INVESTIGATIONS OF PATHOGENIC VIBRIO PARAHAEMOLYTICUS POPULATION DYNAMICS AND VIRULENCE DETERMINANTS OF HAWAIIAN WATERS

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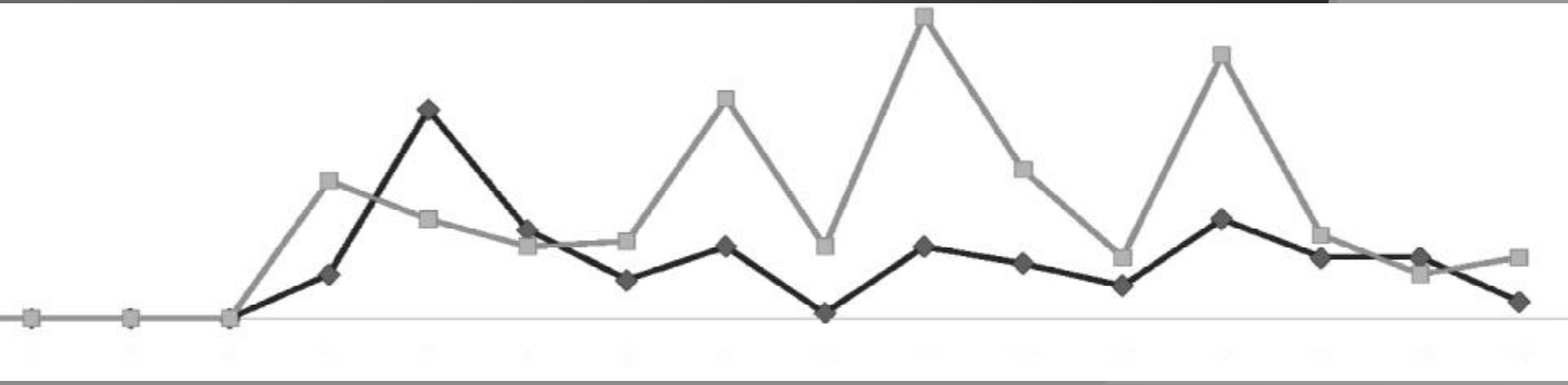
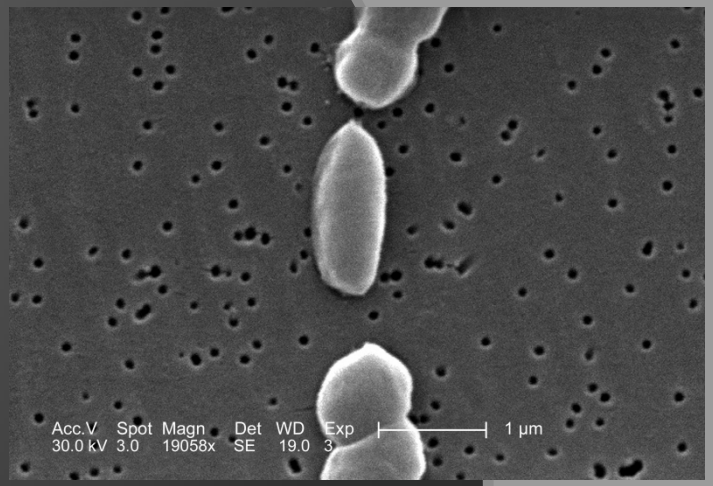


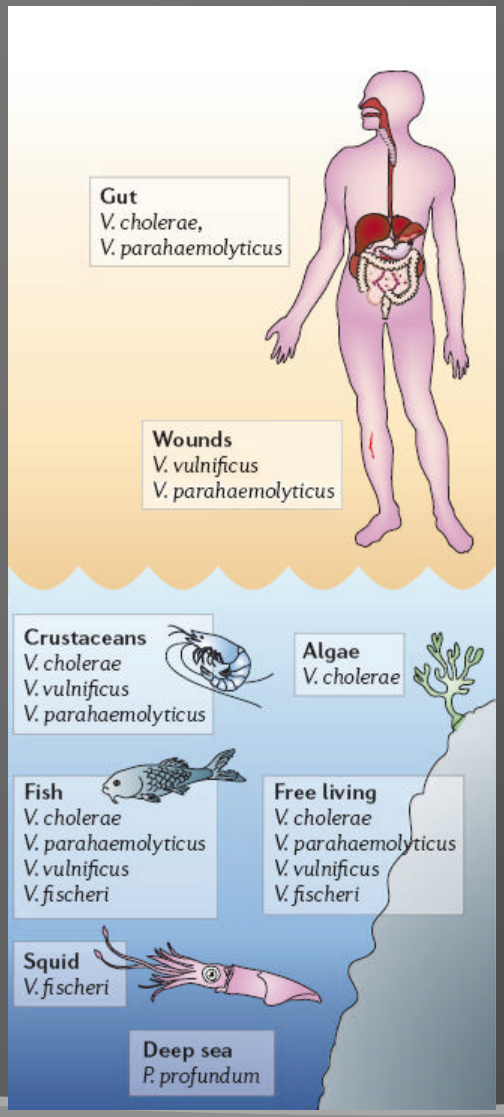
What defines *Vibrio*?



