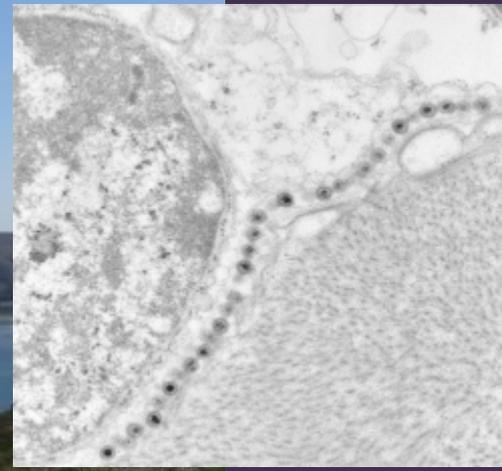
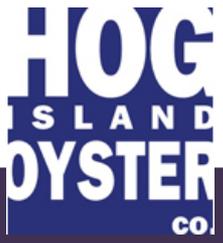


Tools and solutions to enhance shellfish aquaculture production in the face of the emerging Ostreid herpes virus pathogens: OsHV-1 & OsHV-1 μ var



National Aquaculture Extension Conference in Boise, June 5-9, 2017

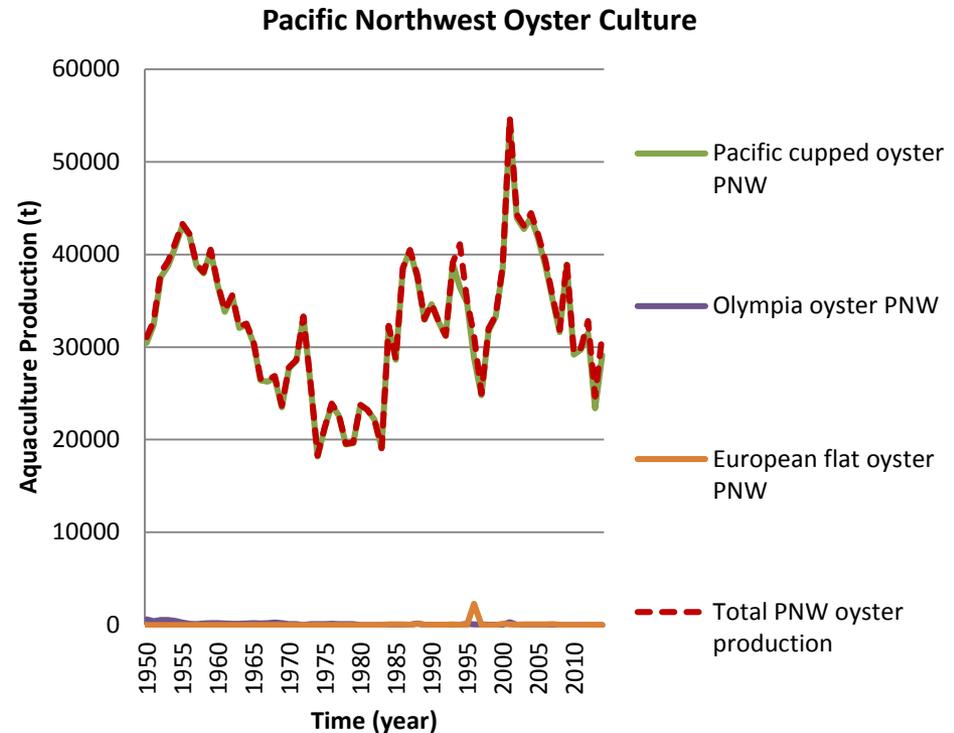


Goals

- **The main aim of this proposal is to:**
 - **Arm the shellfish industry with options and tools to prepare for the introduction and or spread of the lethal Ostreid herpes virus -1 and its variants:**
 - **We will provide educational materials and training to help prevent OsHV-1 spread.**
 - **In addition, we will provide:**
 - **Diagnostic tools,**
 - **Identify oyster herpesvirus-resistant oyster lines/species,**
 - **And determine relative risk of disease transmission among life stages.**

