The background of the slide is a close-up photograph of numerous oysters. The shells are dark, almost black, with some showing a lighter, yellowish-brown interior. The oysters are piled together, creating a textured, natural-looking background.

# How do OsHV-1 $\mu$ vars stack up? A comparison of multiple variants through experimental challenges

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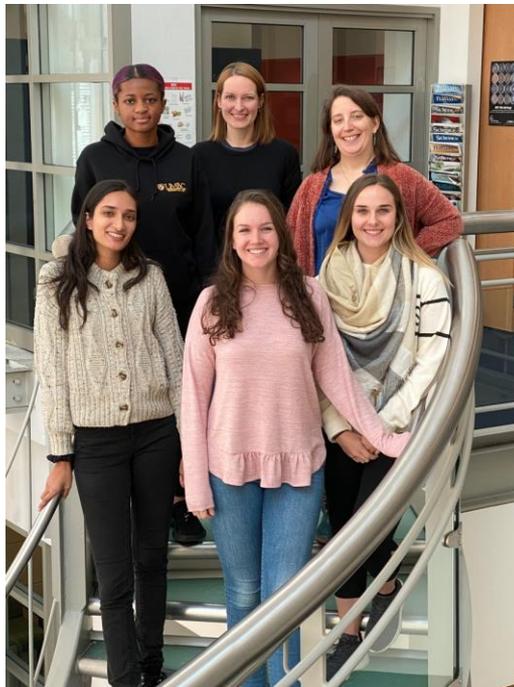
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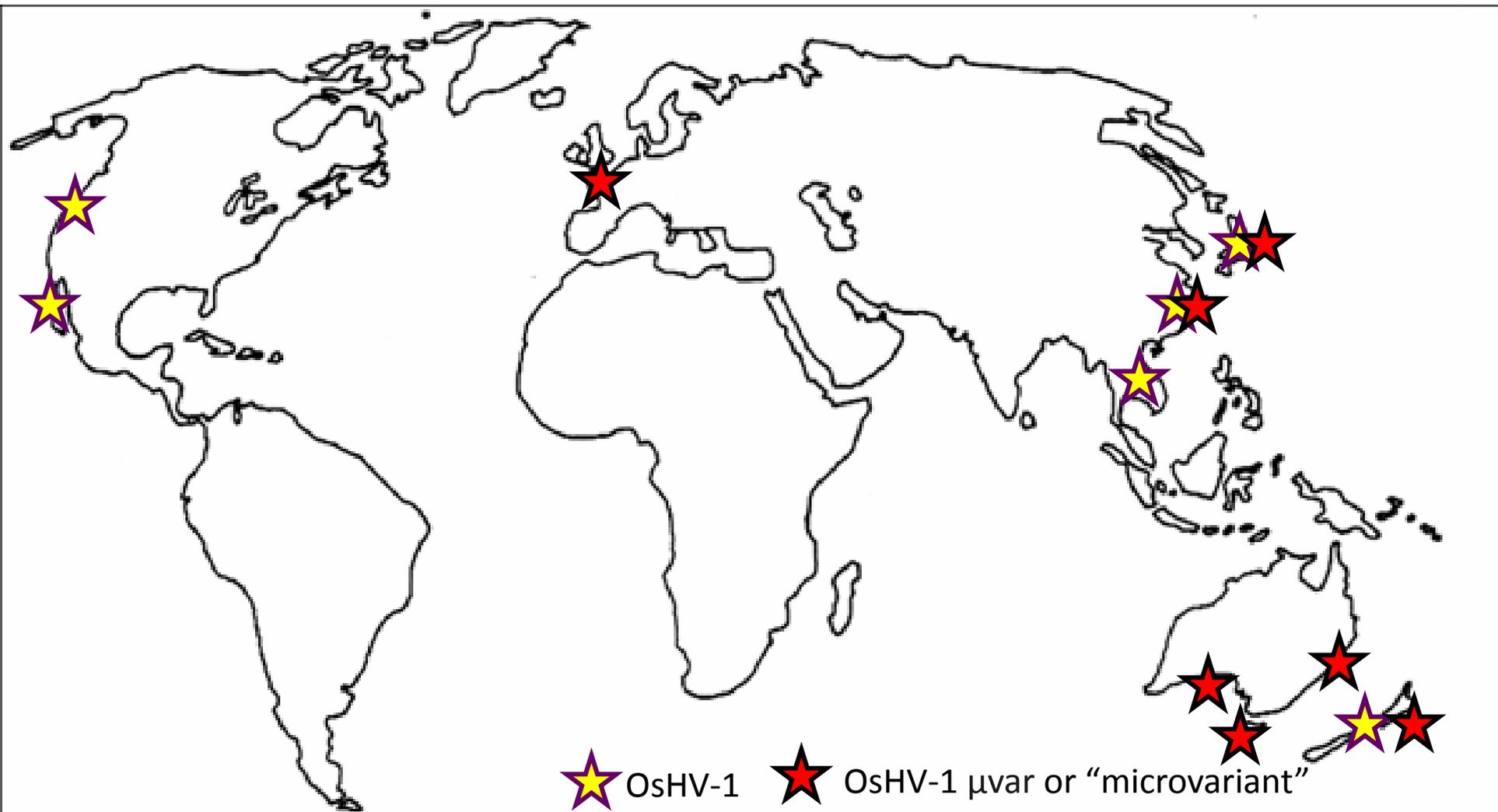
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# What is OsHV-1 and why do we care?

- The Ostreid herpesvirus 1 (OsHV-1) causes mass mortalities of Pacific oysters
- Host range not restricted to Pacific oysters
- Disease progresses rapidly and can kill up to 100%
- Once established OsHV-1 persists in the environment (likely in surviving oysters), causing seasonal mass mortalities
- Virus can be transmitted within species and life stages; both horizontally and vertically
- **Multiple variants of OsHV-1 exist including the emerging OsHV-1  $\mu$ vars; a cause of concern for oyster growers globally**

