

Mortality and oyster herpesvirus infections in Tomales Bay, California, USA

Colleen A. Burge¹, Robyn M. Estes-Strenge¹, Daniel P. Cheney², Frederick J. Griffin³, Kimberly S. Reece⁴, Tristan Renault⁵ & Carolyn S. Friedman¹

¹School of Aquatic and Fishery Sciences, University of Washington, Seattle, USA

²Pacific Shellfish Institute, Olympia, Washington, USA

³University of California, Davis, Bodega Marine Lab, Bodega Bay, California, USA

⁴VIMS, College of William and Mary, Gloucester Point, Virginia, USA

⁵IFREMER, Laboratoire de Génétique et Pathologie, FRANCE

Presentation Outline

- Background
 - Summer seed mortalities (SSM)
 - SSM in Tomales Bay
- Sentinel Field Studies 2000-2003
 - SSM and Oyster herpesvirus (OsHV) in Tomales Bay
- Bivalves species afflicted with OsHV in Tomales Bay
- Current Research

"Summer mortality versus SSM"

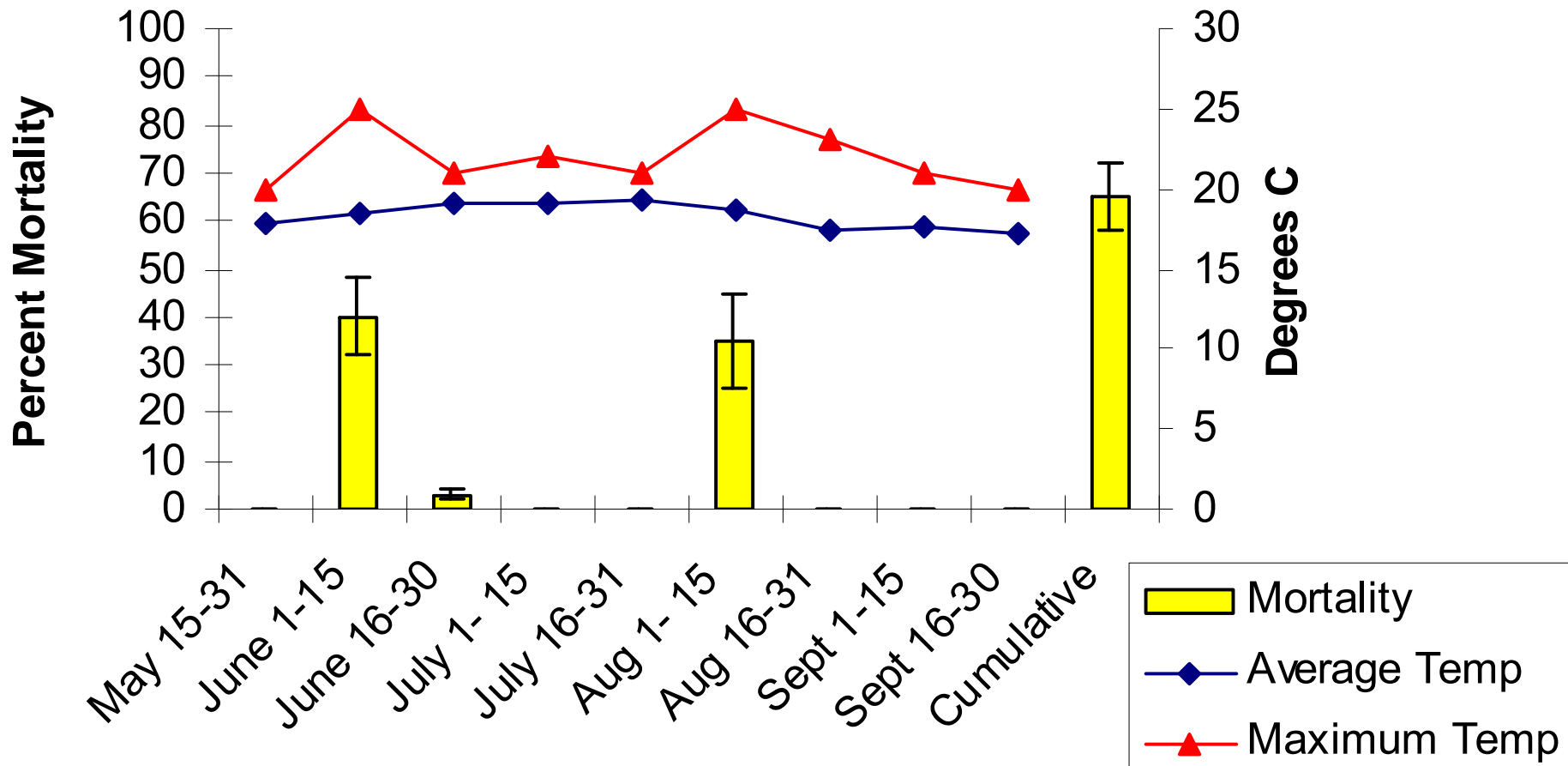
	"Classic" Summer Mortality	Summer seed mortality
Afflicts	mainly older animals	seed
Duration of M(x)	prolonged	short
Associated with:		
Water temperature	X	X
High nutrient loads	X	
Δ in Phytoplankton	X	X
Δ in salinity	X	
Δ in DO	X	