Importance of Oyster Culture

- In the US, oysters represent important economic and ecological species:
- In 2013 sales from 483 farms were worth \$180M.
- The US West Coast leads US shellfish aquaculture with >\$100M (56%) produced annually; \$24M (16%) and \$50M (28%) are grown along the Gulf and East coasts, respectively.
- The Pacific oyster, *Crassostrea gigas*, dominates oyster culture value (48%).
- **We address “Emerging and Re-emerging Diseases Affecting Aquaculture Production”**



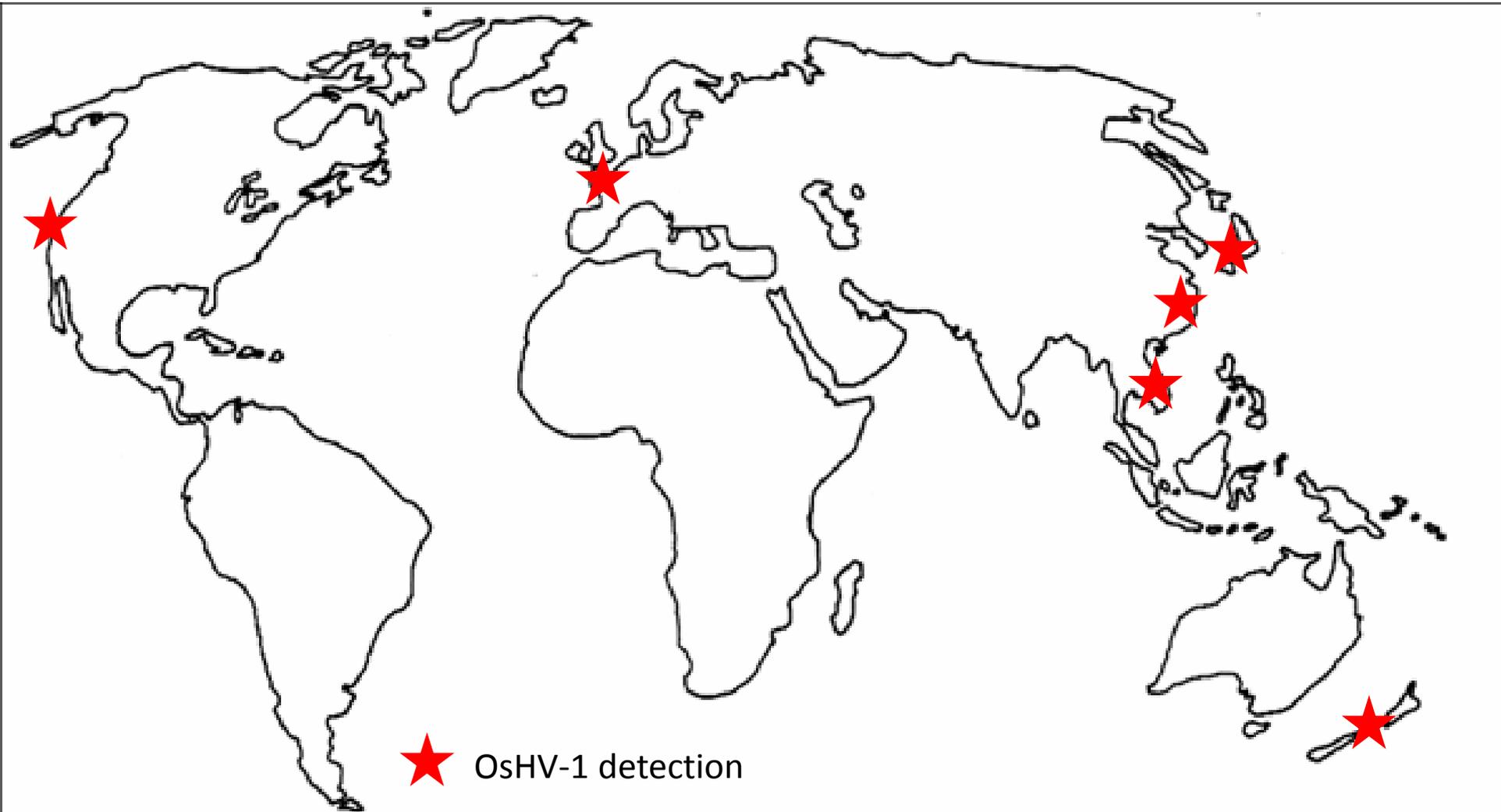
Oyster aquaculture production along the US west coast (Pacific Northwest) (www.fao.org)