# Oyster herpesvirus: 1991-2019



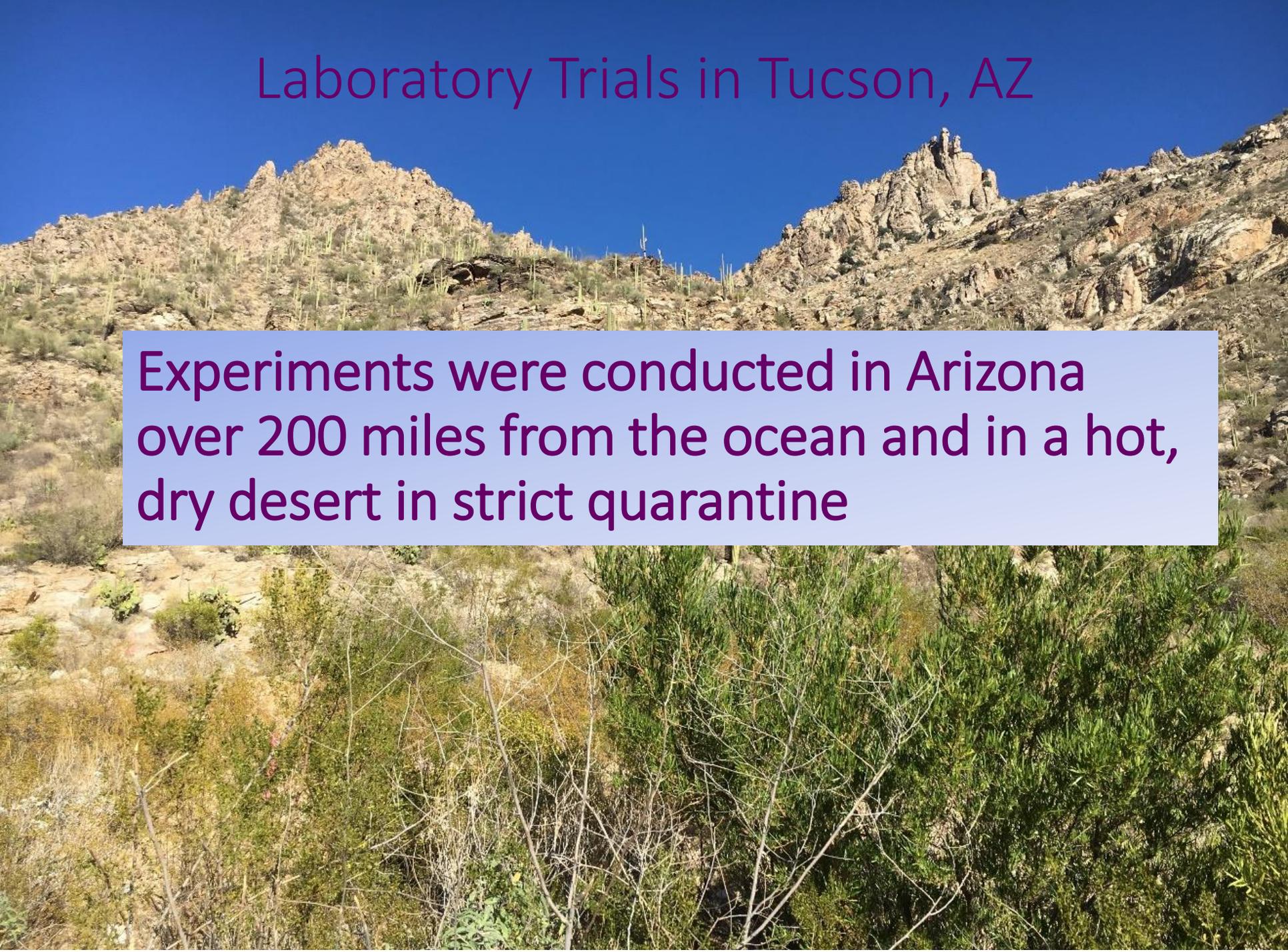


# Research Objectives

- Conduct laboratory trials to examine differential survival of oysters to OsHV-1  $\mu$ vars and OsHV-TB using multiple stocks and species
- Initial characterization of a new OsHV-1 from San Diego, California



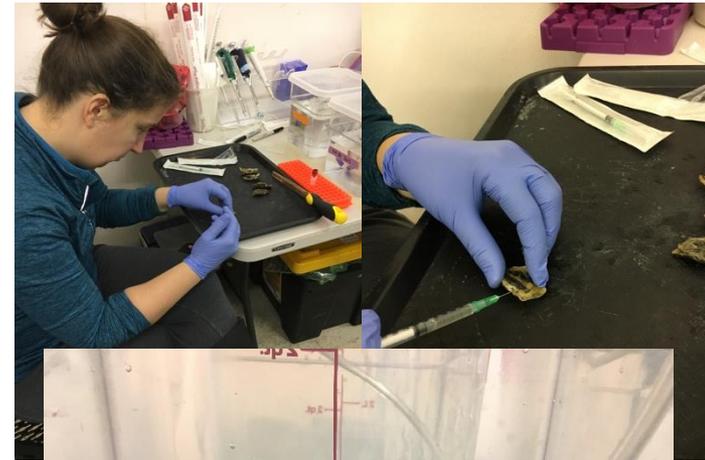
# Laboratory Trials in Tucson, AZ

A photograph of a desert landscape in Tucson, Arizona. The background shows rugged, rocky mountains with several saguaro cacti scattered across the slopes. The sky is a clear, bright blue. In the foreground, there is a dense thicket of green and brown shrubs and trees, some of which appear to be dead or dormant.

Experiments were conducted in Arizona over 200 miles from the ocean and in a hot, dry desert in strict quarantine

# Laboratory methods

- Received infectious virus stocks from France & Australia
- Used susceptible animals to create larger stocks of homogenates
- Sampled moribund animals to create filtered tissue homogenates
- Three challenge types
  - Bath
  - Injection
  - Natural water exposure



