U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

# Handling and Resubmergence
# A Summary of the Latest Science

Victoria Pruente

US FDA, Gulf Coast Seafood Laboratory

2023 ISS - GSASSC

# Oyster Aquaculture Culture Techniques

Manual Tumbling

Manual Desiccation

Tidal Tumbling

Tidal Desiccation

# Handling Techniques & Vibrio Risks

- Handling practices may increase vibrio risks
- Oysters concentrate naturally occurring vibrios
- *Vibrio vulnificus (Vv)*
  - Accounts for 95% of seafood-related deaths
- *Vibrio parahaemolyticus (Vp)*
  - Leading cause of seafood-related infection

# Handling & Vibrio Risks

## 1 Pre-Handling

- Ambient *Vibrio* levels in oysters

## 2 Routine Handling

- *Vibrio* levels increase

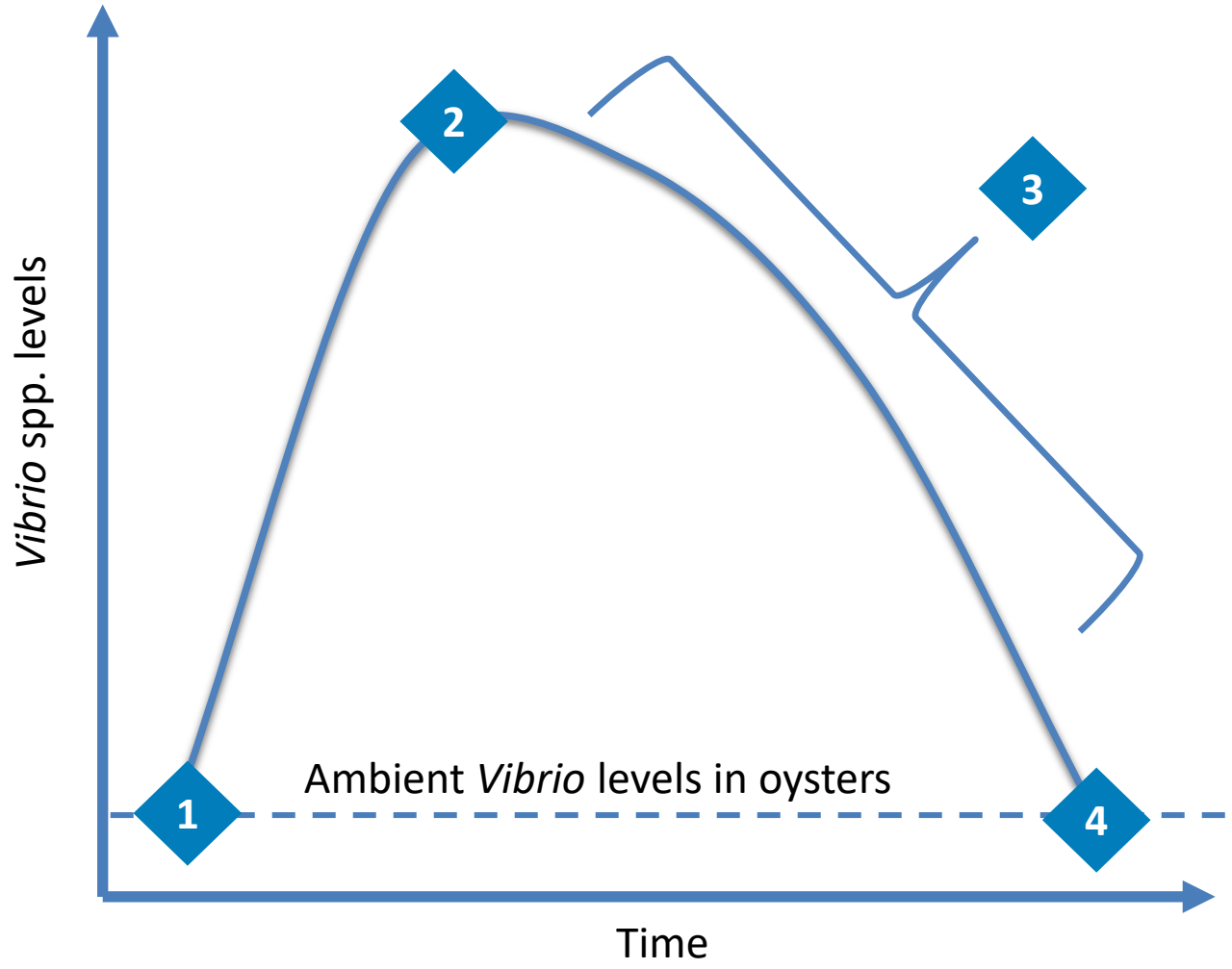
## 3 Resubmersion Period

- Elevated *Vibrio* levels decrease

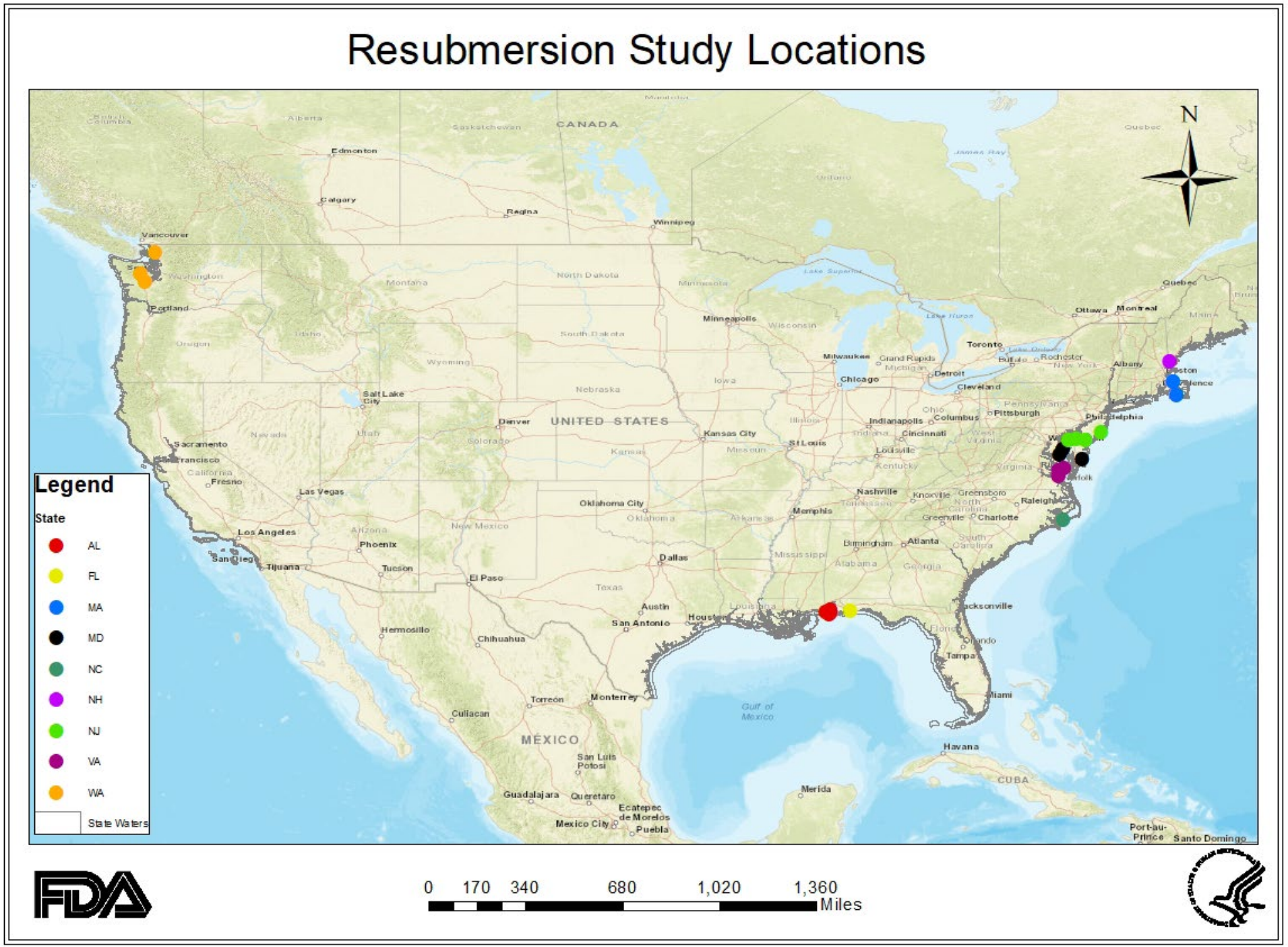
**How long?**

## 4 Harvest

- *Vibrio* levels recovered to ambient levels



- 15 studies
  - Peer-reviewed literature: 9
  - Unpublished, reports available online: 4
  - In prep for publication: 2
- 9 states



