

Environmental Considerations for *Vibrio parahaemolyticus* in Puget Sound Oysters, Water, and Sediments

Russell P. Herwig

School of Fisheries
University of Washington
Seattle, WA

Daniel P. Cheney

Pacific Shellfish Institute
Olympia, WA

Research Support: Interstate Shellfish Sanitation Conference
(ISSC) and School of Fisheries, University of Washington

Other Research Personnel

- Robyn Estes
 - ◆ University of Washington
- Cindy Messey
 - ◆ Pacific Shellfish Institute

Introduction

- Objectives: Enumerate levels of *V. parahaemolyticus* in southern Puget Sound
 - ◆ Oysters, sediment, water
 - ◆ Summer 1999
- Reason: *V. parahaemolyticus* may cause foodborne illness - outbreaks in recent years
 - ◆ Raw product or poor handling of cooked product
 - ◆ Symptoms: diarrhea, cramps
 - ◆ Regulated levels: 10,000 cells per gram

Vibrio parahaemolyticus

■ Ecology

- ◆ Worldwide distribution in temperate and tropical environments; marine and estuarine waters
- ◆ Associated with water, sediment, plankton, fish, shellfish
- ◆ Higher numbers during summer months
 - ✦ Warmer temperatures
 - ✦ Zooplankton blooms

Vibrio parahaemolyticus

■ Physiology

- ◆ Heterotrophic organism
 - ✦ Organic compounds for growth
 - Wide variety metabolized
- ◆ Facultative anaerobe
 - ✦ Capable of growth with or without oxygen
- ◆ Require salt
- ◆ Temperature range of growth: <15 to 40°C
- ◆ Rapid doubling time
 - ✦ 8 - 9 minutes at 37°C

Enumeration Protocols

- Currently accepted protocols
 - ◆ FDA (Bacteriological Analytical Manual)
 - ◆ May take over a week for complete test
 - ◆ Extremely labor intensive - manual manipulation and transfers
- Alternative molecular methods
 - ◆ Supplies and equipment, more expensive
 - ◆ Potentially more rapid; less labor, less expensive

Currently Accepted FDA MPN Protocols

- Samples to laboratory
- Blend and dilute
- Inoculate series of tubes - Alkaline Peptone Water. Incubate overnight
- Streak turbid tubes onto TCBS Agar. Incubate overnight
- Pick suspect colonies from TCBS and perform several tests on isolates

Developing a MPN-Molecular Method

- Start the same
 - ◆ Samples to laboratory
 - ◆ Blend and dilute
 - ◆ Inoculate series of tubes - Alkaline Peptone Water. Incubate overnight
- Molecular steps
 - ◆ Transfer small aliquot from each tube into polymerase chain reaction (PCR)
 - ◆ Evaluate for PCR product on by agarose gel electrophoresis

Processing samples



Collecting oysters, sediment and water

Shucking



Processing samples



Blending samples

Inoculating APW
Deep-well microtiter plates



Processing samples

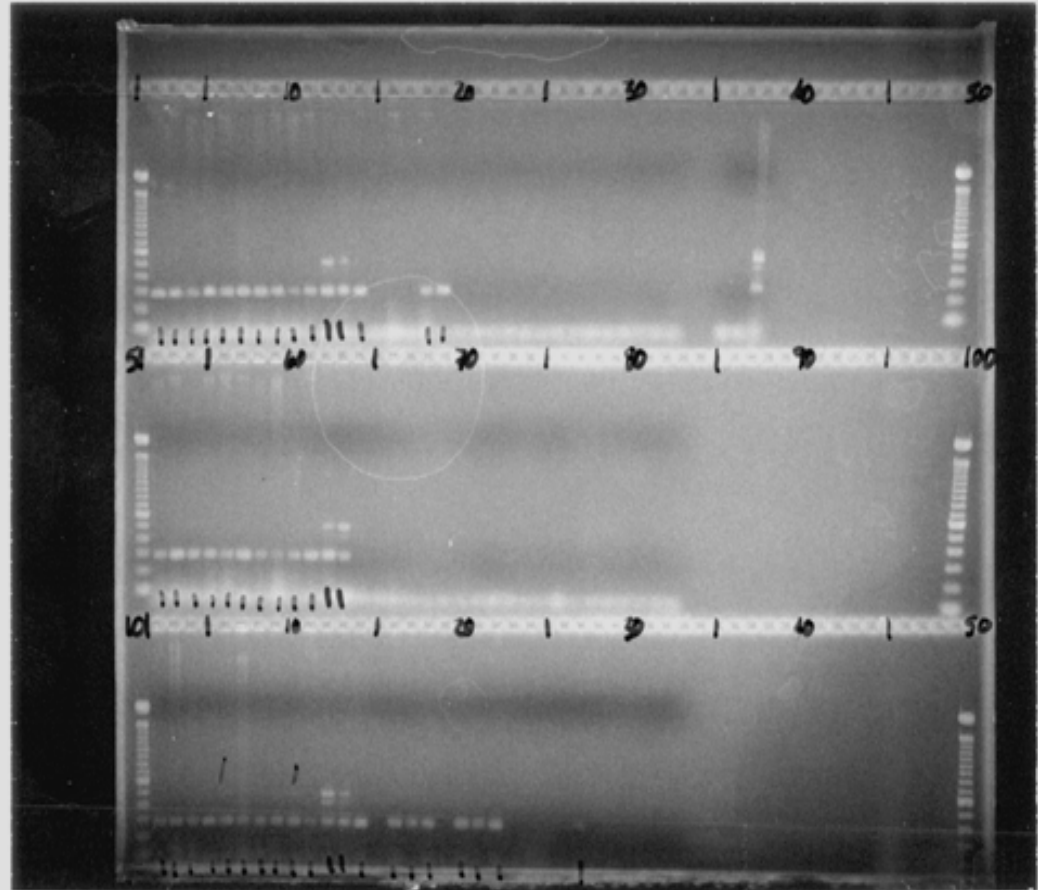


Performing PCR
Aliquots from PCR onto
agarose gel

Molecular Detection Method for *Vp*

- Selection of gene or specific DNA fragment associated with target species
- *gyrB* - Venkateswaran et al. 1998
 - ◆ Gene for DNA gyrase - enzyme for DNA replication
- Polymerase Chain Reaction
 - ◆ Target DNA (*gyrB*) for *V. parahaemolyticus* is amplified
- Detection method
 - ◆ Agarose gel electrophoresis