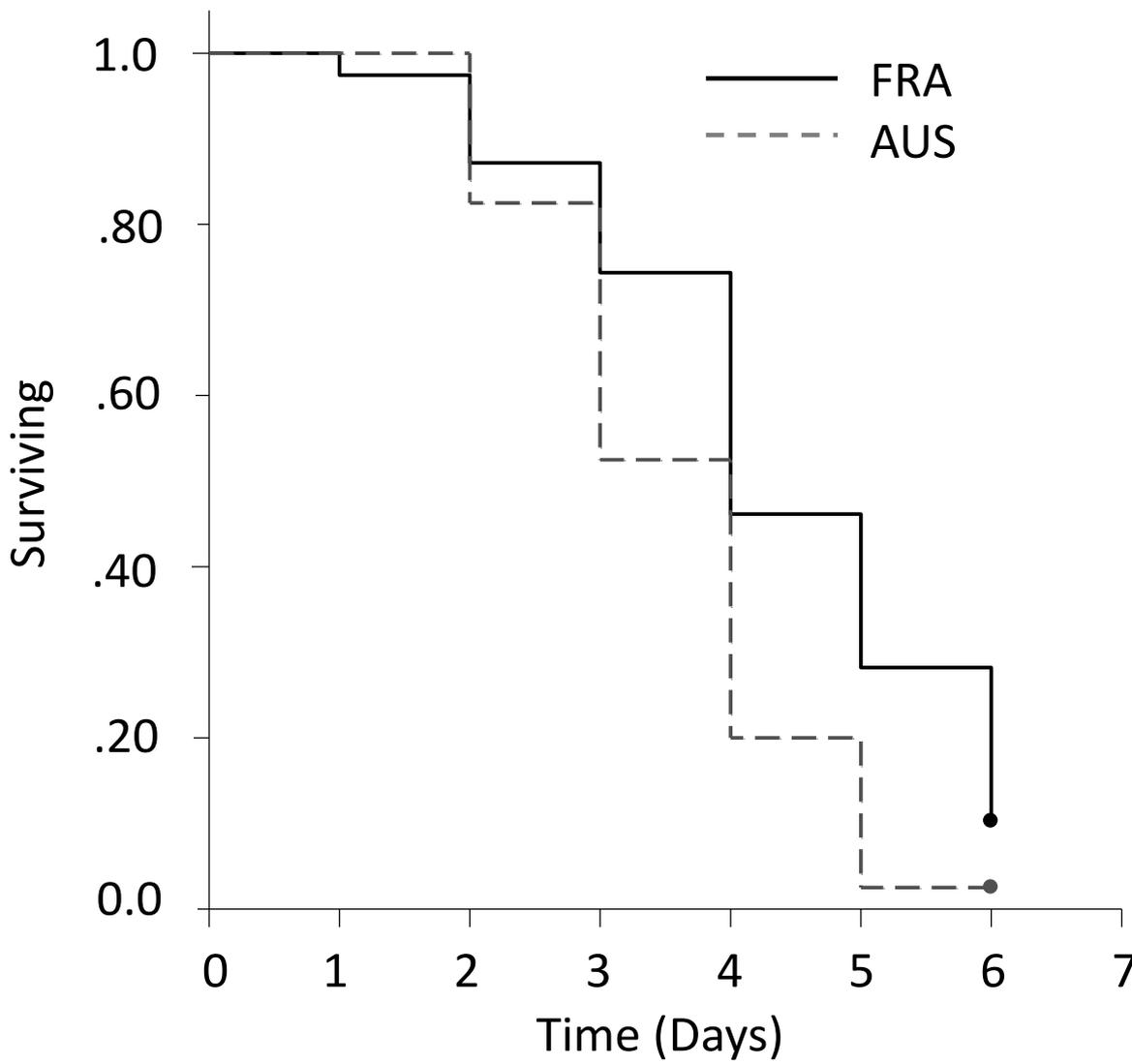


# First comparison of two “μvars” in bath challenge

9 mm naïve Pacific oysters

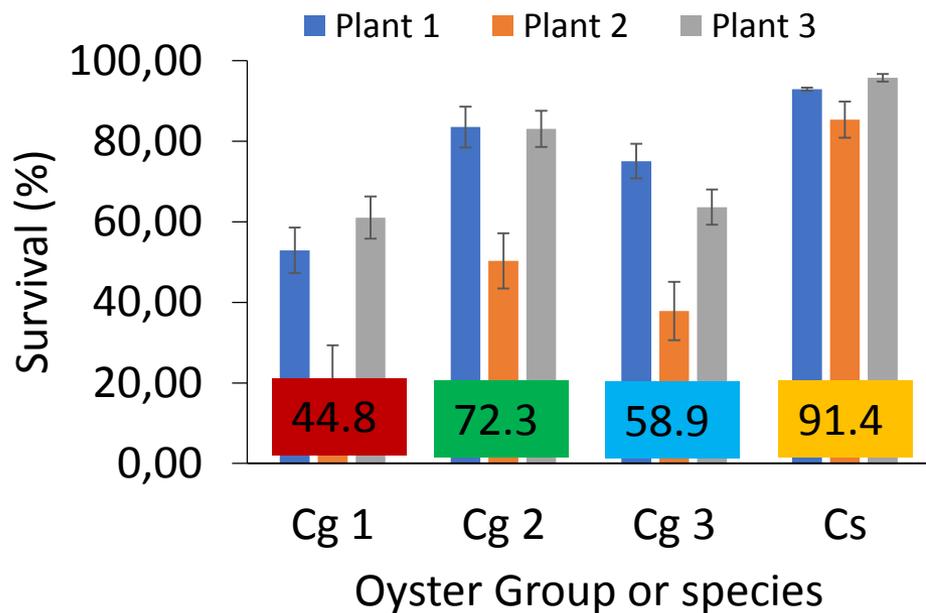
**AUS μvar > FRA μvar**  
**p=0.004**

(Log-rank Chi-Square;  
p<0.05)





# Injection trial (from field to lab)



- *Cg1*=low survival ( $52.9 \pm 0.7$  mm)
- *Cg2*=high ( $53.2 \pm 0.8$ )
- *Cg3*= moderate ( $44.8 \pm 0.6$ )
- *Cs*= high ( $41.5 \pm 0.5$ )
  
- *Cv1*=high ( $40.8 \pm 0.50$ )
- *Cv2*=high ( $39.1 \pm 0.6$ )

*Cg*= *Crassostrea gigas*;  
Tazzie, Midori & Wild X MBP  
*Cs*=*Crassostrea sikamea*  
*Cv*=*Crassostrea virginica*;  
DEBY, LOLA

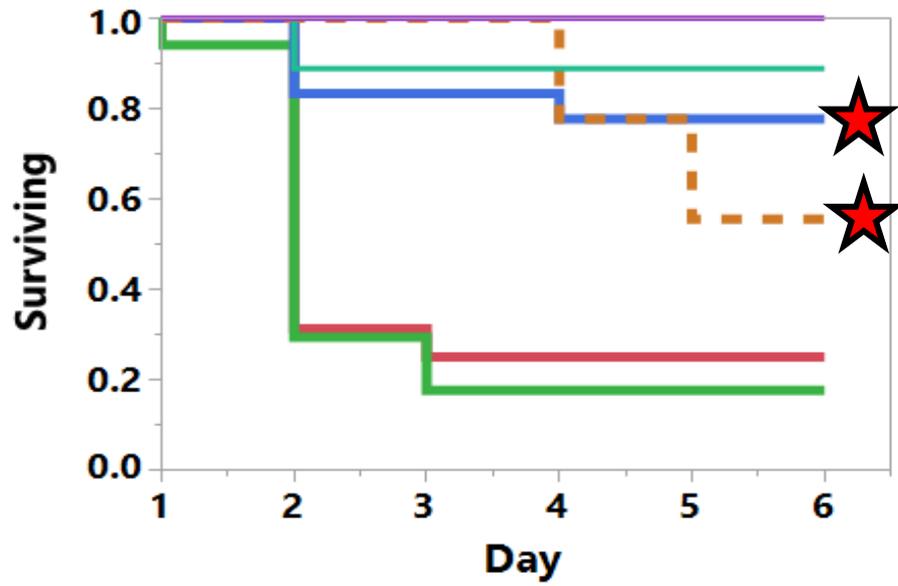
Field data:  
Burge *et al.* in prep



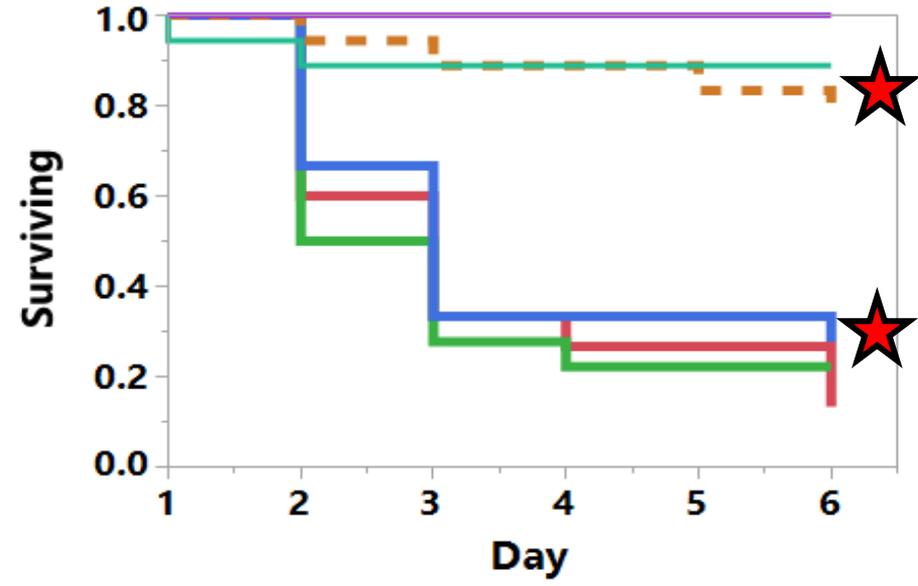
# Injection challenge: Survival of US oyster species and stocks