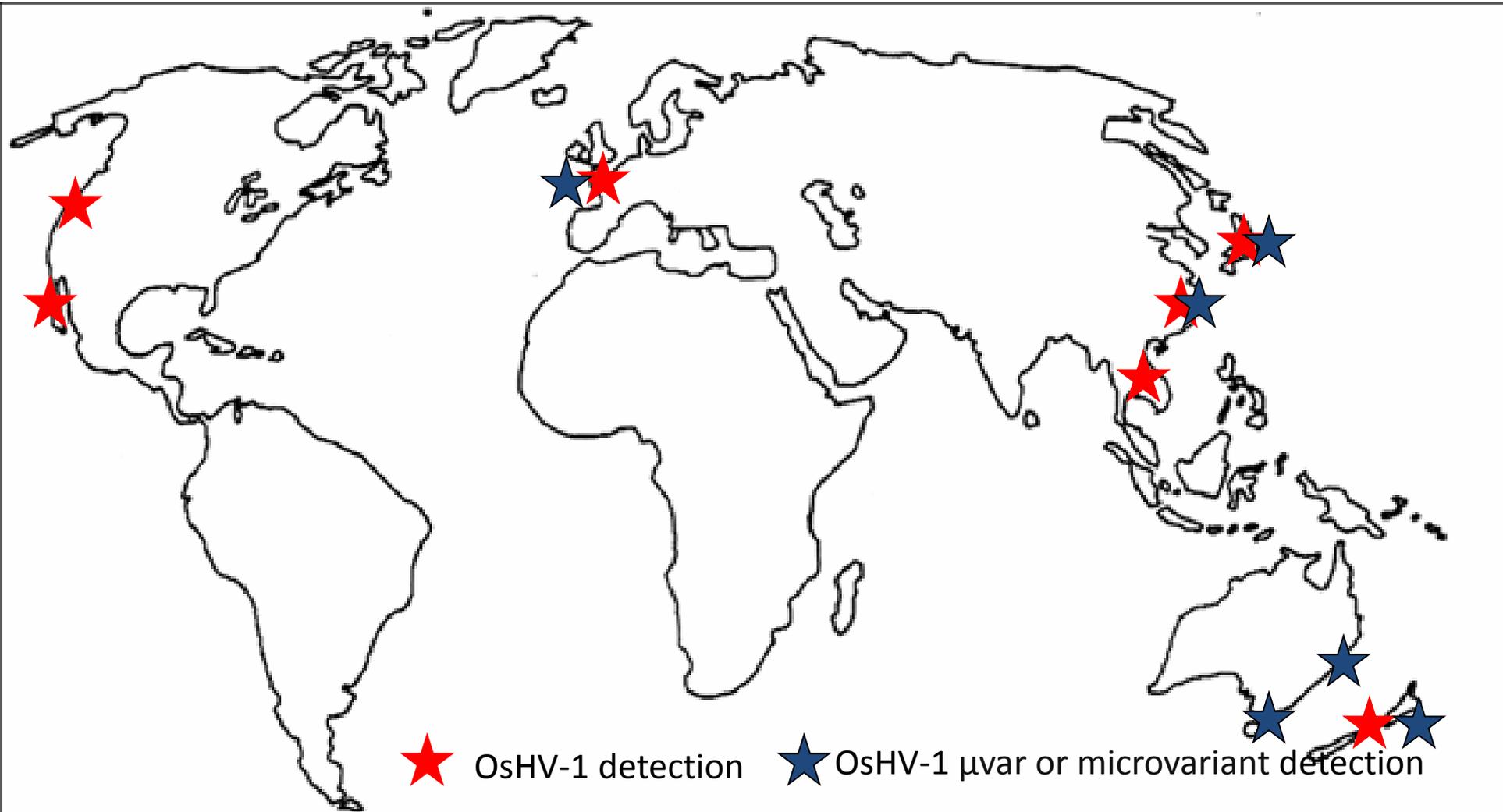
Oyster mortalities in Tomales Bay

- Short pulses of mortality of juvenile Pacific oysters have occurred nearly annually since 1993 (up to 90%)
- Initially no disease agent was identified
- The Oyster Herpesvirus (OsHV) was first detected in 2002 & via examination of archived samples is known to have been present as early as 1995
- OsHV is non-culturable and is difficult to detect with traditional methods – Need rapid molecular assays
- OsHV-1-induced Pacific oyster losses in TB → closure of oyster farms and establishment of oyster farms in other embayments to reduce losses due to OsHV-1 and expand the US west coast shellfish aquaculture industry

Oyster herpesvirus: 1993-2002



Oyster herpesvirus: 2008-2016



Project Background

- OsHV-1 μ var's ability to kill seed and adults heightens concern over this variant relative to its progenitor, OsHV-1, which is lethal to larvae and young oysters.
- OsHV-1 resistance has been shown to confer resistance to μ var in field trials in France.
 - In addition, these resistant oysters were able to both limit infection loads and eliminate virus from their tissues.
- In New Zealand, oysters susceptibility to μ var decreased over time providing hope for development of resistance.
- Collectively this suggests that selection for resistance is possible and that preparing for OsHV-1 μ var is prudent.

2016 PCSGA Shellfish Health & Disease Panel



Major focus of questions:
OsHV-1 and OsHV-1 μ vars,
*NSGO funds proposal for
additional work*

California Sea Grant New Data

- We have demonstrated increased resistance to OsHV-1 in some US west coast Pacific oyster lines
- Summer 2015, we identified two hybrid Pacific oyster lines from an industry breeding program (Taylor Hybrid) with differential survival
- One line (10-15:58x19) experienced moderate (47%), and a second line (10-15:12x52) experienced low (25%), survival after OsHV-1 exposure in TB
- Whole body burden of OsHV-1 was 1.5x higher on average in the family with low survival relative to that with higher survival
- The survival of line 10-15:58x19 was considered exceptional given the young age and very small size (~5 mm) of these spat
- Susceptibility to OsHV-1 is inversely proportional to size