Agarose gel of *gyrB* PCR product from Alkaline Peptone Water MPN Tubes



RME 8-12-99

Summary of UW Molecular Methods

- **Results within 24 - 32 hours**
- **Multiplex PCR possible for:**
 - ◆ Total numbers of *Vp* (*gyrB*)
 - ◆ TDH positive *Vp* (*tdh+*)
 - ◆ TRH positive *Vp* (*trh-*)
- **Detection limits in sample**
 - ◆ Oysters: 0.36 MPN/g
 - ◆ Sediment: 0.90 MPN/g
 - ◆ Water: 0.018 MPN/ml
- **PCR requirement: 1 - 12 cells**

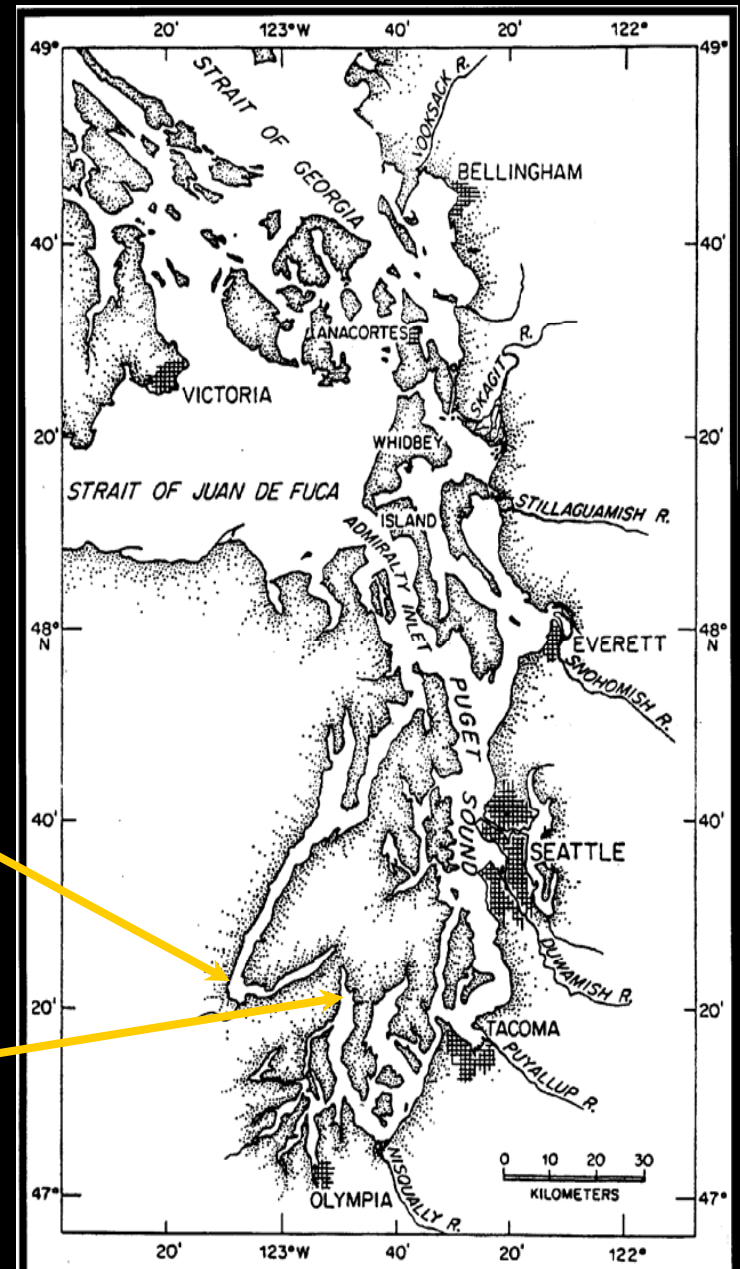
Work Plan

- Samples: oysters, sediment, water
- Sites in Puget Sound, WA
 - ◆ Hood Canal (Sisters Point)
 - ◆ Puget Sound main basin
 - ◆ Case Inlet (Allyn), Totten Inlet, Mud Bay
- Dates: June - September 1999
 - ◆ 2 week intervals - lowest low tide cycles
- Samplers: Pacific Shellfish Institute