# Experimental Design

## Pre-Treatment

- Control samples collected

## Post-Treatment

- Control/Treatment Samples collected
- Oysters returned to water

Sampling days vary depending on study


## Treatment Period

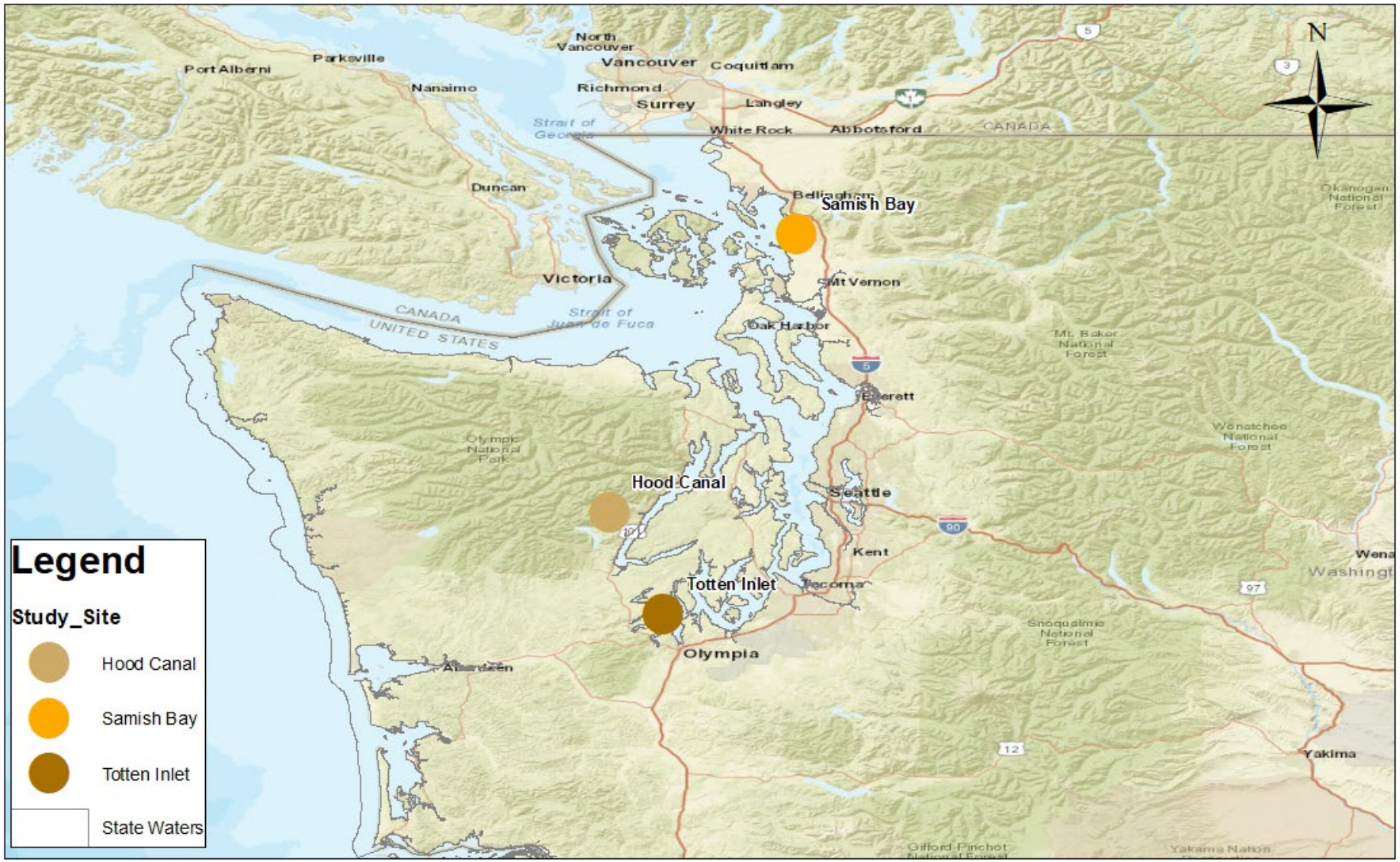
- Oysters removed from water
- Treatments were applied

## Resubmersion Period

- Samples collected each day
- Vibrio levels quantified to determine recovery

x Multiple Trials

# Resubmersion Studies - Washington State



# Effect of intertidal exposure on *Vibrio parahaemolyticus* levels in Pacific Northwest oysters

J.L. Nordstrom, C.A. Kaysner, G.M. Blackstone, M.C.L. Vickery, J.C. Bowers, A. DePaola