SSM in Tomales Bay

- Short pulses of mortality have occurred since 1993 (up to 90% losses).
- In 1993-1994, cumulative losses of 5 farms approached 50-65%
 - Previous to 1993, losses over an 18 month culture cycle were typically 15-35%
- Sentinel field study of 1995 reported mortalities correlated with warm temperatures (up to 25 C) and phytoplankton blooms

Simulated SSM in Tomales Bay



Oyster herpesvirus and SSM

- First observed in France and New Zealand associated with larval mortalities in 1991-1992
- OsHV first associated with SSM in France in 1993

	OsHV mortalities	Tomales Bay SSM
M(x) Affects:		
Life stages	larvae and seed	seed
Larvae	many species	NA
Seed	Flat and Pacific oysters	Pacific oysters only
Duration of M(x)	short	short
Associated with:		
Water Temp	X	X

Sentinel Field Studies, 2000-2003

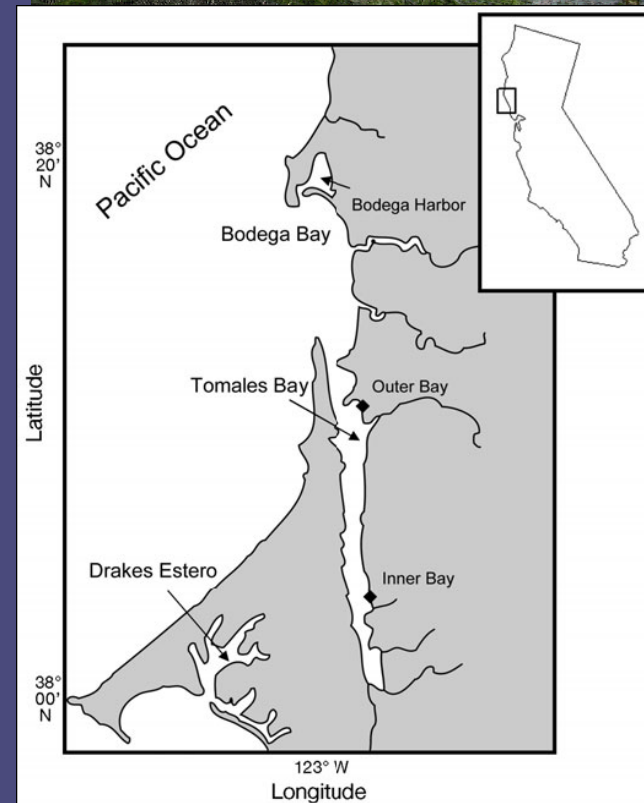
Objectives

- What is causing SSM in Tomales Bay?
 - Does survivorship vary
 - between family lines?
 - with environmental stress?
 - with outplant time?

Or is it caused by disease?