Puget Sound Sample Sites

Hood Canal

Case Inlet



Hood Canal



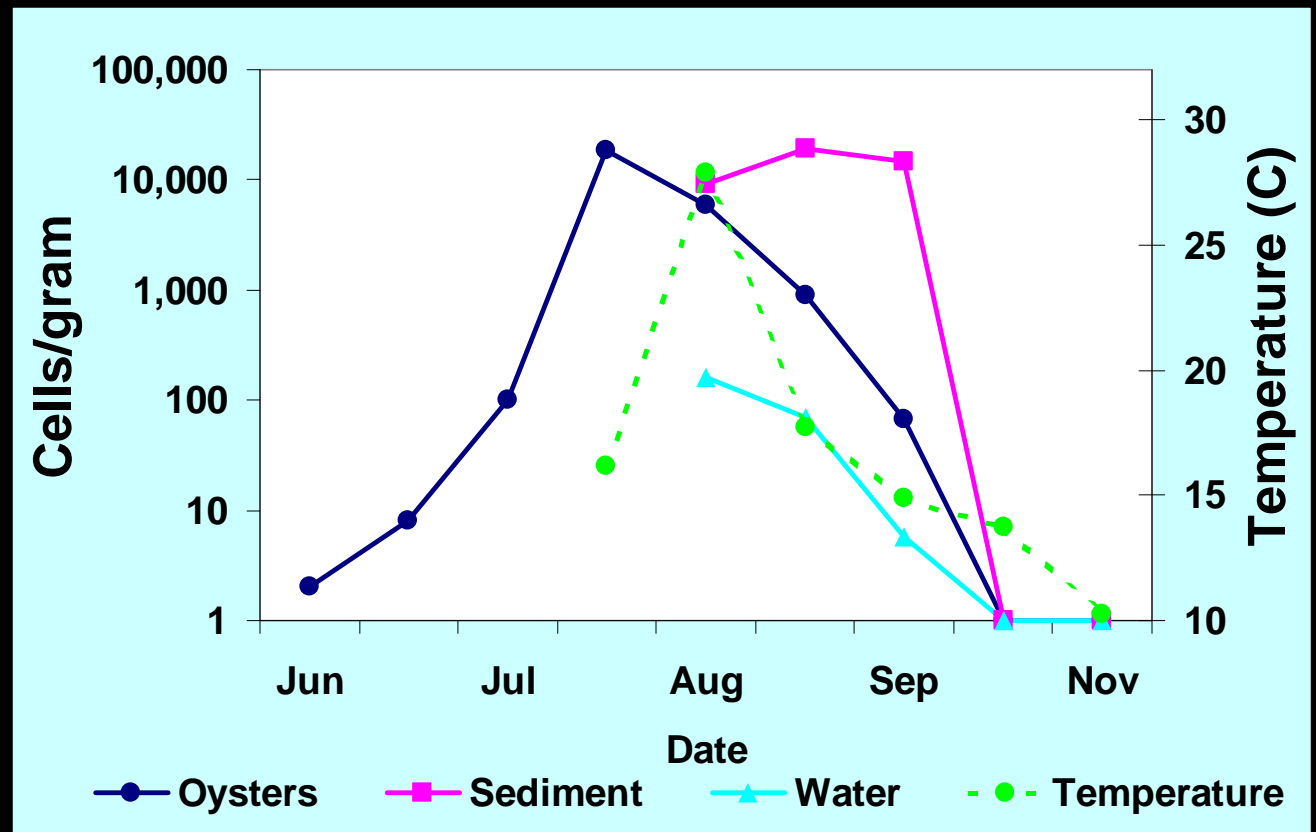
Hood Canal



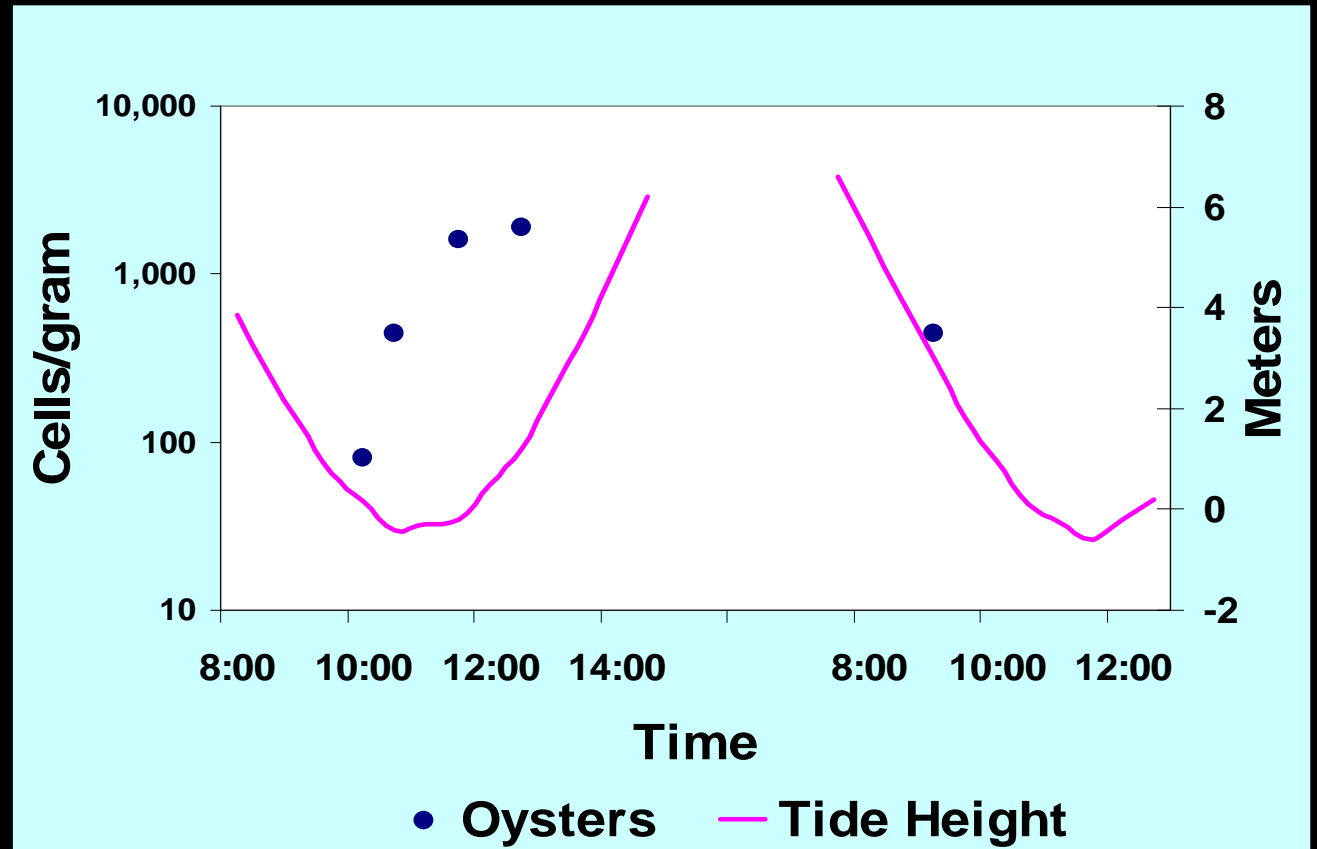
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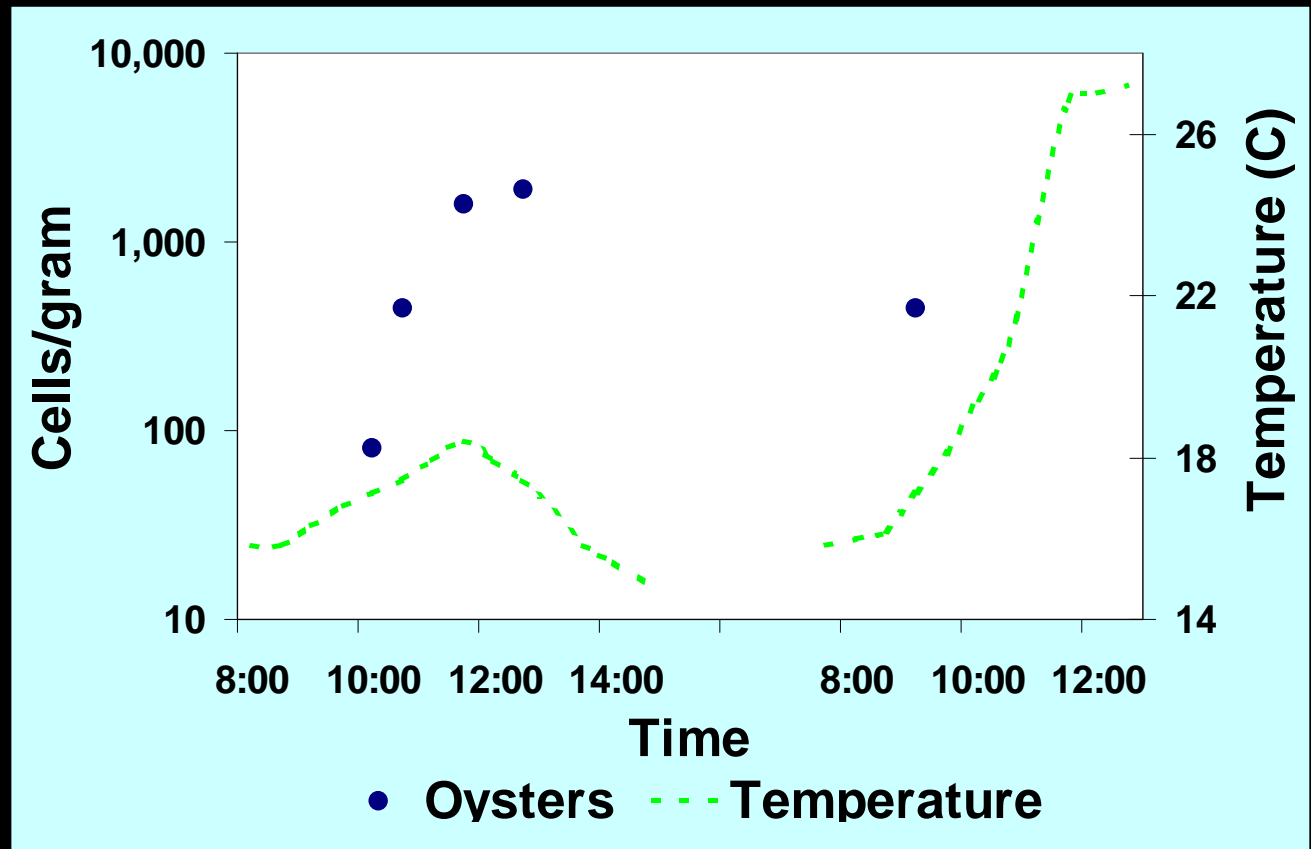
Hood Canal - Oysters, Sediments, Water and Temperature