■ Cg 1   ■ Cg 2   ■ Cg 3   ■ Cs   ■ Cv 1   ■ Cv 2

### AUS $\mu$ var

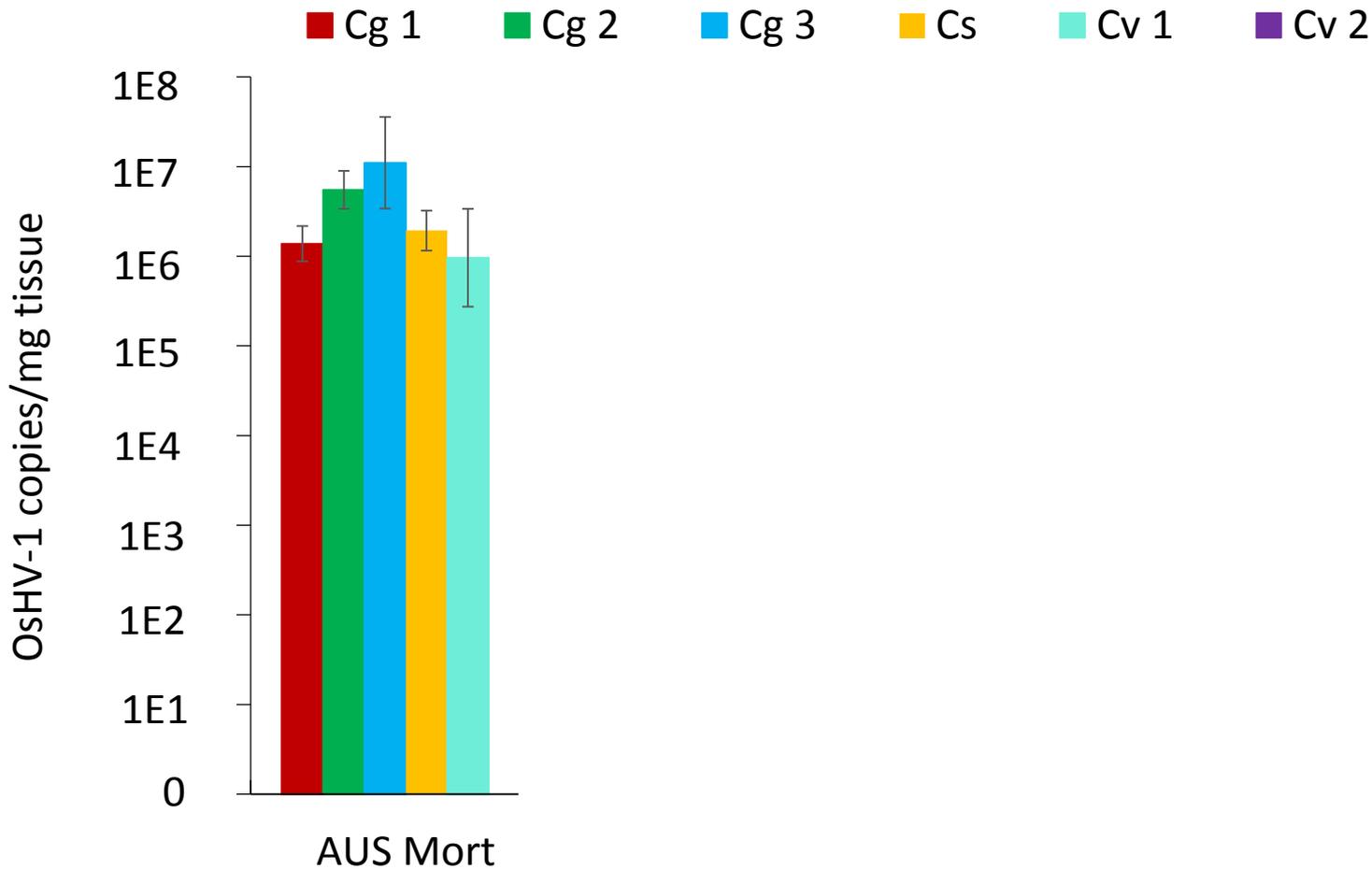


### FRA $\mu$ var





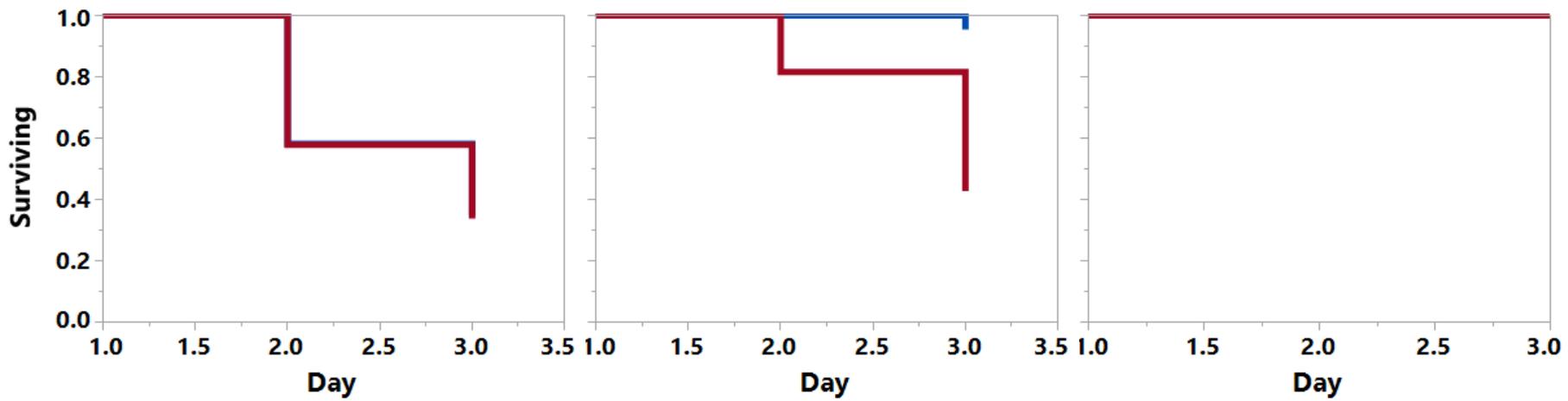
# Injection challenge: Viral load





# Injection challenge 2: survival

**AUS  $\mu$ var**  
**FRA  $\mu$ var**



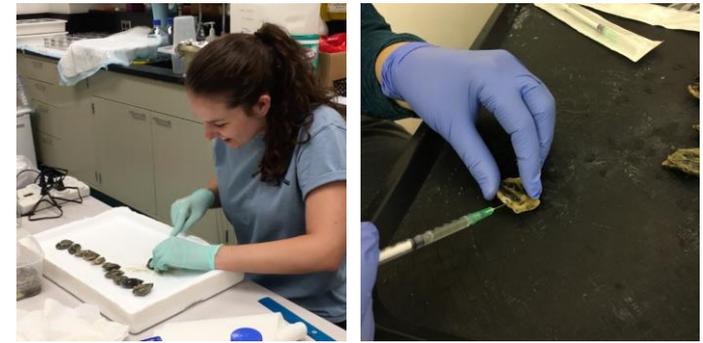
- Cg1 highly susceptible to both  $\mu$ vars

- Cg3 more susceptible to FRA  $\mu$ var

- Cv 2 no mortality

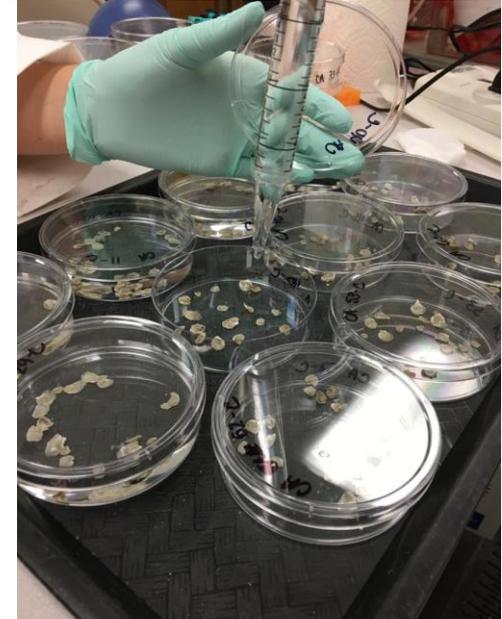
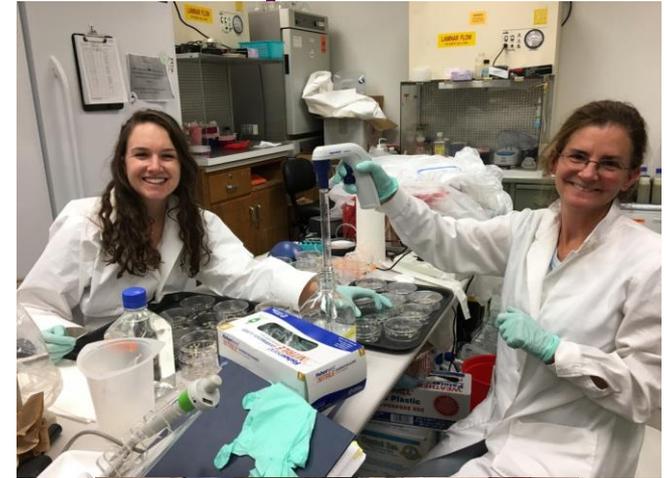
# 'Natural' exposure technique

- Injected 20 juvenile (~1 yr old) *C. gigas* with 1 million copies of virus