Sentinel Field Studies 2000-2003

- Seed planted on racks +1 ft tide level: Fall 2000 to Spring 2003
- Cohorts of 3-5 stocks per year
 - Low and high performing family lines, and diploid and triploid hatchery stocks
- Environmental monitoring: temperature, salinity, & phytoplankton
- Health status, growth, and survival were monitored
 - 2002 OsHV Exploratory Survey
 - 2003 OsHV Monitoring

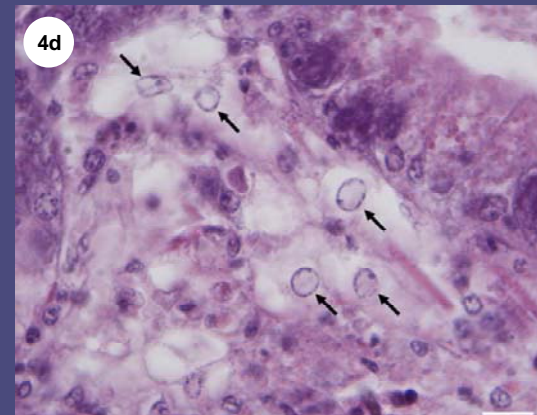
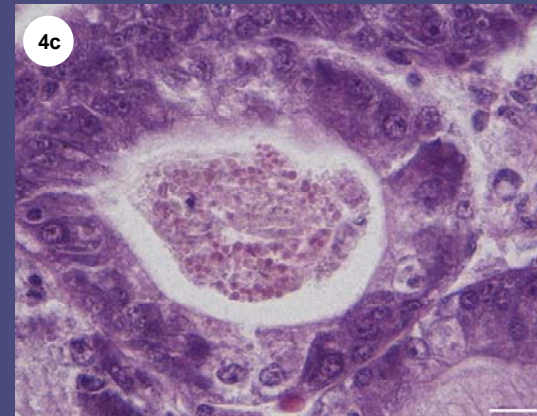
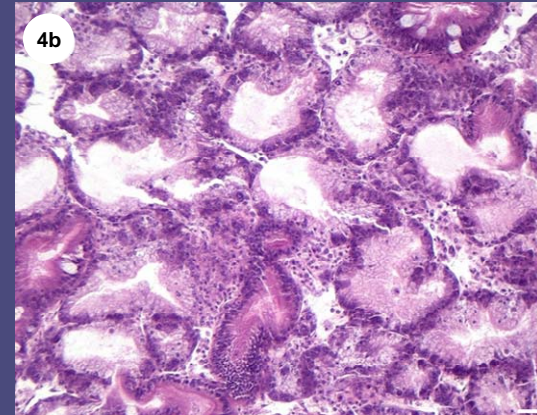


Results: 2000-2002

- Mortality Results:
 - Bodega Harbor: 0%
 - Outer Bay: 0-15%
 - Inner Bay: 47-100%
- Spring plants are more susceptible than those that overwintered in the bay ($p < 0.05$)
- Smaller oysters are more susceptible to mortality
- Environmental Monitoring:
 - Max. temp correlated with mortality ($r = 0.949$, $p < 0.05$)
 - Phytoplankton blooms unrelated to mortality ($p > 0.05$)
- Stocks were identified that are less susceptible to mortality