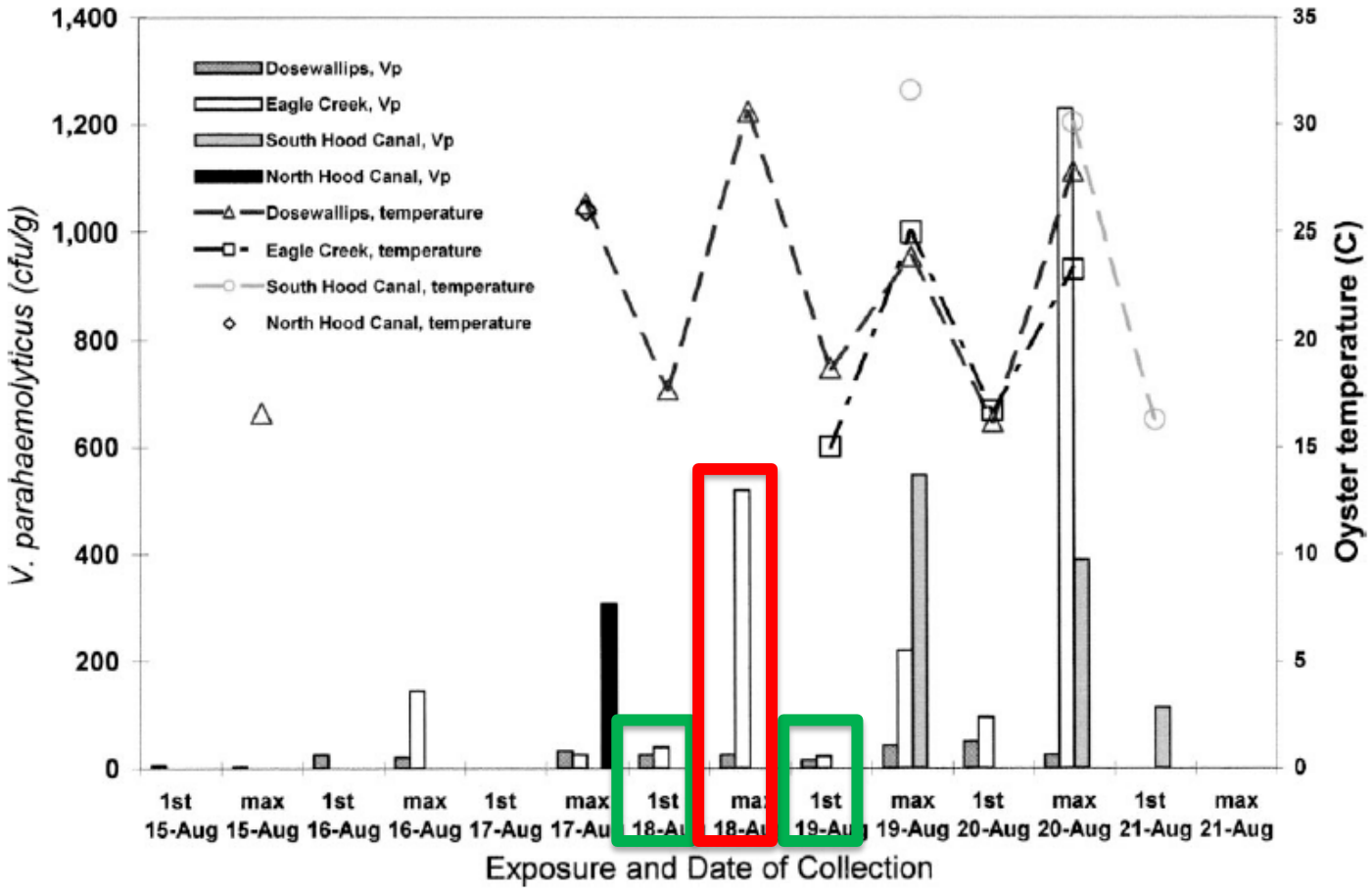



FIGURE 2. *Total* Vibrio parahaemolyticus *levels at first and maximum exposure versus oyster temperature over time.*

Journal of Food Protection, 2004

# Effects of intertidal harvest practices on levels of *Vibrio parahaemolyticus* and *Vibrio vulnificus* bacteria in oysters

J.L. Jones, T.P. Kinsey, L.W. Johnson, R. Porso, B. Friedman, M. Curtis, P. Wesighan, R. Schuster, J.C. Bowers

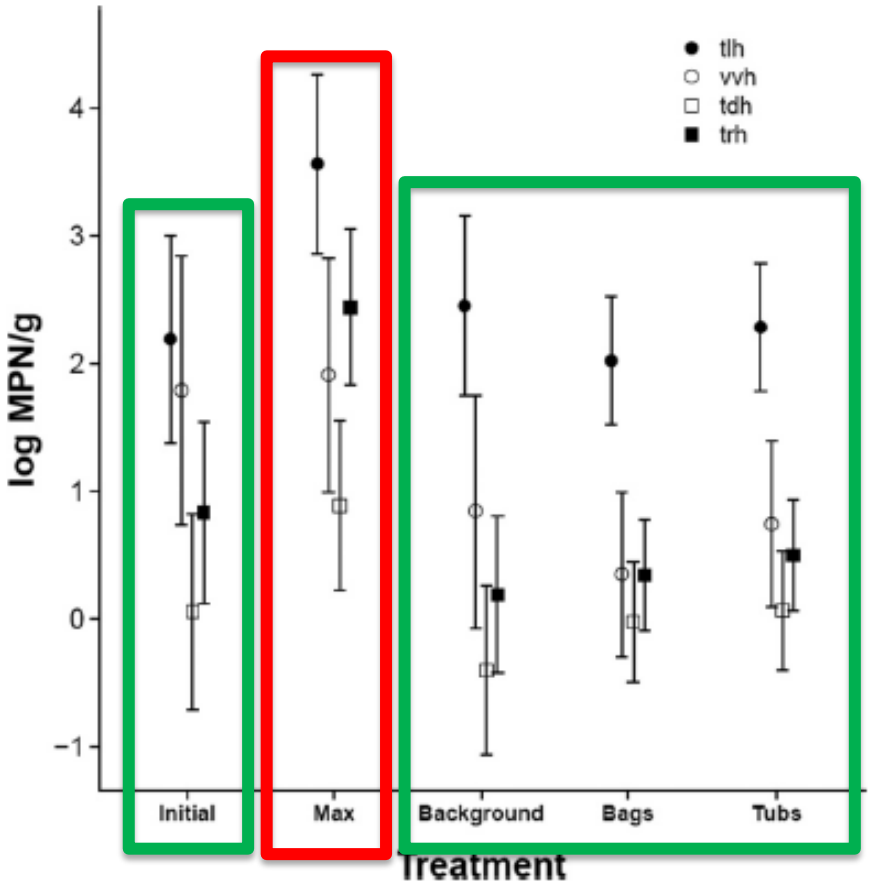



FIG 3 Mean levels of total (*tlh*) and pathogenic (*tdh* and *trh*) *V. parahaemolyticus* and *V. vulnificus* in oysters collected from each treatment in Washington. The error bars represent 95% confidence intervals (2 standard errors estimated by ANOVA).

Appl Environ Microbiol, 2016

# Effects of culture methods in the Pacific Northwest on the levels of *Vibrio* spp. in farm-raised oysters (*Crassostrea gigas*)