Hood Canal - Exposure during low tide - 25 & 26 August



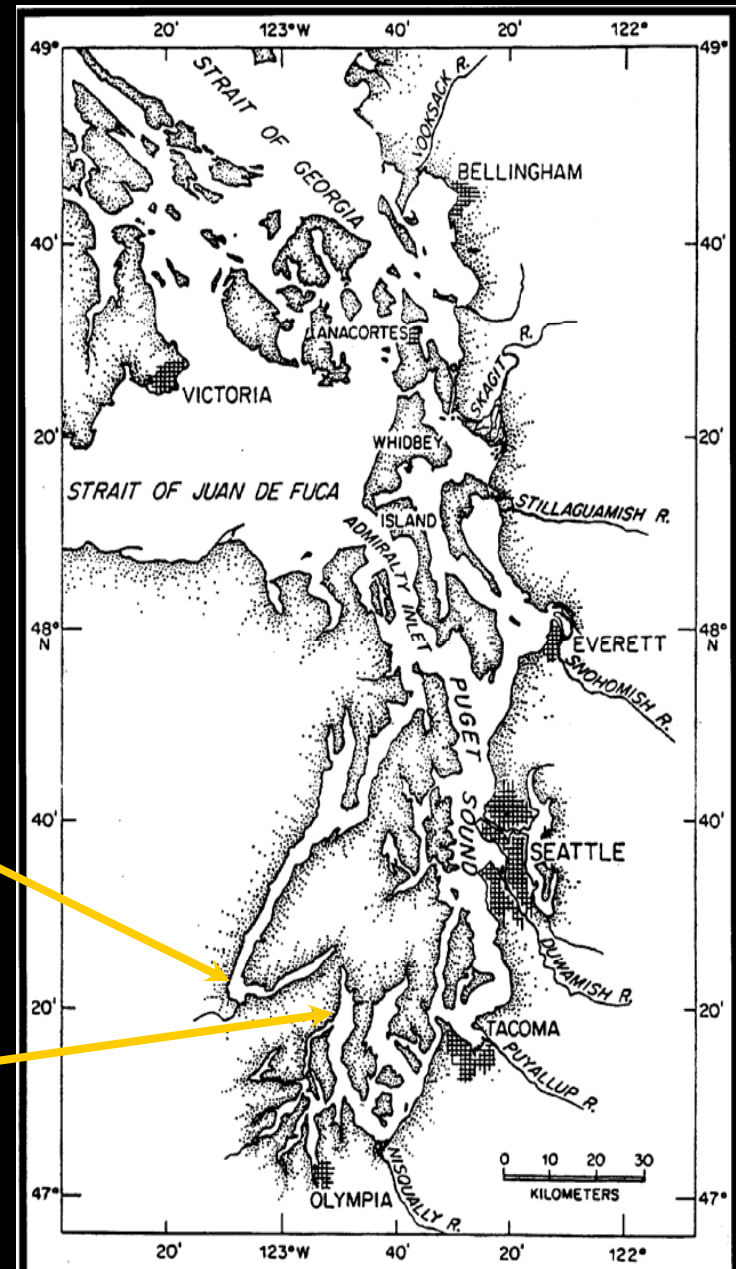
Hood Canal - Exposure during low tide - 25 & 26 August