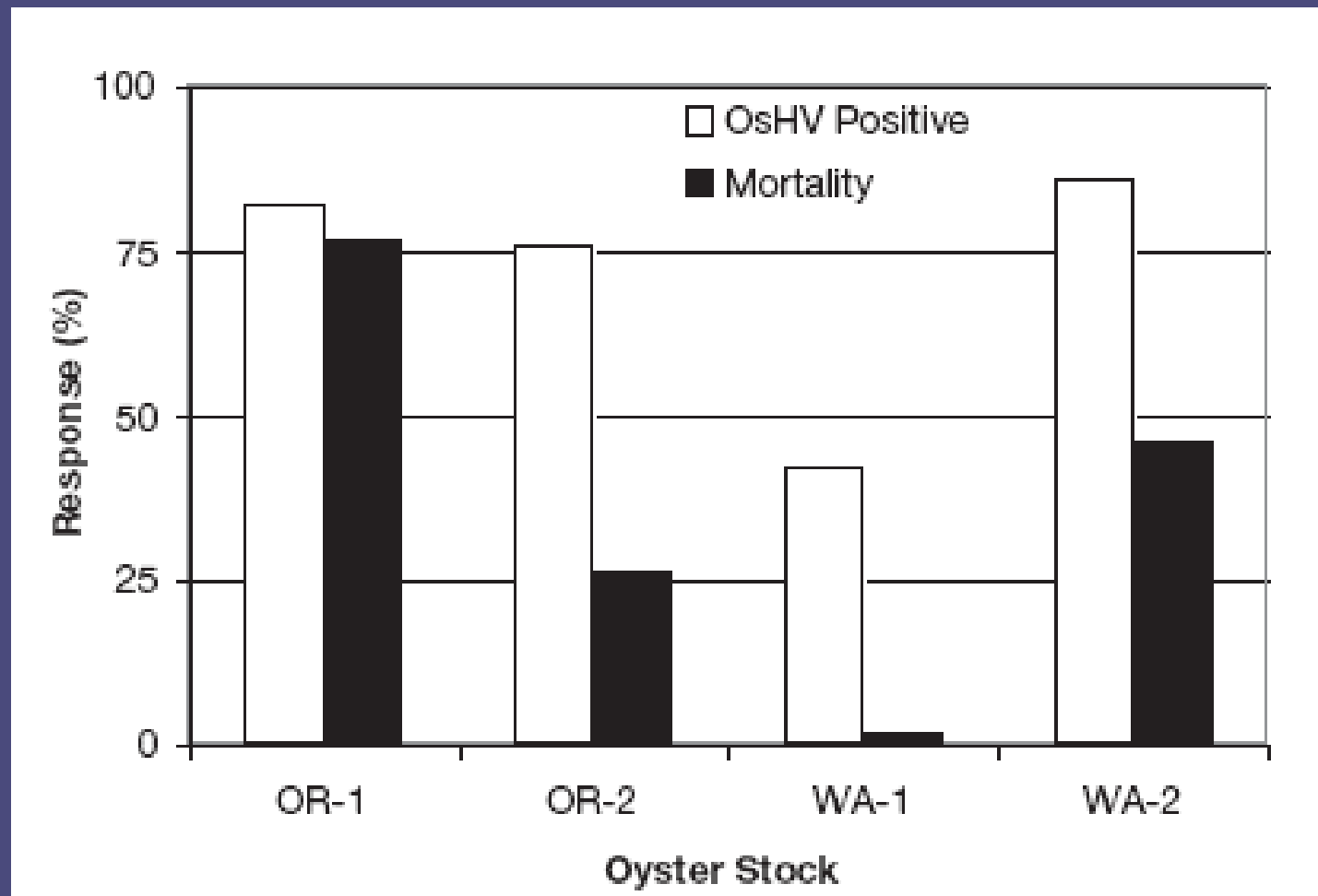
Results: Health Status

- Routine paraffin histology 2000-2001 survey
 - Bodega Harbor and Outer Bay oysters had normal tissue and cellular architecture
 - Oysters collected during a mortality event at the Inner Bay site had abnormal tissue and cellular architecture
 - Dilated digestive tubules with infiltration of hemocytes around tubules (inflammation)
 - Dilated digestive tubules containing cellular debris
 - Cowdry Type A inclusions suggestive of herpesvirus infection