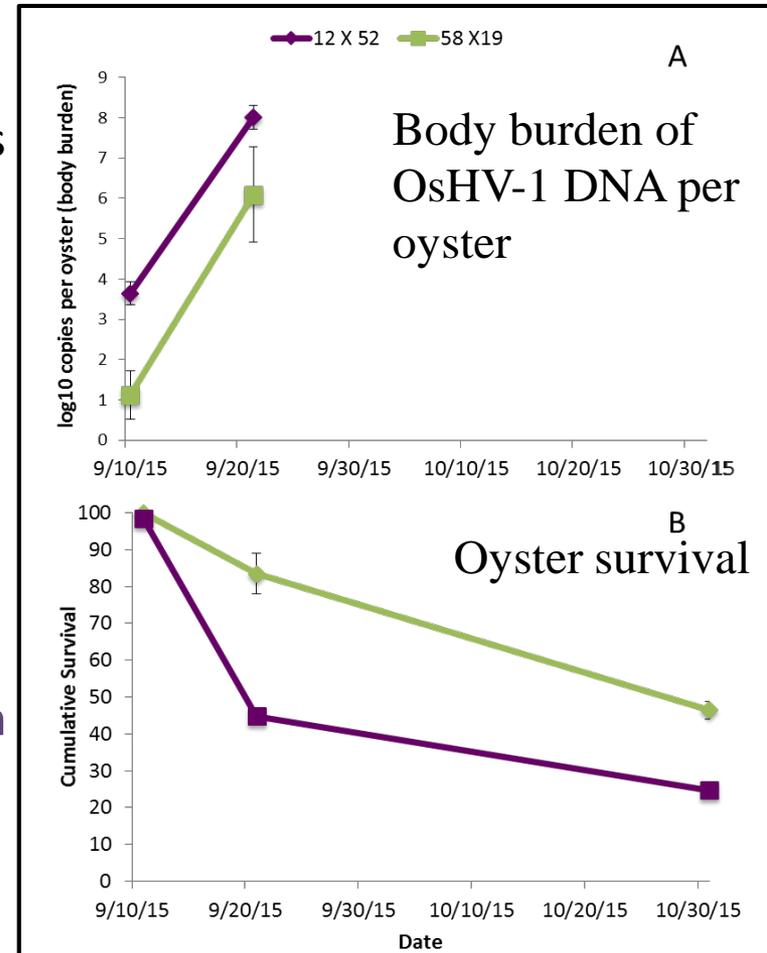


Figure 2. Differential performance of seed from two hybrid Pacific oyster families planted in Tomales Bay summer 2015. A. Body burden (log₁₀ copies of OsHV-1 DNA per oyster). B. Oyster survival.