Puget Sound Sample Sites

Hood Canal

Case Inlet



Case Inlet



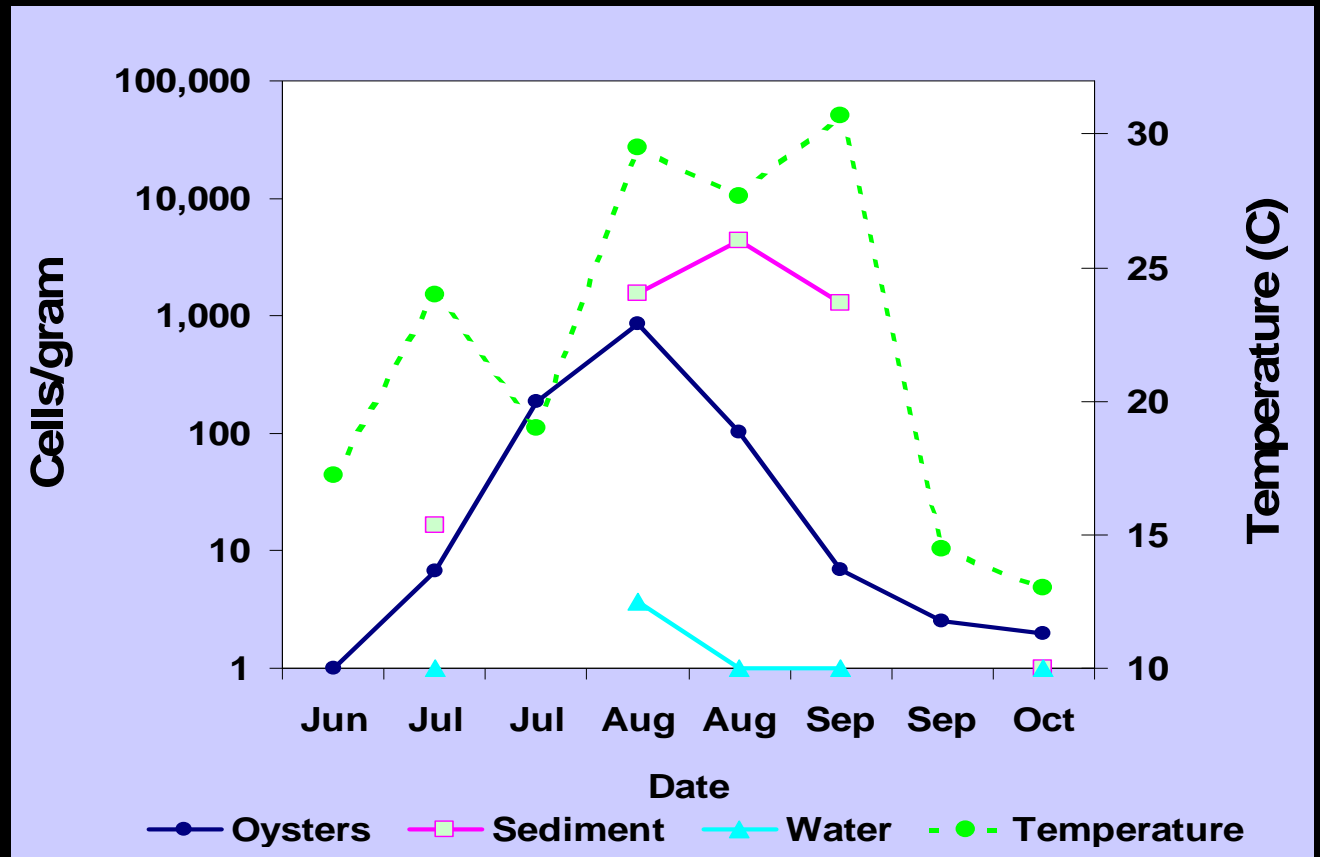
Case Inlet