J.F. Kelly, W.C. Walton, J.L. Jones

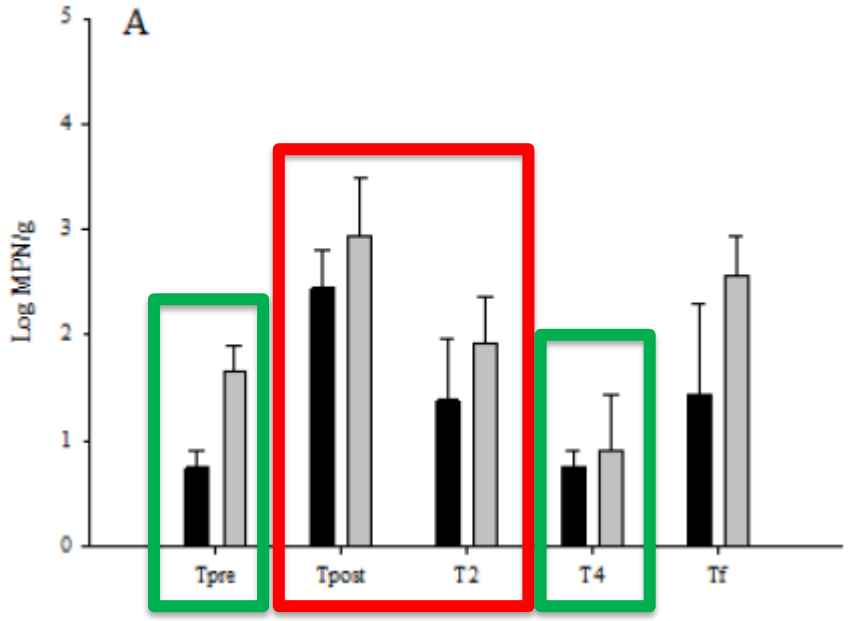



Figure 2.4. Effect of culture method and sampling time on mean log-transformed total *V. parahaemolyticus* levels in oysters from Trial A (A; July 16-17, 2019), Trial B (B; July 19-20, 2019), Trial C (C; July 21-22, 2020), Trial D (D; July 31-August 1, 2020), and Trial E (E; August 4-5, 2020) prior to tidal desiccation ($T_{pre}$), following maximum air exposure ($T_{post}$), 2-h following resubmersion from the incoming tide ($T_2$), 4-h following resubmersion ($T_4$), and 24-h

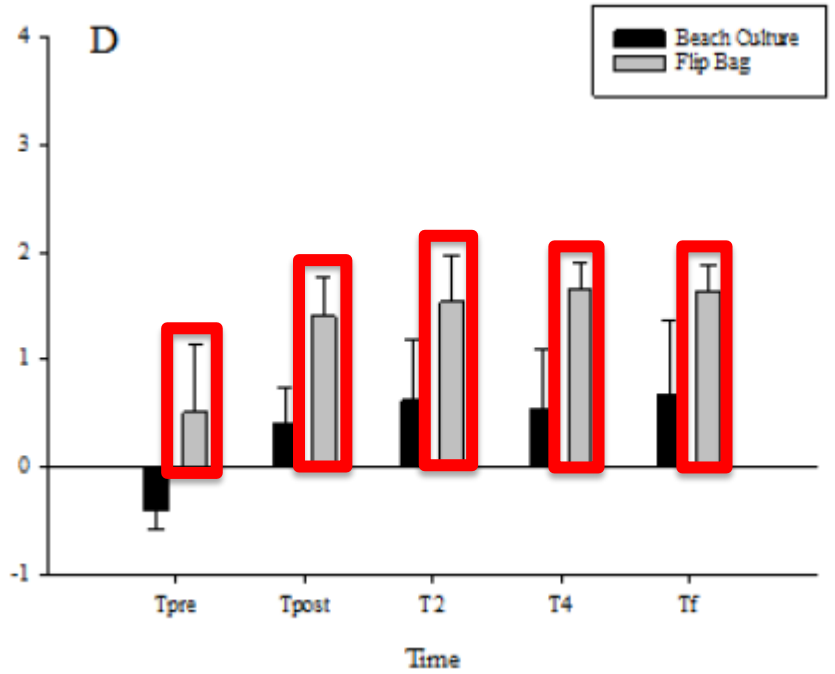
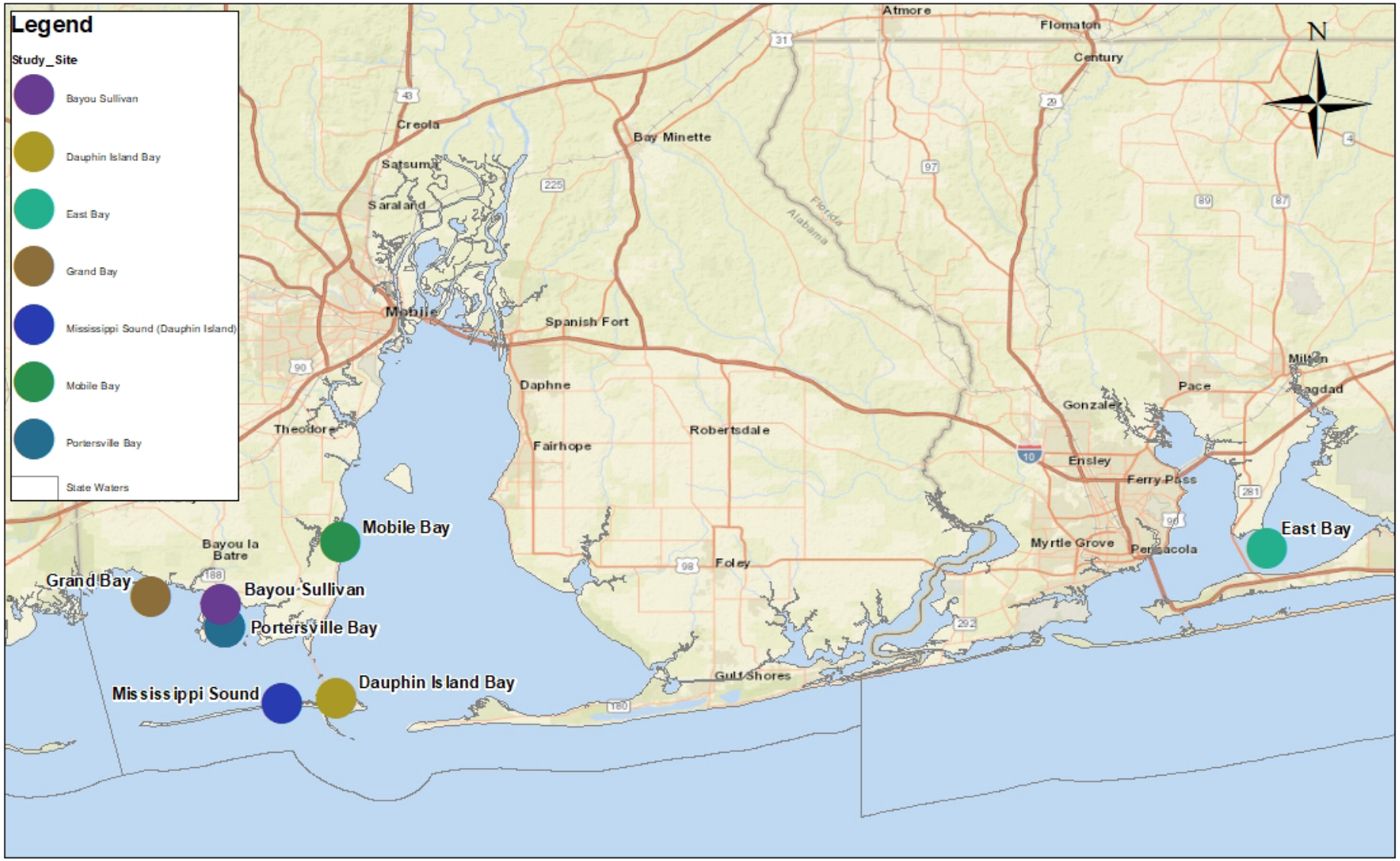