2002 OsHV Exploratory Survey

- Oysters collected 10/02 and tested for OsHV



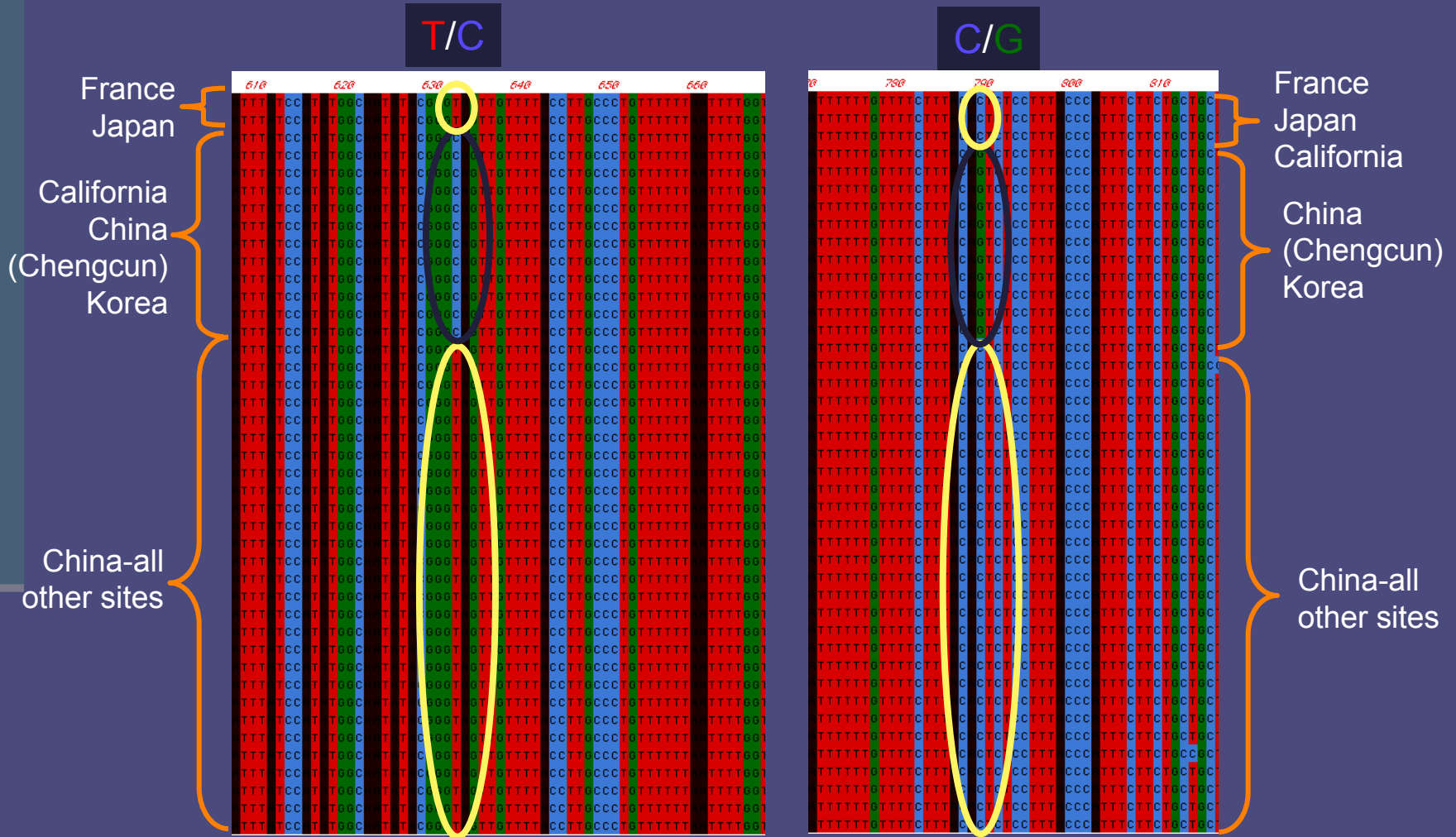


Geographic Distribution of OsHV in the US

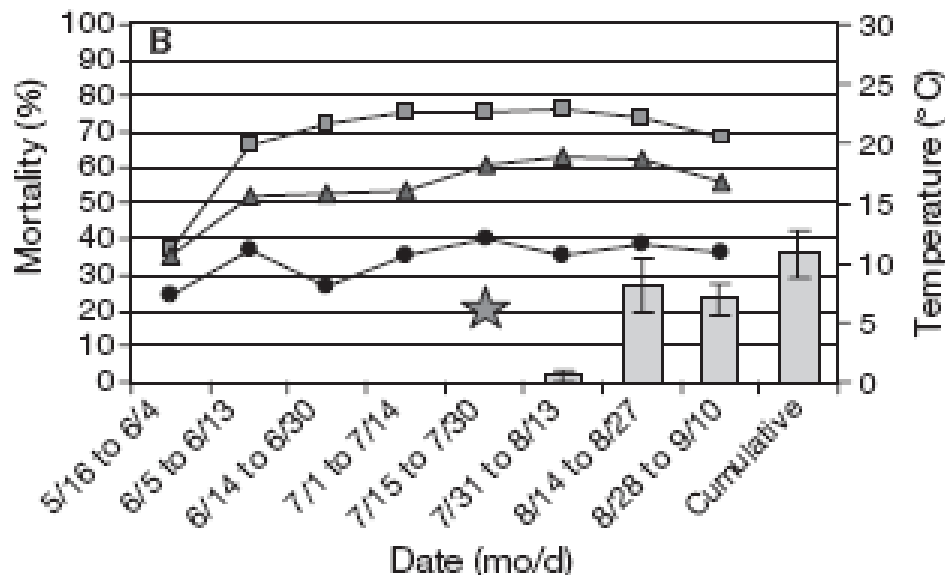
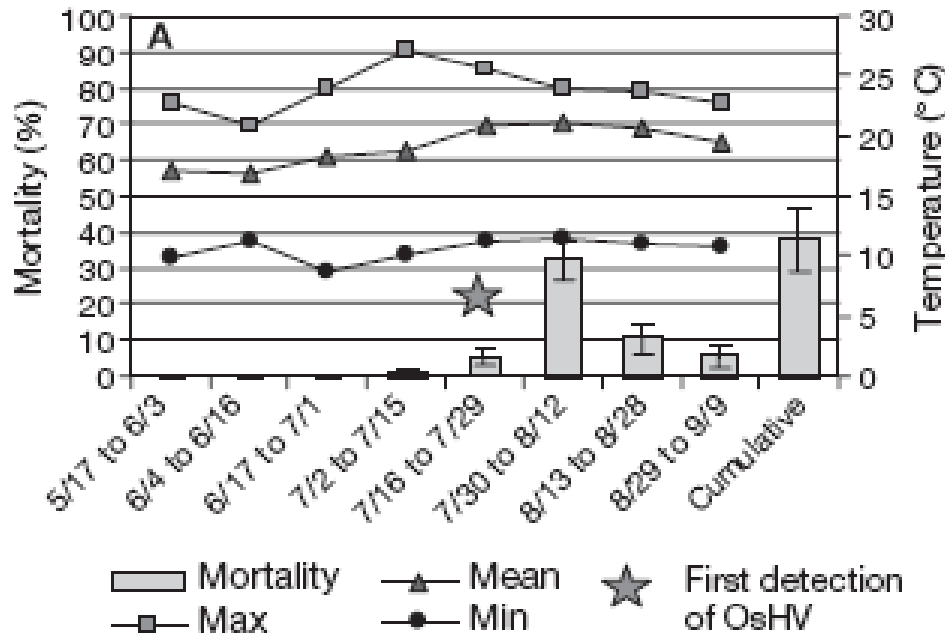
- Larval and seed Pacific and eastern oysters were collected from:
 - Tomales Bay, California
 - Newport, Oregon
 - Totten Inlet and Quilcene, Washington
 - Louisiana, Florida, Virginia, and Maine
 - **Only oysters from Tomales Bay were PCR + for OsHV; Oregon and Washington hatcheries that produced California seed were uninfected based on PCR examination**



Known OsHV Variation in Asia and North America



2003 Mortality/OsHV Surveys



- Mean temperatures were similar between sites and predicted OsHV presence ($p < 0.005$)
- OsHV presence predicted mortality ($p < 0.01$)