  - OsHV-1  $\mu$ var FRA
  - OsHV-1  $\mu$ var AUS
  - OsHV-1 TB (California)
- Virus replicated for ~24 hours
  - $> 5.25 \times 10^6$  copies of virus added per plate
  - Relative concentration: FRA>AUS>TB at 24 hours



# 'Natural' exposure technique

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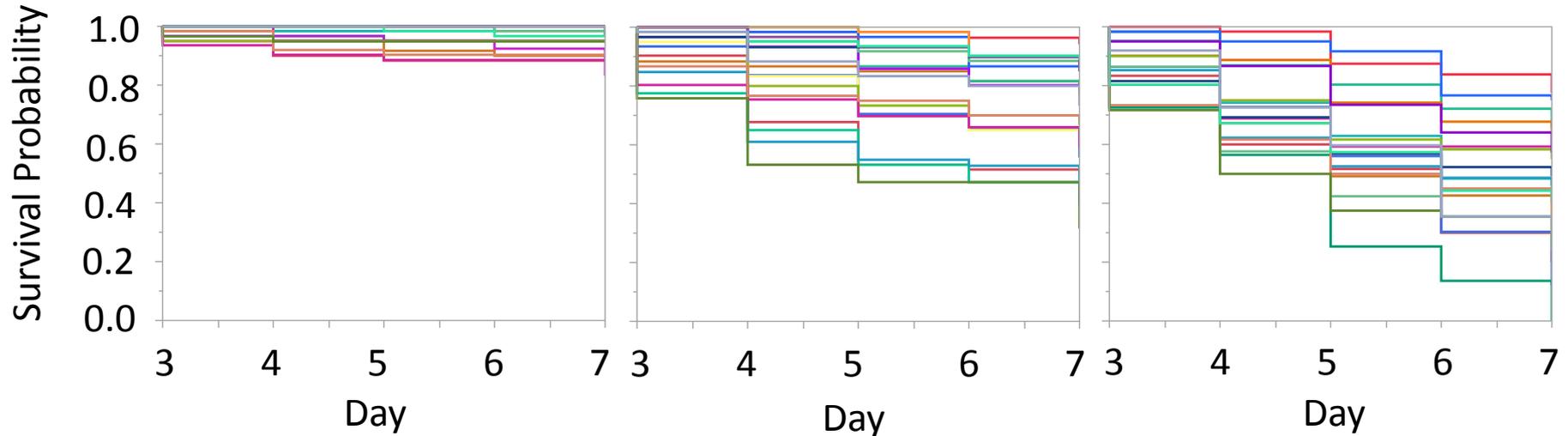


## Increased transmission in Pacific oysters

**OsHV-1 TB**

**AUS  $\mu$ var**

**FRA  $\mu$ var**



20 Molluscan Broodstock Program families

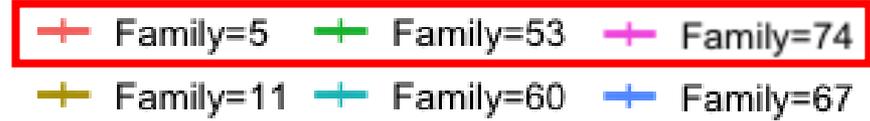
10 low/10 high based on data from Divilov *et al* in press

