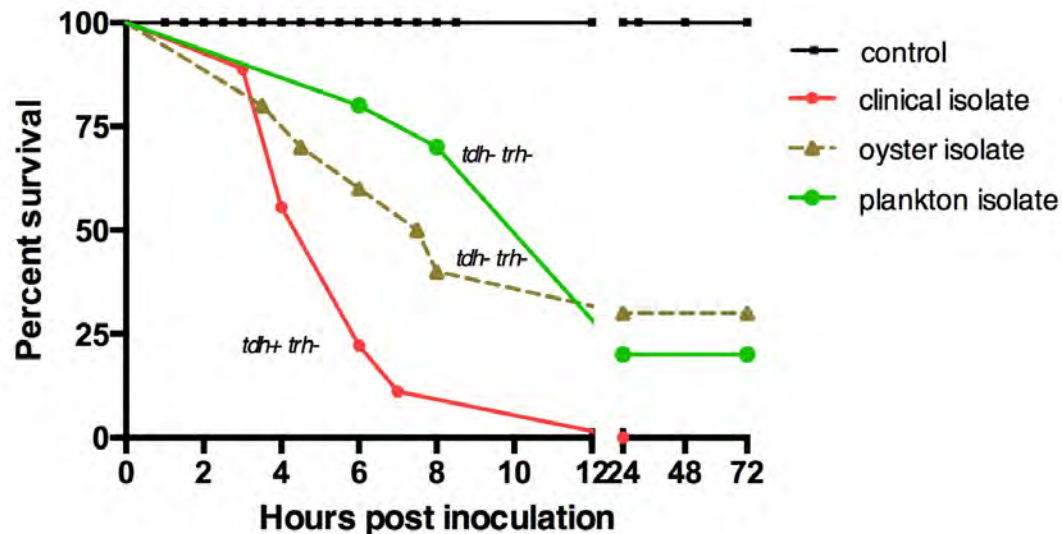
- PNW population semi-clonal: 2 large clades
- Environmental clade clonally related to the pandemic strain
 - *tdh+*/*trh-* strains
 - clinical strains from other geographic regions
- PNW clinical clade
 - endemic strains from PNW (*tdh+*, *trh+*)
- At least 5 other clinical sequence types identified
- 3 new ST's
- Sequenced 23 representative strains

Are the *tdh+* *trh-* environmental isolates likely to be pathogenic?



Comparison of virulence profiles of strains using zebrafish model of infection

Virulence based on survival time



*Poster: Zebrafish as a model for *Vibrio parahaemolyticus* virulence. Paranjpye *et al.*

Publication: Paranjpye *et al.* 2013. *Microbiology*. 129:2615.

Comparison of virulence of *V. parahaemolyticus* isolates*

| Source | Genotype | Survival time (h) | MLST(ST) |
|---------------------------------------|---------------------------|-------------------|----------|
| Water (WA) | <i>tdh+</i> / <i>trh-</i> | 22.0 ± 0 | 3 |
| Plankton (WA) | <i>tdh+</i> / <i>trh-</i> | 22.0 ± 0 | 3 |
| Clinical RIMD2210633 (pandemic) | <i>tdh+</i> / <i>trh-</i> | 6.15 ± 1.11 | 3 |

- Strains with the same genotype or ST may vary in virulence
- Significant concentration of *tdh+*/*trh-* environmental isolates from the PNW may not be pathogenic

*using the zebrafish model

Summary

- Majority of strains causing clinical infections in the PNW distinct from pandemic complex
- Strains with profiles similar to that of the pandemic complex present in the PNW environment
- Presence of *tdh+* by itself is not an adequate predictor of virulence

Future directions

- Assess the correlation of the potentially pathogenic strains from the PNW with specific environmental factors
- Identify and validate the genetic and virulence markers that differentiate *V. parahaemolyticus* strains from the PNW and those of the pandemic strain
- Utilize the information to develop more refined
 - risk assessment models
 - early warning systems

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