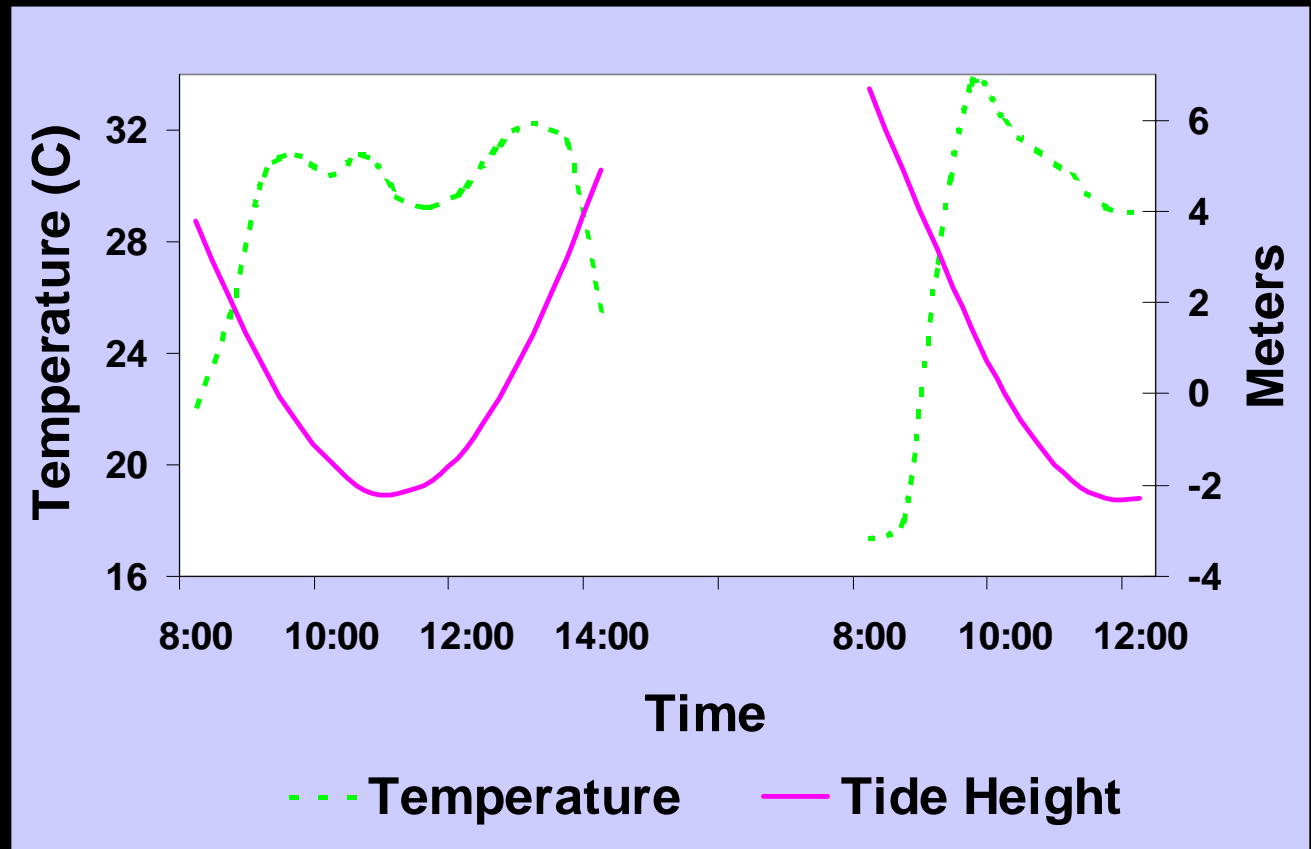
Case Inlet



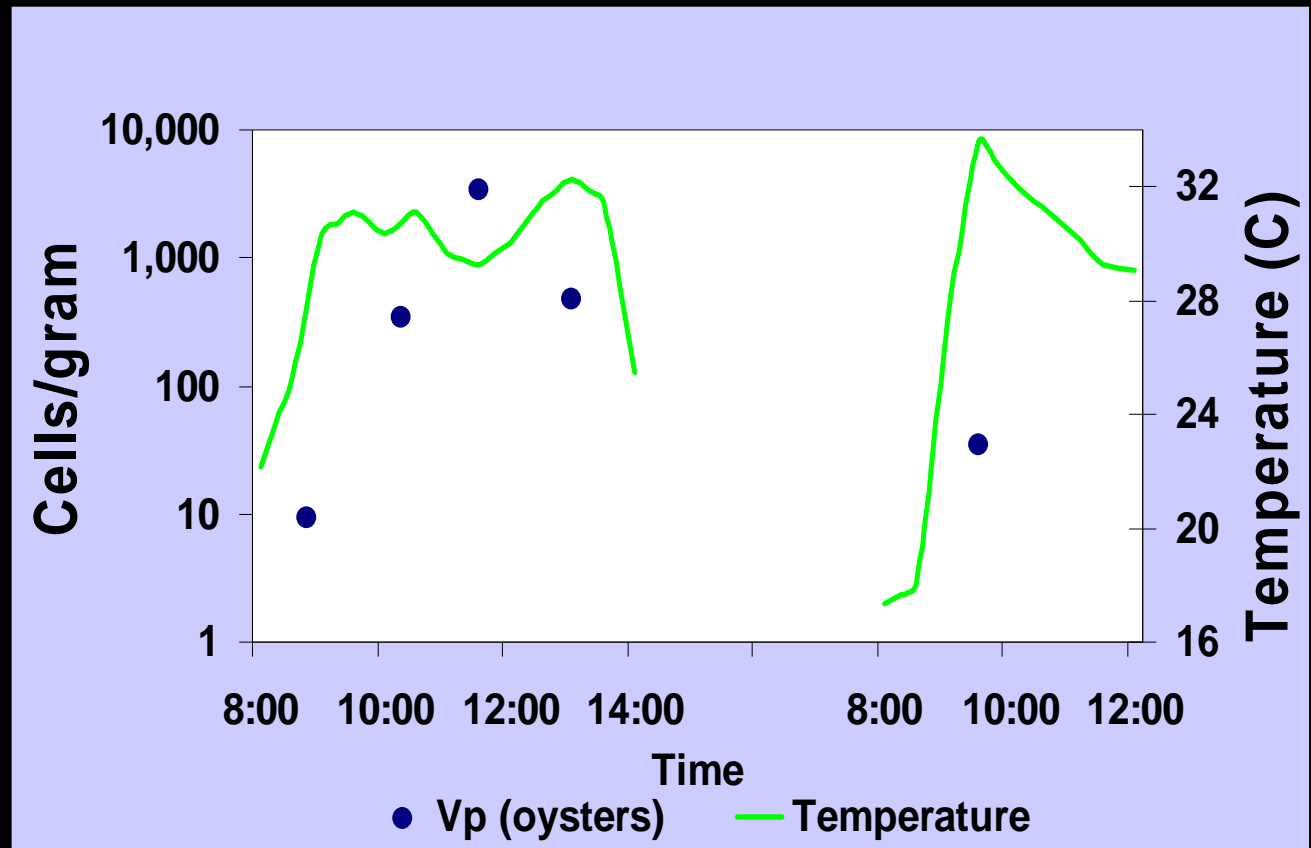
Case Inlet - Oysters, Sediments, Water and Temperature