**OsHV-1 TB < AUS  $\mu$ var < FRA  $\mu$ var (p<0.001)**

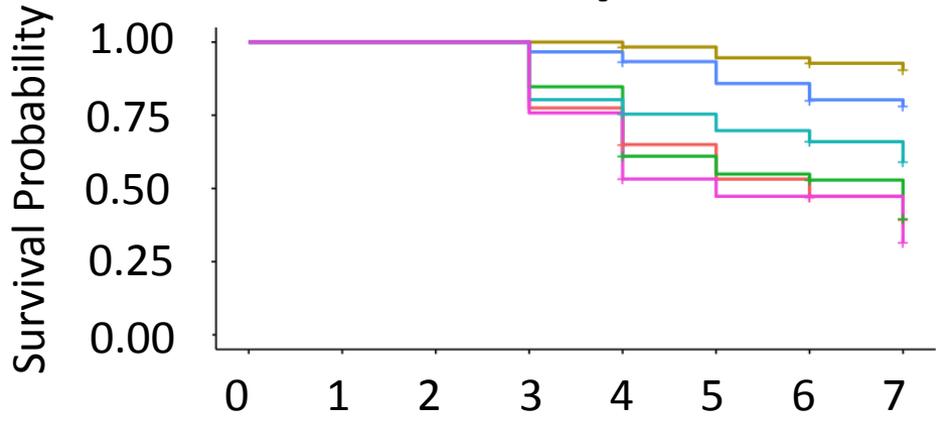
Agnew, Burge, Friedman *et al* unpub data



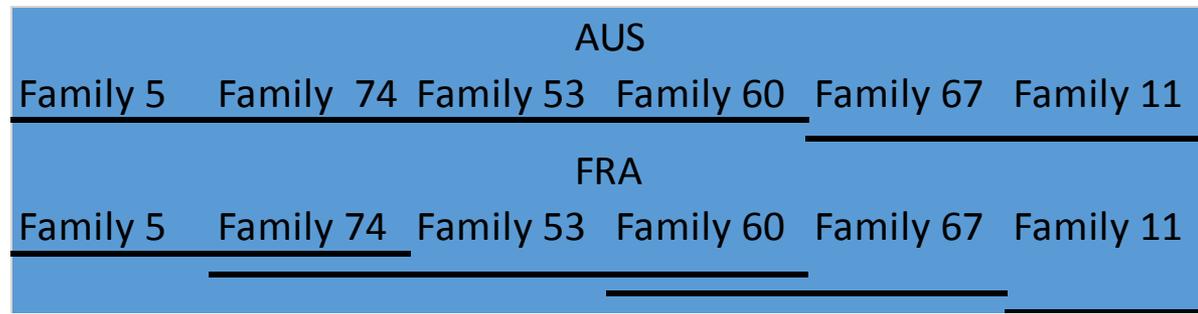
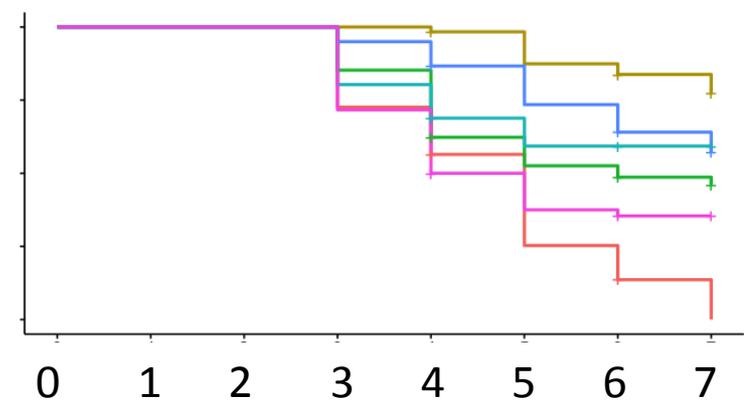
# Six low and high performing families



### AUS $\mu$ var



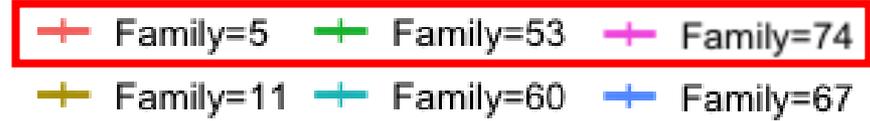
### FRA $\mu$ var



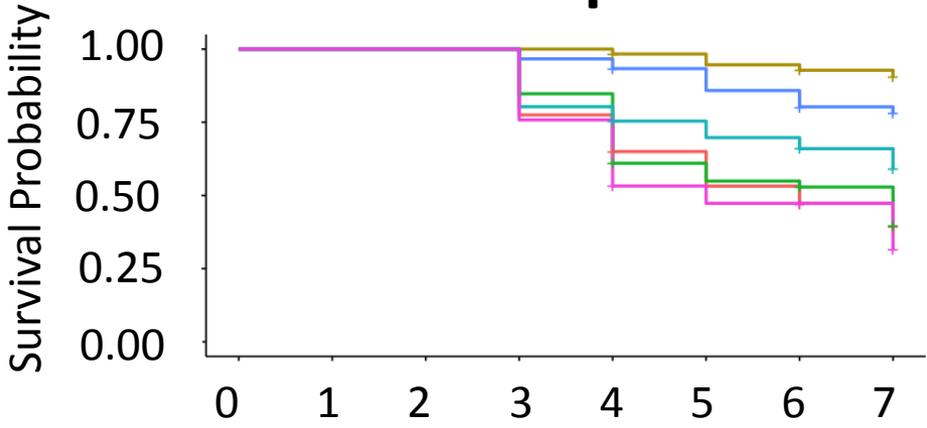
Log-rank Chi-Square statistics  $p < 0.05$   
Using R packages survival & survminer



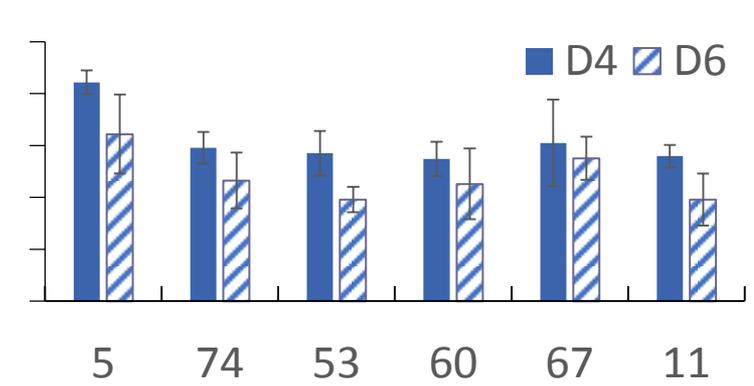
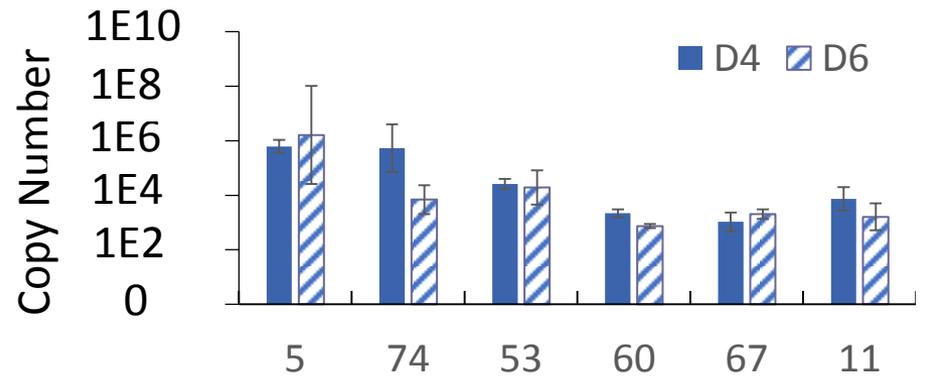
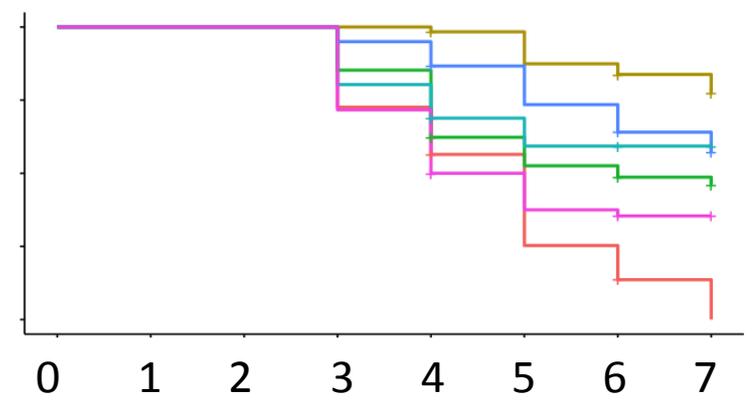
# Six low and high performing families