Figure 2.5. Effect of culture method and sampling time on mean log-transformed total *trh+ V. parahaemolyticus* levels in oysters from Trial A (A; July 16-17, 2019), Trial B (B; July 19-20, 2019), Trial C (C; July 21-22, 2020), Trial D (D; July 31-August 1, 2020), and Trial E (E; August 4-5, 2020) prior to tidal desiccation ($T_{pre}$), following maximum air exposure ($T_{post}$), 2-h following resubmersion from the incoming tide ($T_2$), 4-h following resubmersion ($T_4$), and 24-h

Auburn University
Master's Thesis, 2020
Pub in prep

# Resubmersion Studies - Gulf Coast



# Effects of dry storage and resubmersion of oysters on total *Vibrio vulnificus* and total and pathogenic (*tdh*+/*trh*+) *Vibrio parahaemolyticus* levels

T.P. Kinsey, K.A. Lydon, J.C. Bowers, J.L. Jones

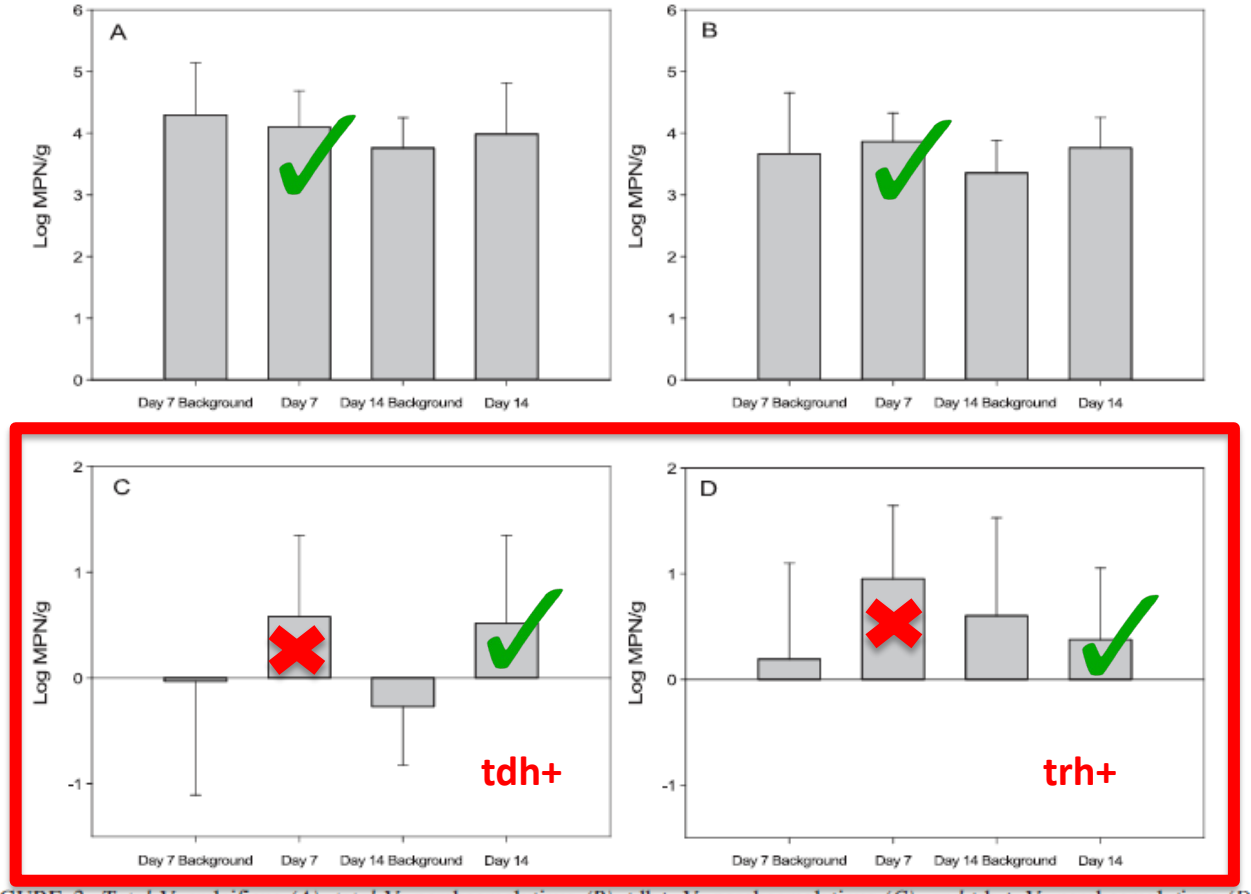



FIGURE 3. *Total* V. vulnificus *(A), total* V. parahaemolyticus *(B),* tdh+ V. parahaemolyticus *(C), and* trh+ V. parahaemolyticus *(D) levels in oysters after 7 and 14 days of resubmersion following 24-h dry storage and levels in concurrent background samples at 7 and 14 days. Bars are representative of mean levels, with error bars indicating the standard deviation.*

Journal of Food Protection, 2015

# Effects of desiccation practices of cultured Atlantic oysters (*Crassostrea virginica*) on *Vibrio* spp. in Portersville Bay, Alabama, USA

S.M. Grodeska, J.L. Jones, C.R. Arias, W.C. Walton

TABLE 2. *Days at which* V. parahaemolyticus *levels returned to background level*

| | Day[b] | |
|---|---|---|
| Trial[a] | Air dried[c] | Freshwater dipped[d] |
| I | 2 | 1 |
| II | 1 | 7 |
| III | 2 | 1 |