Case Inlet - Oyster exposure during low tide - 9 August



Case Inlet - Oyster exposure during low tide - 9 August

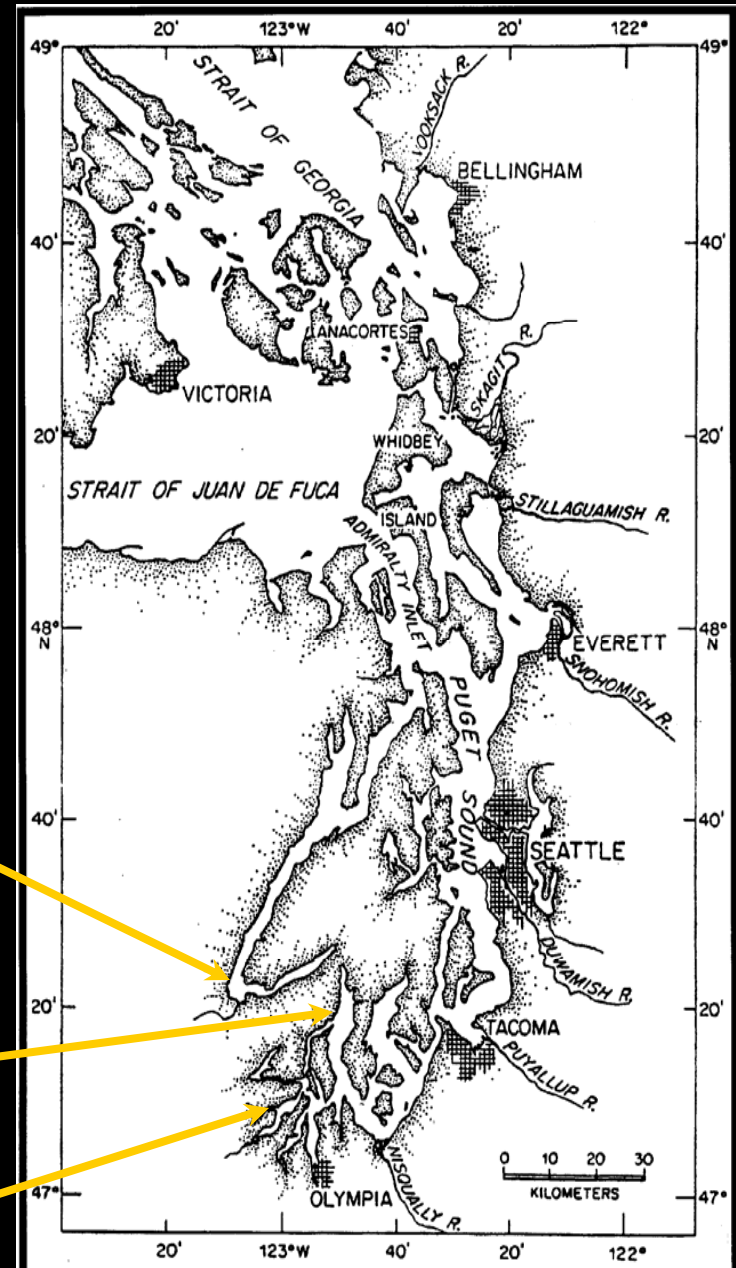


Puget Sound Sample Sites

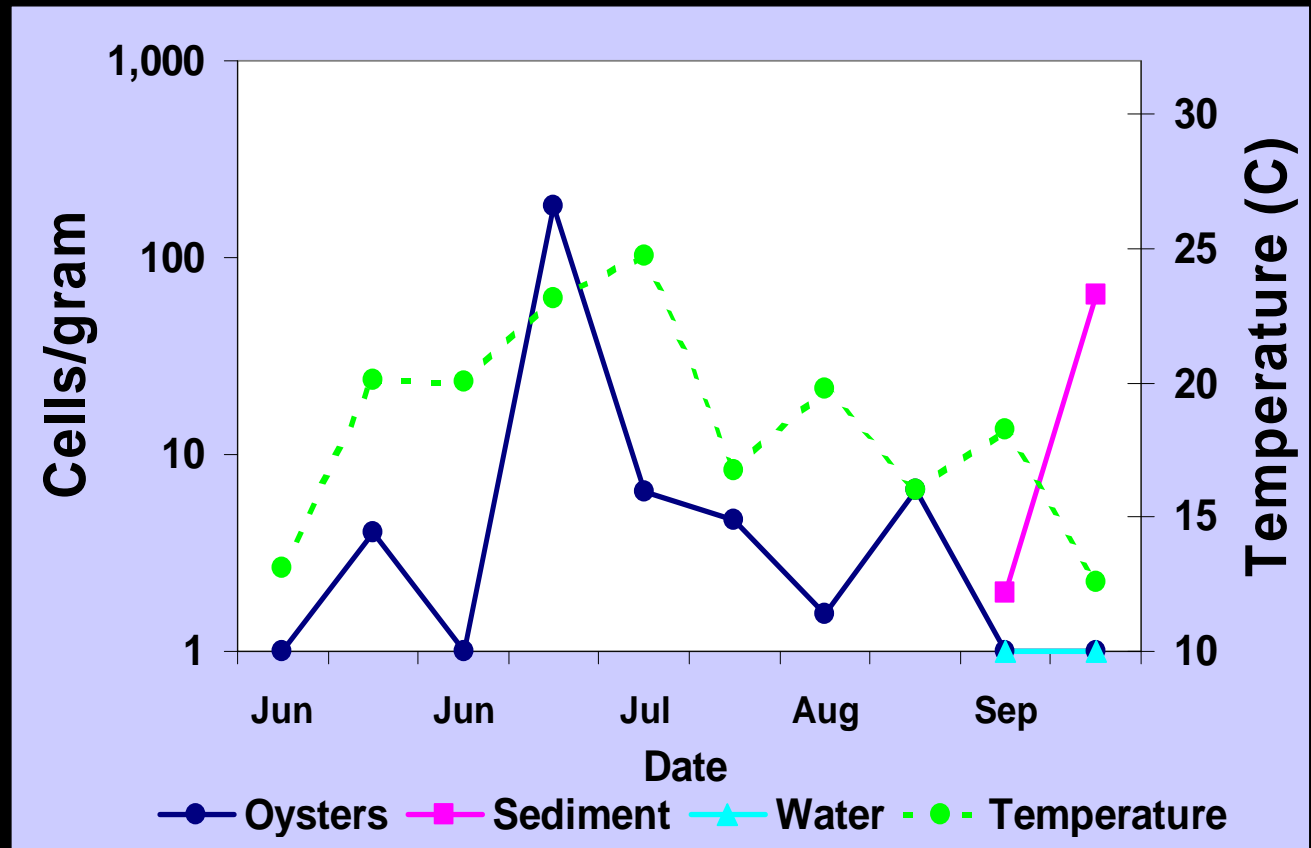
Hood Canal

Case Inlet

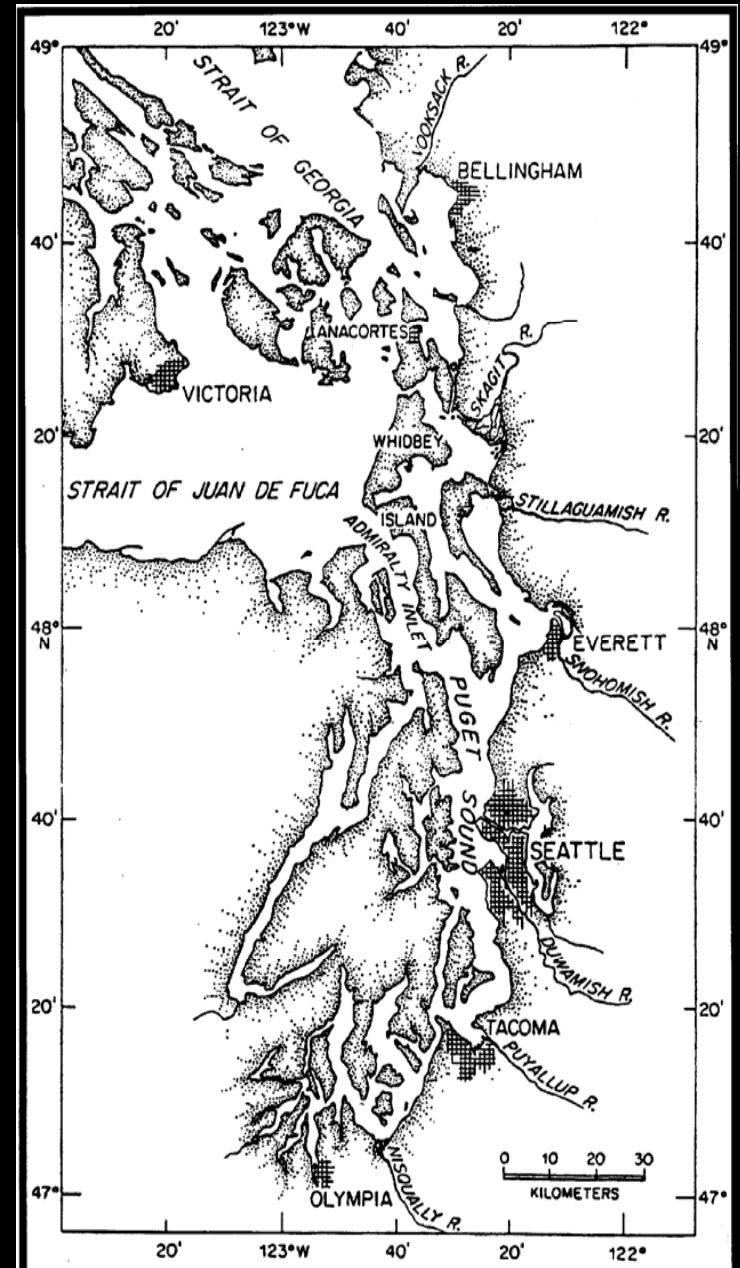
Totten Inlet



Totten Inlet - Oysters, Sediments, Water and Temperature



Puget Sound



Generation Times for *V. parahaemolyticus* at Puget Sound Sites Exposed During Low Tide Episodes

Location	Date	Sample	Generation Time (min)
Case Inlet	8/9	Oysters	23
		Sediments	37
Hood Canal	8/25	Oysters	33
		Sediments	45
Hood Canal	9/7	Oysters	31
		Sediments	33

Generation Times to Real Problems?

Generation Time (minutes)	Starting Number (Vp per gram)	2 Hours	4 Hours	6 Hours
30	1	16	256	4,096
30	10	160	2,560	40,960
30	100	1,600	25,600	409,600

Summary of Results

- Highest numbers in late July, early August
- Hood Canal - levels higher than other sites in southern Puget Sound
- Sediment - highest numbers
- Water - lowest numbers
- Substantial increase in V_p numbers in oysters during low tide episodes in late summer

Conclusions & Future Work