### AUS $\mu$ var



### FRA $\mu$ var

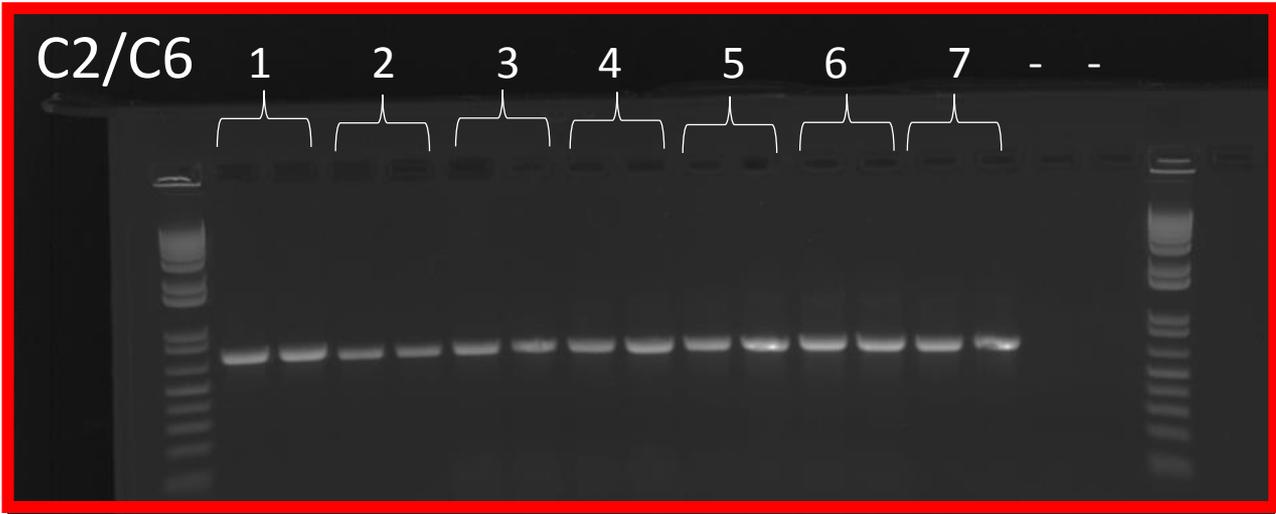




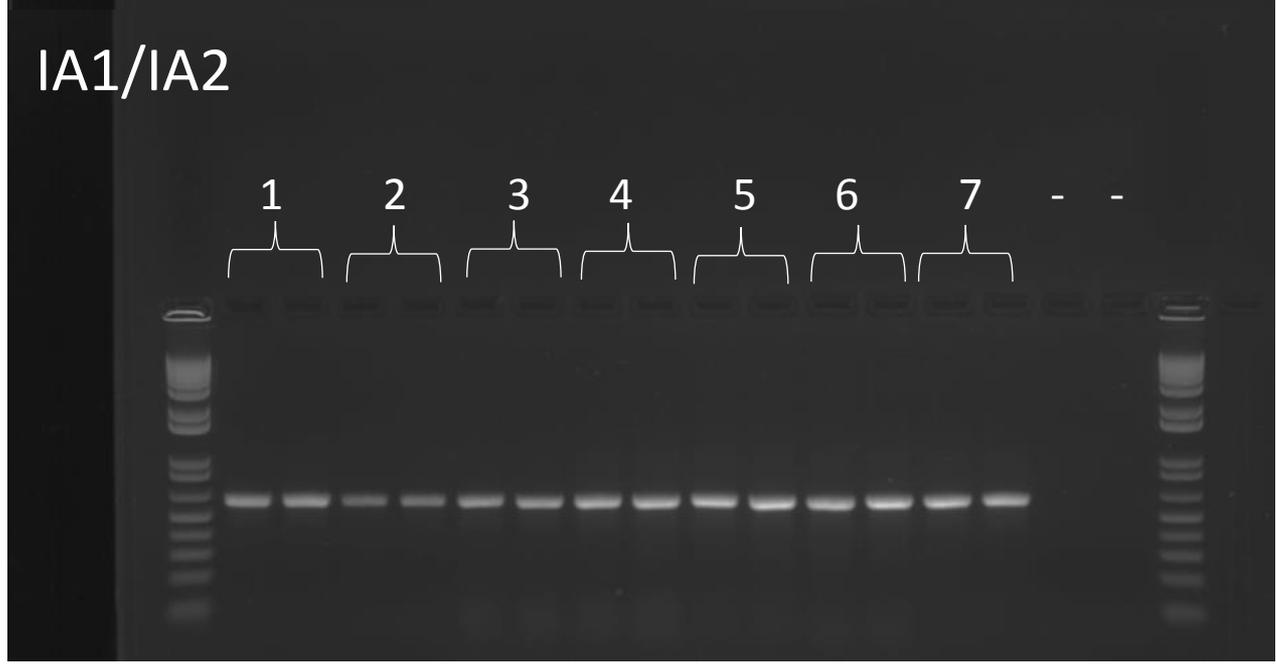
# OsHV-1 in San Diego: late 2018

- First detection of OsHV-1 outside of Tomales Bay area in the US (since likely establishment in early 1990's)
- In new aquaculture venture establishing health history
- Nursery depopulated and shutdown
- Original seed source tested and “heat” test of juveniles coming from source bay
- Ongoing “sentinel” outplants by California Department of Fish and Wildlife both in San Diego & S. California