| IV | 2 | 2 |
| V | NA[e] | NA |

[a] Two-week-long trials. Trials I and II took place in 2014, and trials III to V took place in 2015.
[b] Day post-resubmersion on which *Vibrio* spp. levels were not significantly different from background levels.
[c] Ambient air dried for 27 h.
[d] Freshwater dipped for 3 h and then ambient air dried for 24 h.
[e] NA, not available because of the lack of significant increases in treated samples.

TABLE 3. *Days at which* V. vulnificus *levels returned to background level*

| | Day[b] | |
|---|---|---|
| Trial[a] | Air dried[c] | Freshwater dipped[d] |
| I | 2 | 2 |
| II | 1 | 7 |
| III | 3 | 3 |
| IV | 2 | 2 |
| V | NA[e] | NA |

[a] Two-week-long trials. Trials I and II took place in 2014, and trials III to V took place in 2015.
[b] Day post-resubmersion on which *Vibrio* spp. levels were not significantly different from background levels.
[c] Ambient air dried for 27 h.
[d] Freshwater dipped for 3 h and then ambient air dried for 24 h.
[e] NA, not available because of the lack of significant increases in treated samples.

Journal of Food Protection, 2017

# Effects of desiccation practices and ploidy in cultured oysters, *Crassostrea virginica*, on *Vibrio* spp. abundances in Portersville Bay (Alabama, USA)

S.M. Grodeska, J.L. Jones, W.C. Walton, C.R. Arias

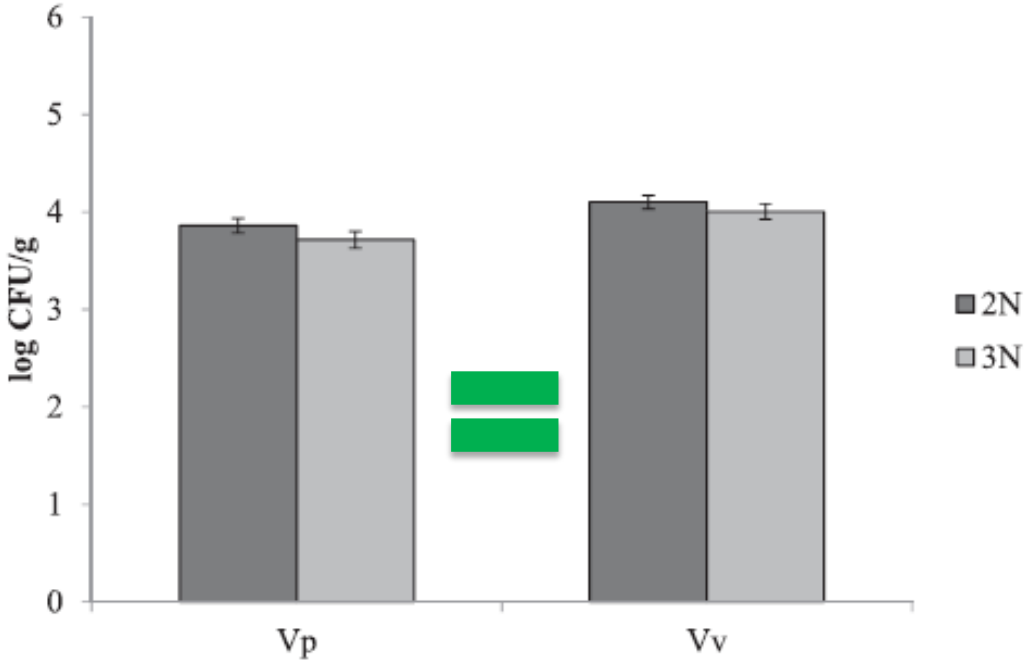



Fig. 3. Comparison of mean log transformed CFU/g of *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv) by ploidy standard error bars (combined across treatments and time). The key describes ploidy: diploids (2 N) and triploids (3 N). No significant differences were found between ploidy.