CDC, 2005

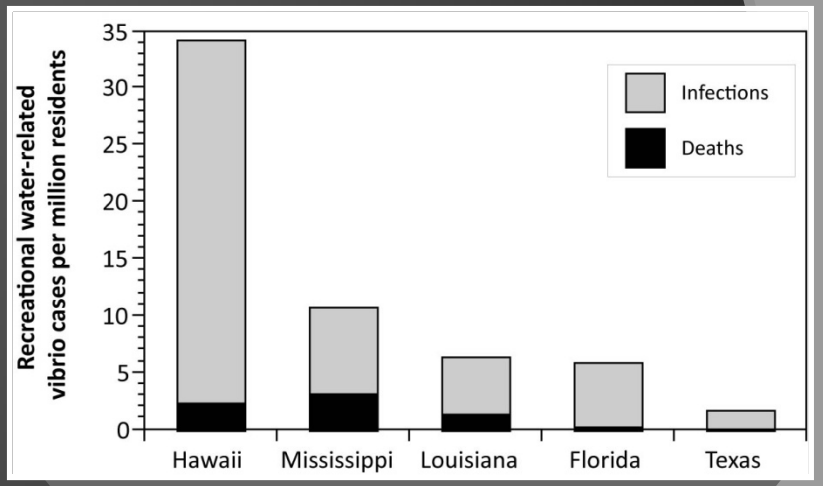
- ✓ Genus of Gram-negative bacteria
- ✓ Halophilic (Requires Saltwater)
- ✓ Free-swimming
- ✓ Naturally occurring in coastal regions around the world
- ✓ 65 known species: only 12 harmful to humans.
- ✓ **BIG 3:** *V. cholerae, V. vulnificus, V. parahaemolyticus*
- ✓ Pathogens detrimental to *both* humans and marine animals

Human Health



F. Jerry Reen et. al. 2006

Most disease causing strains are associated with **gastroenteritis** but can also infect open wounds and cause septicemia. **Not** all strain cause disease.



Hawai'i has the **highest** *per captia* infectious cases of *Vibrio* of all US states

✓ Year-round tropical climate

✓ Visitor Influx

Vibrio parahaemolyticus

para-haemo-lyticus

hemo (BBlood)

lyses

Blood Agar Media

The disease mechanism of *V. parahaemolyticus* infections has not been fully elucidated. However, most clinical disease results from strains that carry either the **thermostable direct hemolysin** gene (*tdh*) or the **thermolabile haemolysin** gene (*tlh*).

Ala Wai:

Google Earth © 2008

Objectives:

1. **Determine** the abundance
2. **Characterize** and screen for the presence of virulence associated genes.
3. **Understand** how environmental conditions influence abundance and the relative proportion.
4. **Relationship** to the Ala Wai watershed and the surrounding marine ecosystems of Waikiki, Hawaii.

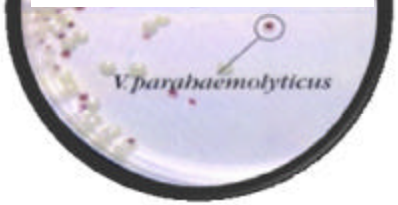
Approach:

Water Sample

3

Filter

Grow Isolates



PCR

tlh

tdh

2

1

4

Temp

Salinity

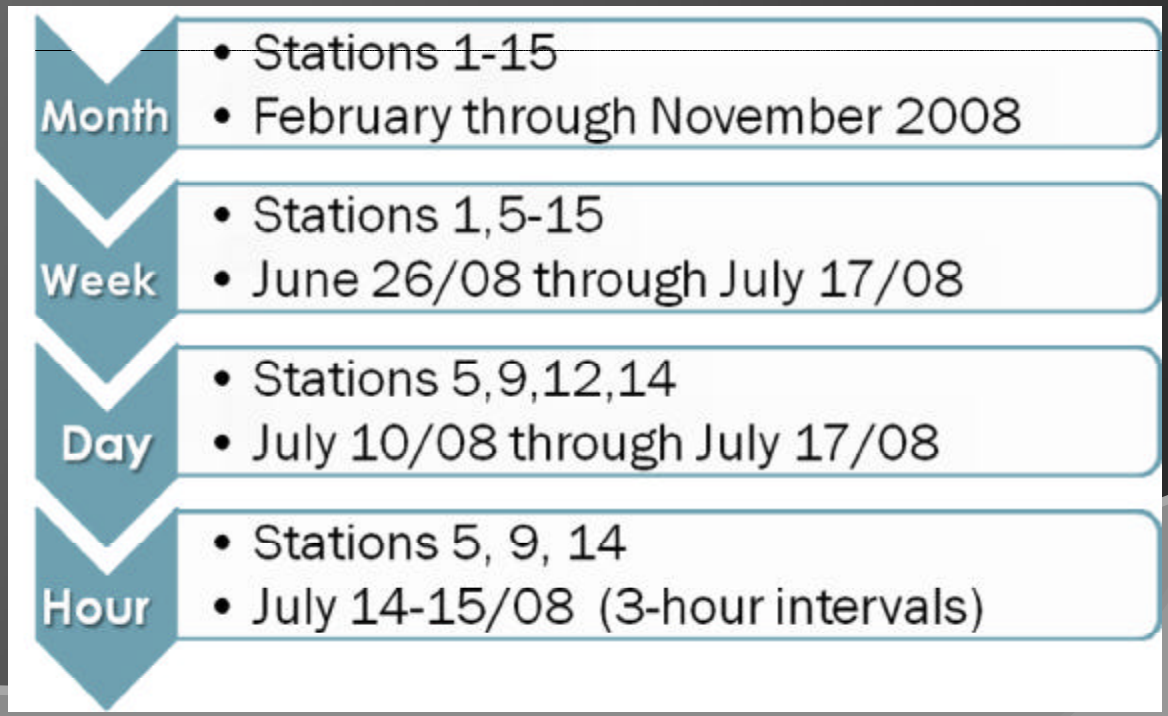
Nutrients, Dissolved iron, TOC, POC, and Chlorophyll *a*

Incomplete analysis – will be included in future analysis.

Time Scheme:

- Started in February 2008
- Monthly time series
- Variations on shorter timescales
- Collected during the morning to early afternoon hours.

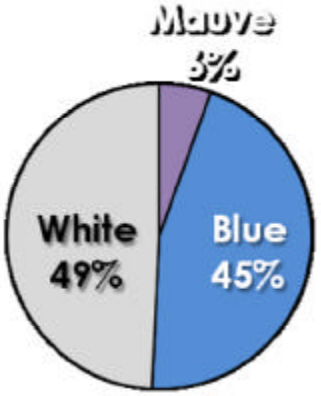
Nested hierarchical time series scheme:



Results:

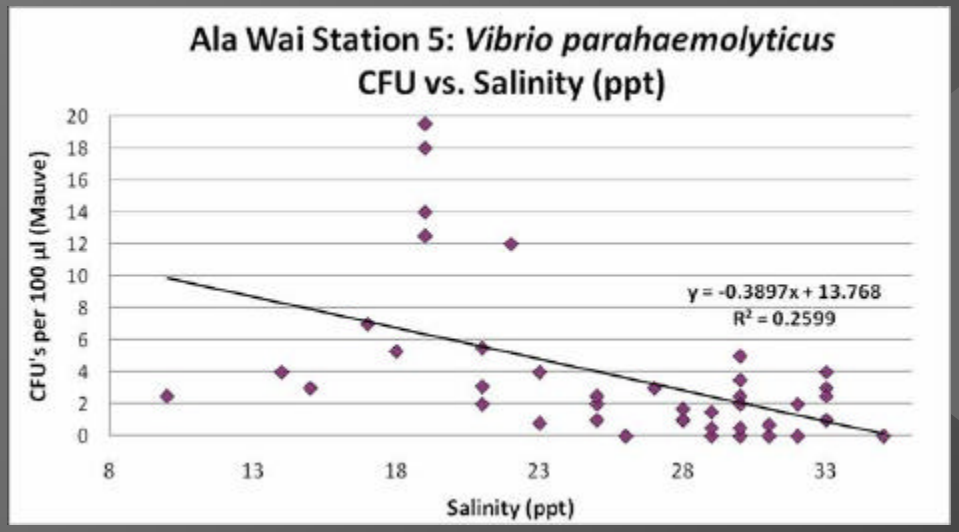
Geographical Representation:

Mean CFU Ratio:



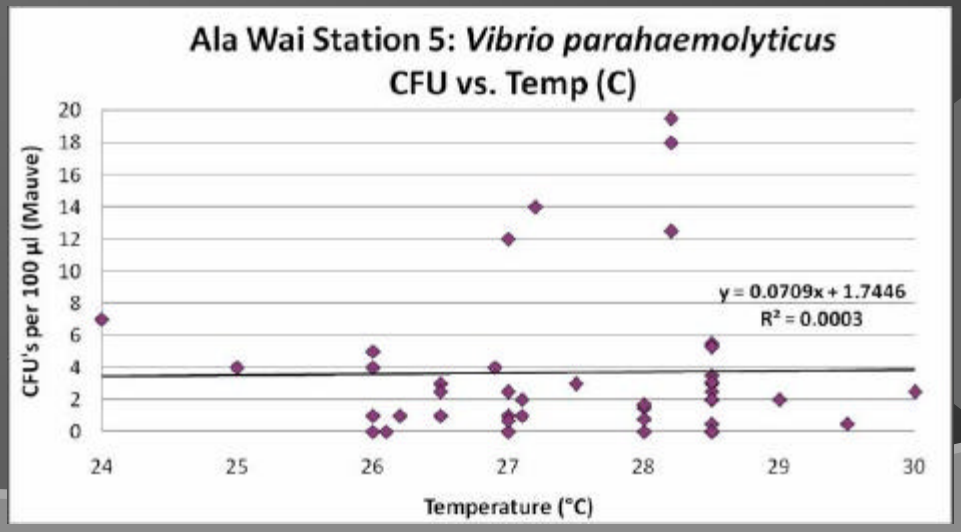
Mauve = *V. parahaemolyticus*
Blue = *V. cholera*
White = *V. alginolyticus*

Results:



SALINITY (‰)

Salinity compared with *Vibrio parahaemolyticus* (Mauve, CHROMagar *Vibrio* Media) CFU's

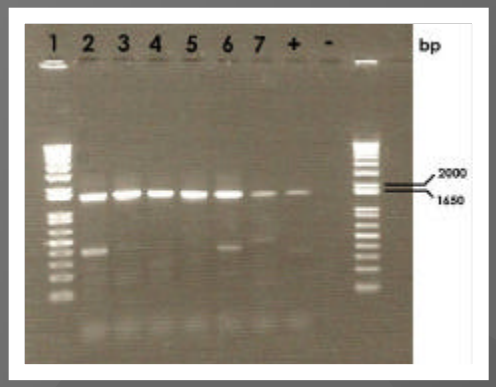


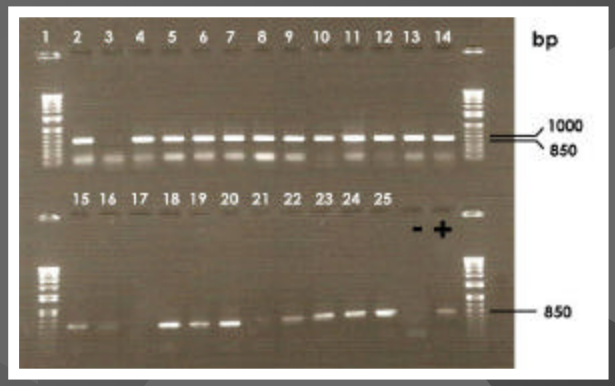
TEMPERATURE (°C)

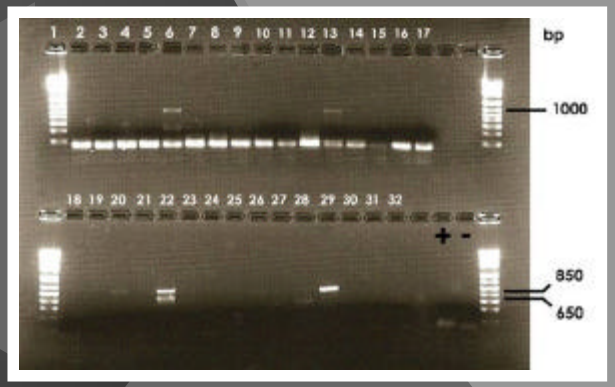
Temperature compared with *Vibrio parahaemolyticus* (Mauve, CHROMagar *Vibrio* Media) CFU's

PCR:

Gel-electrophoresis Assays:







16s

Gene sequences contain hypervariable regions which can provide species-specific signature sequences useful for bacterial identification

tlh

Amplification of species specific primers targeted to *Vibrio parahaemolyticus*

POSTIVE

tdh

Virulence determinate primers targeted to *Vibrio parahaemolyticus.*

NEGATIVE

Discussion:

We found that...

1. *V. parahaemolyticus* **is present** in the Ala Wai.

2. Although present, **no virulent** strains were found.

3. *V. parahaemolyticus* abundance varied geographically, however most probable correlation was dependent on **salinity.**

4. Temperature and salinity are dependant on incoming solar radiation, precipitation, and location along the canal.

Conclusion:

The spread of infectious diseases by water-borne human pathogens is a serious problem.

Vibrio parahaemolyticus, is especially a concern in tropical regions of the world.

Our analysis of the data substantiates that the waters surrounding Waikiki and the Ala Wai Canal carry the *Vibrio parahaemolyticus* bacterium, however confirmation of virulence has yet to be proven.

Acknowledgements:

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Ms. Olivia Nigro

PCRMB, CMORE, professors and fellow peers.

Questions?