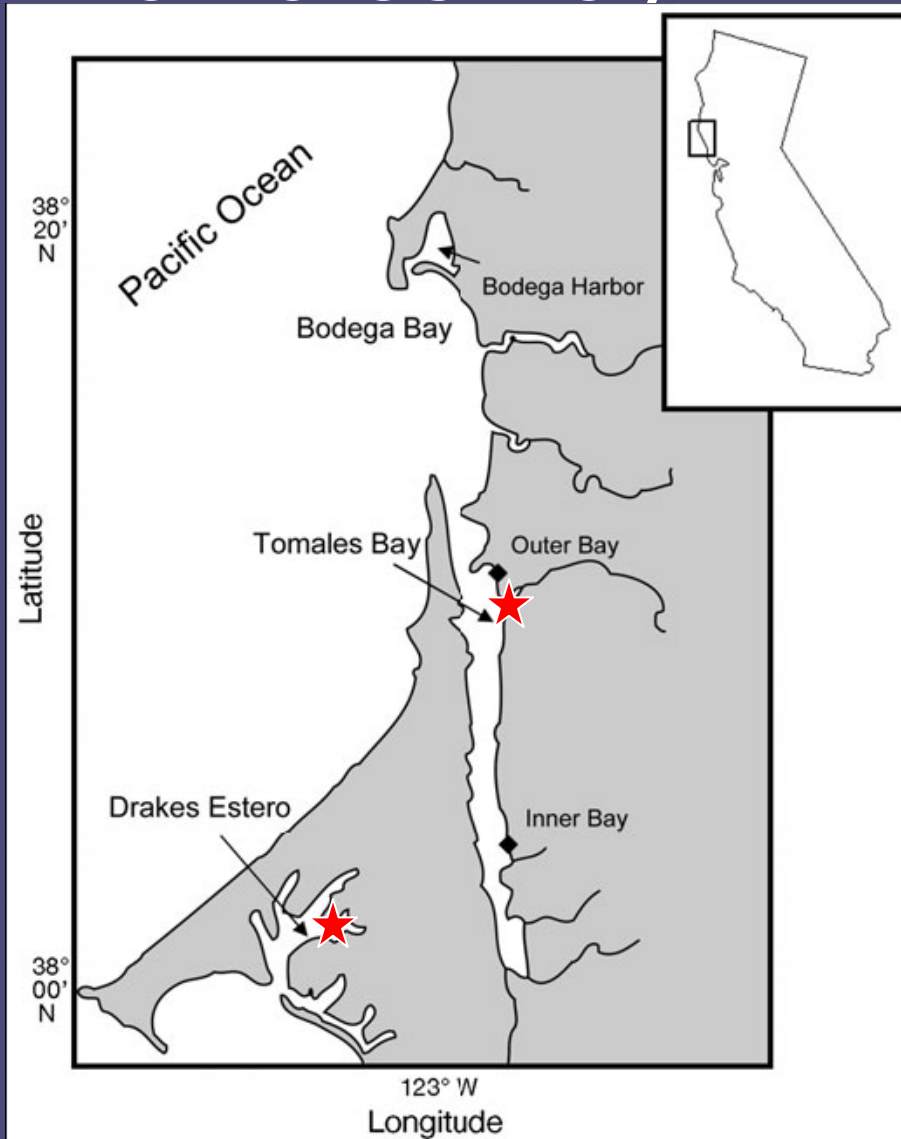
Mortality trends in Tomales Bay

- Mortalities in 1995, 2001, 2002, and 2003 followed temperature maximums of 25 °C **except: Outer Bay mortalities in 2003**
 - Temperature may be related to rate of viral replication
- Mortalities at the Inner Bay site (using data from 2001, 2002, and 2003), were significantly correlated ($p < 0.05$) with total exposure and degree hours greater than 24 °C and 25 °C (but not temperatures between 16-23 °C)

How are oysters being infected?