- *V. parahaemolyticus* live in complex and diverse microbial communities
 - ◆ Background of billion organisms per gram in sediments
 - ✦ *Vp* may represent 0.001% of the total population of bacteria
 - ◆ Heterogeneous distribution in Puget Sound
- Biological interactions
 - ◆ Zooplankton, phytoplankton
- Tidal cycle population fluctuations
- Fate and ecology of TDH⁺ and TRH⁺ strains
- Continued development of molecular methods for rapid enumeration
 - ◆ *In situ* biological sensors

Samples with *tdh+* and *trh+* *V. parahaemolyticus*

Location	Sample	Date	<i>V p(gyrB)</i>	<i>trh+</i>	<i>tdh+</i>
Sisters	Pacific oyster	7/29/99	18,360	0	2.14
Allyn	sediment	8/9/99	3,500	2.24	0
Allyn	sediment	8/9/99	2,465	16.75	0
Allyn	water	8/9/99	0.33	0.23	0
Allyn	water	8/9/99	0.33	0.23	0.002
Allyn	water	8/10/99	11	0.02	0
Totten	Pacific oyster	8/11/99	5	0.40	0
Sisters	Pacific oyster	8/25/99	80	0.40	0
Sisters	Pacific oyster	8/25/99	1,584	0.40	0
Sisters	Pacific oyster	8/25/99	1,584	0.40	0
Sisters	Pacific oyster	8/25/99	1,888	0.40	0
Sisters	sediment	8/26/99	2,465	1.00	0
Sisters	sediment	9/7/99	396	1.00	0
Totten	Pacific oyster	9/7/99	7	1.56	0
Allyn	sediment	9/8/99	116	2.24	0