OsHV-1 in California

PACIFIC OYSTERS

**Disease
resistance?**

**Role of
alternate hosts?**

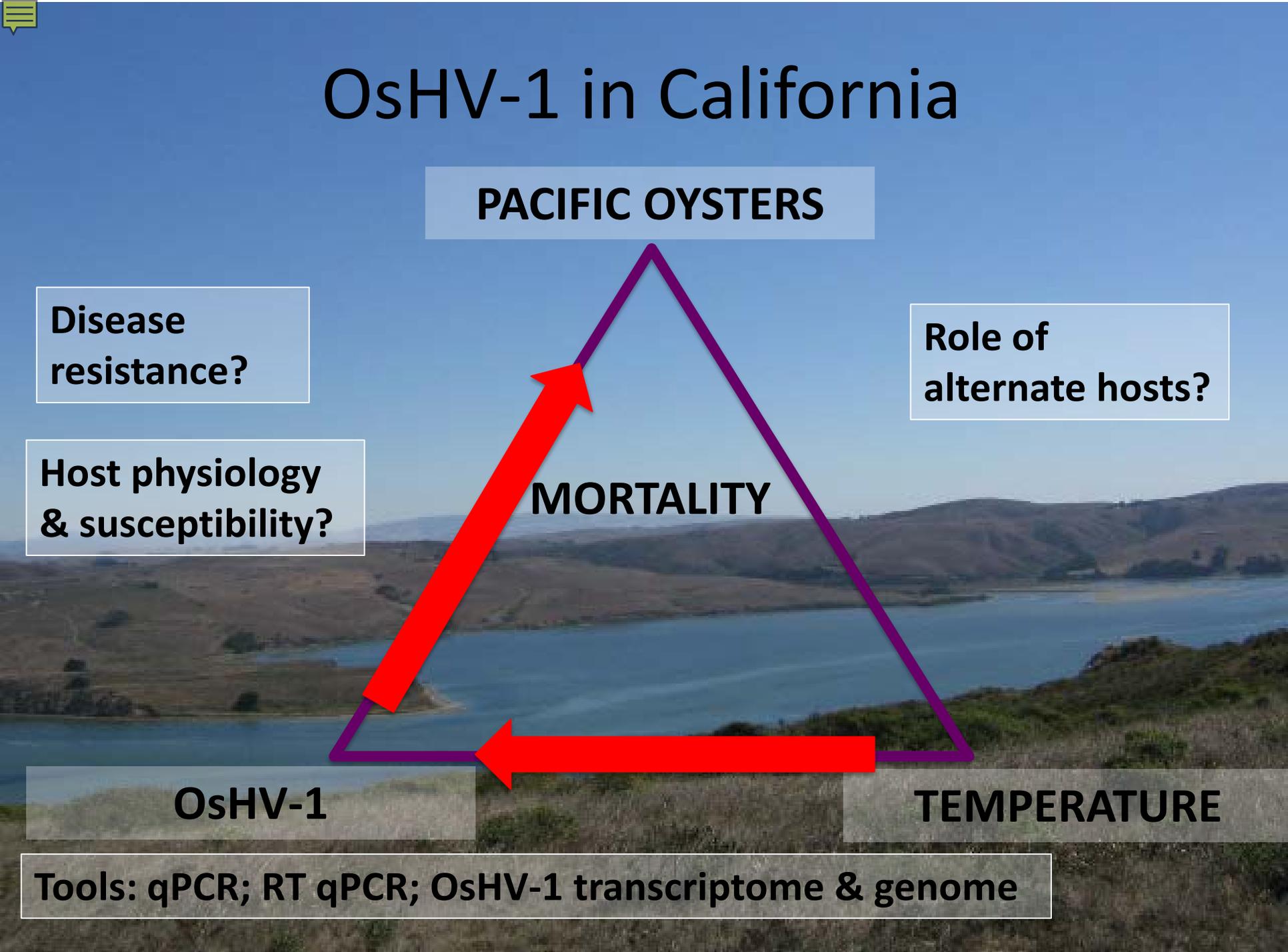
**Host physiology
& susceptibility?**

MORTALITY

OsHV-1

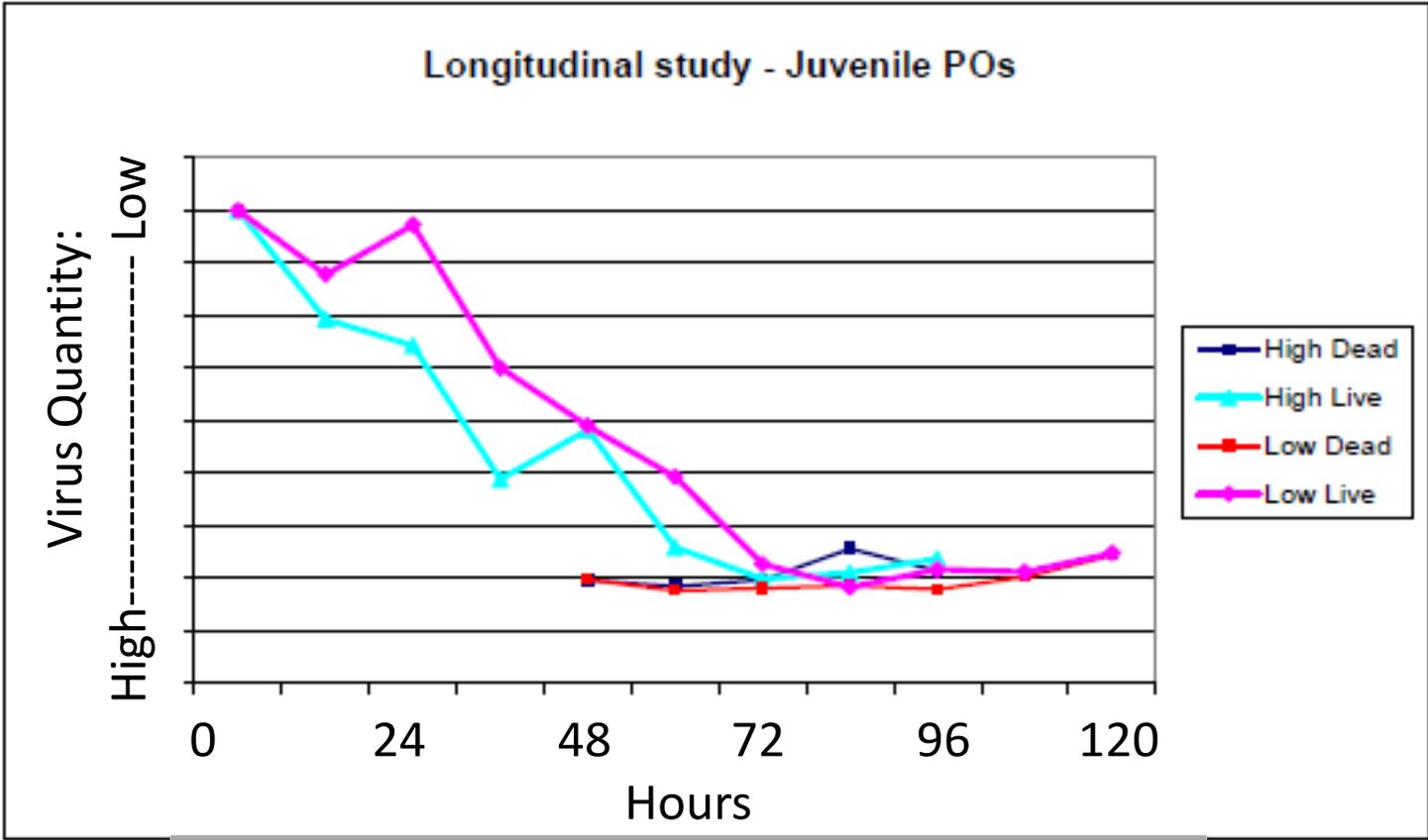
TEMPERATURE

Tools: qPCR; RT qPCR; OsHV-1 transcriptome & genome



OsHV-1 μ var replicates & kills QUICKLY!

Figure 3: Virus concentrations in the tissues of both live and dead juvenile Pacific Oysters after infection with 2 different doses of OsHV-1.



This publication (and any information sourced from it) should be attributed to Kirkland, P.D., Hick, P.M, and Gu X. Elizabeth Macarthur Agriculture Institute, 2015 *Development of a laboratory model for infectious challenge of Pacific Oysters (Crassostrea gigas) with ostreid herpesvirus type-1* Sydney, May, 2015. CC BY 3.0]

In order to reach our objectives (described below), we will test the following hypotheses:

- *H1: Species and family lines will demonstrate differential survival in the face of OsHV-1 and OsHV-1 μ var exposure.*
- *H2: Oyster families or species resistant to OsHV-1 are also resistant to OsHV-1 μ var.*
- *H3: Genome variation of viral strains (i.e. the California OsHV-1 and OsHV-1 μ var strains) can explain differences in virulence.*



Objectives:

- **Objective 1:** *Develop a (multi-plex) real-time, quantitative polymerase chain reaction (qPCR) assay.*
- **Objective 2:** *Conduct field trials to oyster families/species with differential susceptibility to OsHV-1.*
- **Objective 3:** *Conduct laboratory trials to examine differential seed survival to OsHV-1 μ var.*
- **Objective 4:** *Sequence the viral genomes used in infection trials (California OsHV-1 and OsHV-1 μ vars strains)*

Conclusions

- OsHV-1 and its μ vars are serious pathogens of oysters
- Our goal is to limit spread by education and outreach
- We aim to develop tools (molecular assays and oyster lines) to better enable management of this disease, early detection and adaptive management of our oyster stocks