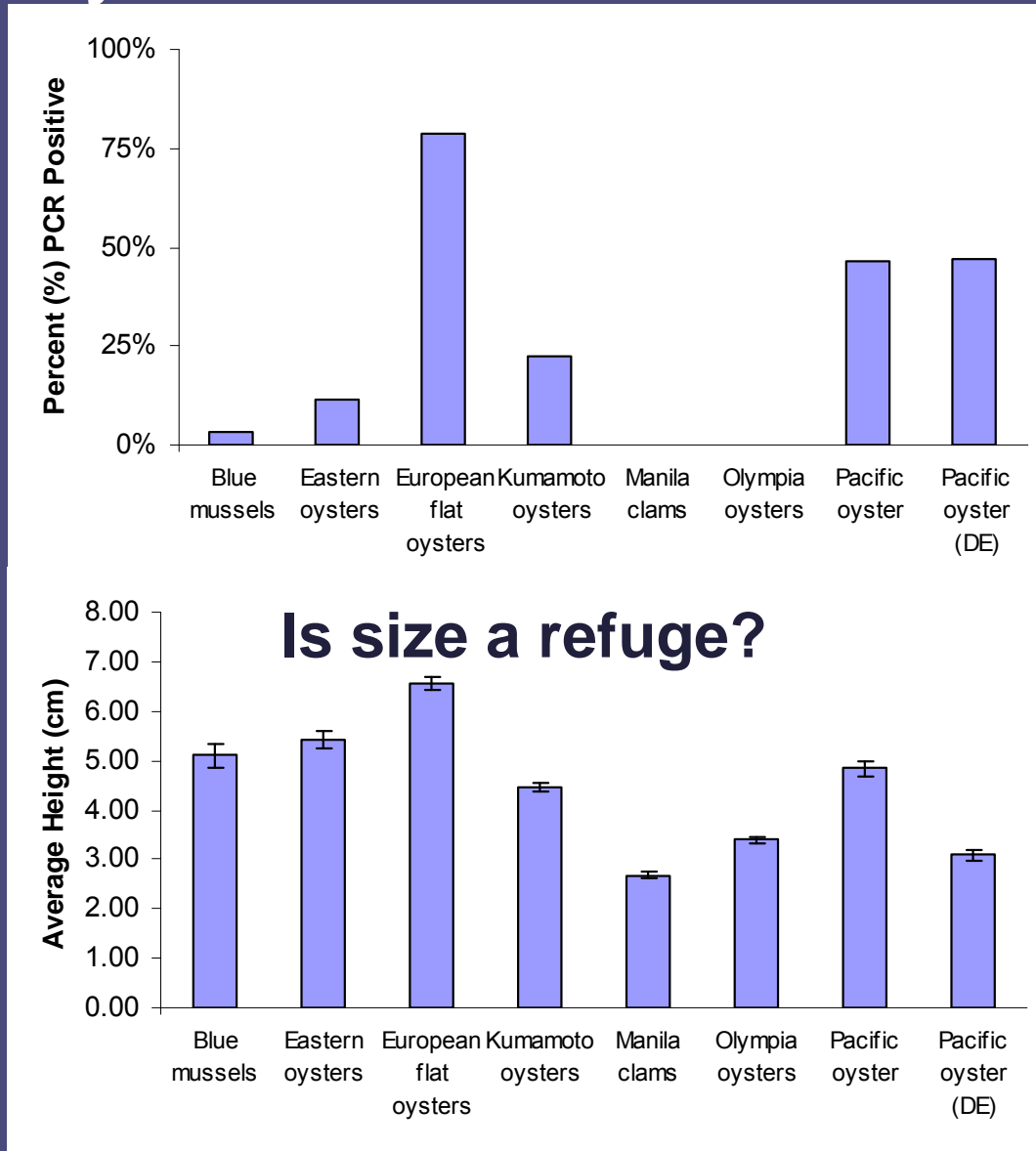
- Uninfected seed are outplanted each year
 - OsHV has not been detected in any hatcheries or nurseries to date in the US
- Mortality occurs only after temperature extremes
 - Temperature (>25C) has been related to larval mortalities in France.
 - 25 C may trigger viral OsHV viral replication
- Adult oysters in the bay may have latent infections
 - Including other bivalve species grown in the bay
 - OsHV has been detected in several adult bivalve species in France

What is the source of OsHV in Tomales Bay?



- In August 2003, after OsHV presence/mortality occurred in Pacific oysters, multiple species of bivalve were collected to test for the presence of OsHV
- Pacific oysters were also collected from Drakes Estero (Fall 2002 and Spring 2003 outplants)

Species of bivalves infected in Tomales Bay and Drakes Estero



- Prevalence only measures the percent of animals with viral DNA
- Viral load and infection status may be different between species
- Asymptomatic animals may be in the processing of shedding the virus

Current Research

- Was OsHV present in Tomales Bay before initial detection?
 - We are in the process of using an OsHV specific *in situ* hybridization to test individuals from mortalities pre-2002
- How similar is the virus in Tomales Bay to OsHV-1 in France?
 - Currently using PCR primers designed from OsHV-1 genome to amplify and sequence OsHV from Tomales Bay
 - Host susceptibility

Acknowledgements

- **Bodega Marine Lab, University of California Davis**
 - Toxicology lab
 - Fish Disease Lab
 - Animal Care Unit
 - Shellfish Health Lab
- **Virginia Institute of Marine Science**
 - Nancy Stokes
- **IFREMER at La Tremblade**
 - Nicole Faury
 - Inmaculata Lopez
 - Jean Francois Pepin
 - Isabelle Arzul
- **Molluscan Broodstock Program**
- **Taylor Shellfish**
- **Lummi Shellfish**
- **Hog Island Oyster Company**
- **Tomales Bay Oyster Company**



Research was supported by the National Sea Grant – Oyster Disease Research Program, California Sea Grant College and University of Washington