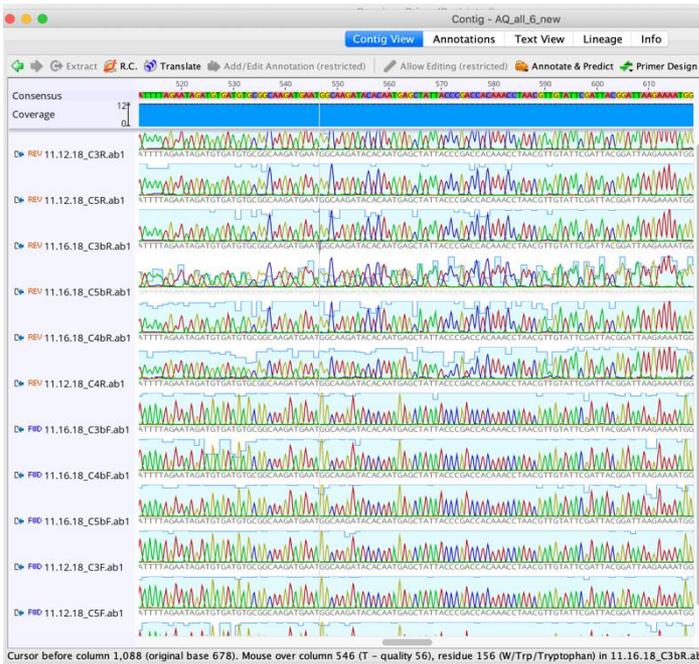


- 1) OsHV-1 TB (2017)
- 2) OsHV-1  $\mu$ var AUS
- 3) **San Diego**
- 4) **San Diego**
- 5) **San Diego**
- 6) OsHV-1  $\mu$ var FRA
- 7) OsHV-1 TB (2003)





# Percent Identity Matrix from Clustal 2.1 “C region”/ORF 4



<b>San Diego (n=3; 3-5)</b>	
Japanese variant	99.36
New Zealand $\mu$ var	99.68
<b>OsHV-1 <math>\mu</math>var FRA</b>	<b>99.84</b>
Italy $\mu$ var full genome	99.84
<b>OsHV-1 <math>\mu</math>var AUS</b>	<b>99.84</b>

OsHV-1 ref full genome

**OsHV-1 TB (2003)**

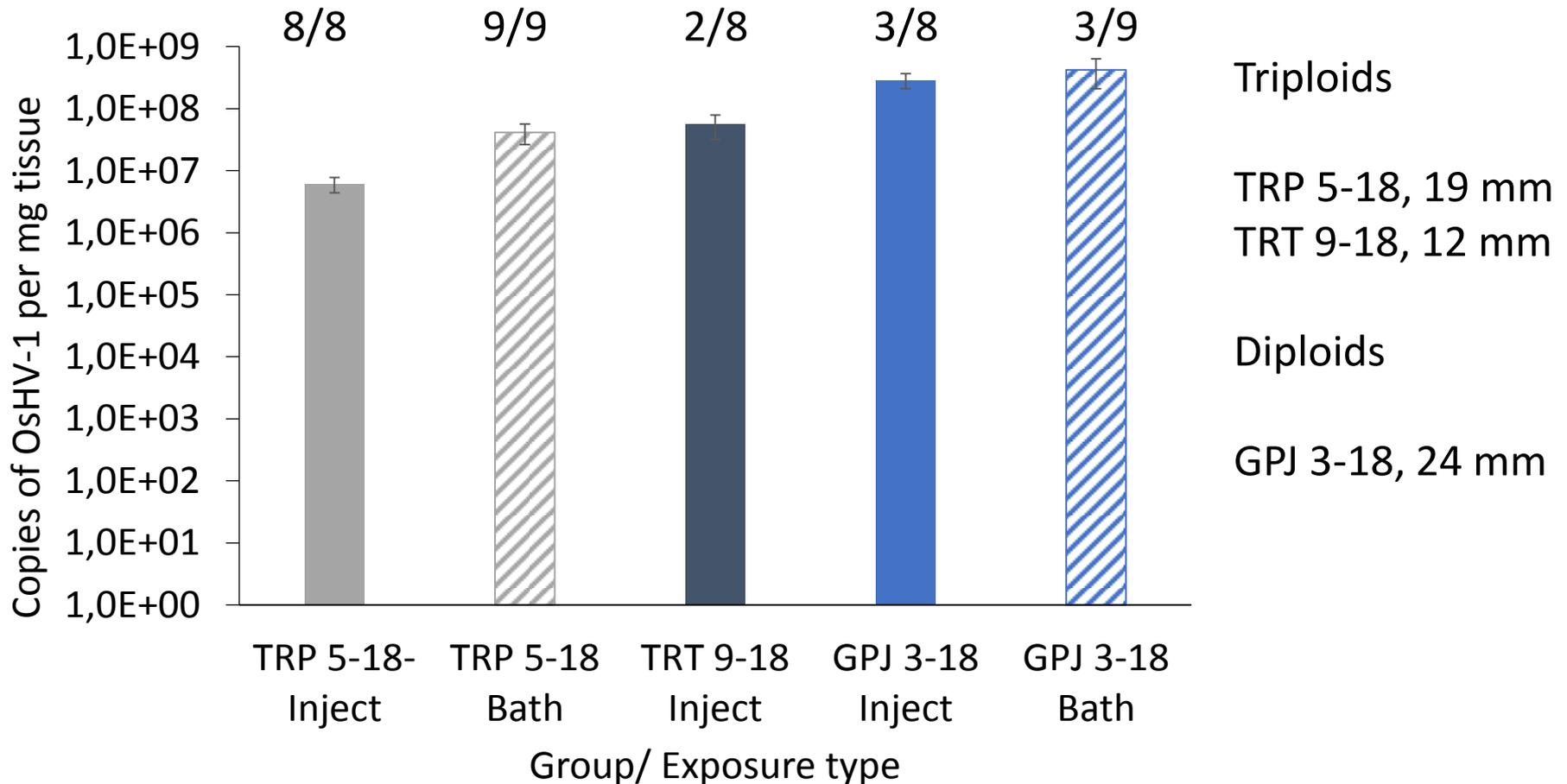
La Cruz, Mexico

99.36	★
99.68	★
<b>99.84</b>	★
99.84	★
<b>99.84</b>	★
95.30	★
<b>94.86</b>	★
95.06	★



# Pilot transmission study: February 2019

Injected with 25,000 copies\* or exposed by bath with 260,000\* total copies; 8-9 animals per trt





# Next Steps Needed

- Though data indicate non-Pacific oyster species are less susceptible, more life stages and lines of oyster species should be tested & assessed as potential vectors
- **Additional characterization of OsHV-1 from San Diego**
- Development of a high-throughput assay to differentiate variants
- **Gene expression analysis of Eastern vs. Pacific oysters**
- Pair laboratory/field trials with quantitative genetic studies to help identify markers of resistance/susceptibility for QTL development
- **Education – Goal to reduce potential for introduction**



# Acknowledgements



Oysters provided by:

Taylor Shellfish, Hawaiian Shellfish, Baywater Inc, Puget Sound Restoration Fund, Molluscan Broodstock Program & Hog Island Oyster Company



Thank you!



# Results: CA OsHV-1