Aquaculture, 2019

# AL VARB Request 2017

| Gear Type | Trial | Total *Vp* | Path. *Vp* (*tdh*+) | Path. *Vp* (*trh*+) | Total *Vv* |
|---|---|---|---|---|---|
| Adjustable Longline System | 1 | NR* | 7 | 7 | 7 |
| | 2 | 7 | 7 | NR* | NR* |
| | 3 | 7 | NR* | 7 | 7 |
| | 4 | NR* | NR* | NR* | 7 |
| OysterGro | 1 | NR* | 7 | 7 | 7 |
| | 2 | NR* | 7 | 7 | 7 |
| | 3 | 7 | 7 | 7 | 7 |
| | 4 | NR* | NR* | NR* | 7 |
| Bottom Cages | 2 | 7 | 7 | 7 | 7 |
| | 3 | 7 | NR* | 7 | 7 |
| | 4 | NR* | NR* | NR* | NR* |

Table 1. Recovery times, in days, for desiccated oysters in three gear types. Note: NR* = target did not return to background levels by day 7, no data past day 7

# Effects of refrigeration and subsequent resubmersion on the abundance of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in cultured oysters (*Crassostrea virginica*)

V.L. Pruente, J.L. Jones, T.D. Steury, W.C. Walton

**Table 3**
Number of days for *Vibrio* spp. levels to return to control levels in oysters of each treatment, determined by mixed effects models.

| Days[a] | | | | |
|---|---|---|---|---|
| Vibrio spp. | TR[b] | TNR[c] | NTR[d] | NTNR[e] |
| V. vulnificus | 2 | 4 | 2 | 2 |
| Total V. parahaemolyticus | 4 | 4 | 4 | 4 |
| Pathogenic V. parahaemolyticus (tdh+) | 7 | 7 | 7 | 7 |
| Pathogenic V. parahaemolytics (trh+) | 4 | 7 | 7 | 7 |

**Table 4**
Number of days for *Vibrio* spp. levels to return to control levels in oysters of each treatment determined by general linear models.

| Trial | Day[a] | | | | |
|---|---|---|---|---|---|
| | Vibrio spp. | TR[b] | TNR[c] | NTR[d] | NTNR[e] |
| I | V. vulnificus | 1 | 2 | 2 | 2 |
| | Total V. parahaemolyticus | 1 | 2 | 2 | 2 |
| | Pathogenic V. parahaemolyticus (tdh+) | 1 | 2 | 1 | 1 |
| | Pathogenic V. parahaemolyticus (trh+) | 4 | 7 | 4 | 7 |
| II | V. vulnificus | 1 | 4 | 4 | 1 |
| | Total V. parahaemolyticus | 2 | 2 | 2 | 2 |
| | Pathogenic V. parahaemolyticus (tdh+) | 2 | 2 | 2 | 4 |
| | Pathogenic V. parahaemolyticus (trh+) | 2 | 1 | 4 | 4 |
| III | V. vulnificus | 2 | 2 | 2 | 4 |
| | Total V. parahaemolyticus | 2 | 7 | 7 | 7 |
| | Pathogenic V. parahaemolyticus (tdh+) | 1 | 4 | 7 | 4 |
| | Pathogenic V. parahaemolyticus (trh+) | 2 | 4 | 4 | 4 |
| IV | V. vulnificus | 2 | 2 | 2 | 2 |
| | Total V. parahaemolyticus | 2 | 2 | 2 | 2 |
| | Pathogenic V. parahaemolyticus (tdh+) | 2 | 2 | 2 | 7 |
| | Pathogenic V. parahaemolyticus (trh+) | 4 | 1 | 4 | 4 |
| V | V. vulnificus | 2 | 1 | 2 | 1 |
| | Total V. parahaemolyticus | 2 | 4 | 2 | 2 |
| | Pathogenic V. parahaemolyticus (tdh+) | 2 | 2 | 2 | 2 |
| | Pathogenic V. parahaemolyticus (trh+) | 2 | 2 | 2 | 2 |

Int Journal of Food Microbiol, 2020

# Effect of gear type on *Vibrio* spp. levels in farm-raised oysters (*Crassostrea virginica*) after routine handling and resubmersion

V.L. Pruente, W.C. Walton, J.L. Jones

TABLE 4. *Number of days for* Vibrio *spp. levels to return to control levels*[a]

| | Days | | | |
|---|---|---|---|---|
| *Vibrio* spp. | ALS TR | ALS des | OG TR | OG des |
| May trials (2018–2019) | | | | |
| *V. vulnificus* | 7 | 7 | 7 | 7 |
| Total *V. parahaemolyticus* | 7 | 7 | 14 | 14 |
| Pathogenic *Vp* (*tdh*+) | 7 | 14[b] | 7 | 7 |
| Pathogenic *Vp* (*trh*+) | 7 | 14[b] | 7 | 7 |
| July trials (2018–2019) | | | | |
| *V. vulnificus* | 3 | 3 | 3 | 3 |
| Total *V. parahaemolyticus* | >14 | 7 | 14 | 7 |
| Pathogenic *Vp* (*tdh*+) | 7 | 7 | 7[b] | 7 |
| Pathogenic *Vp* (*trh*+) | 7 | 7 | 7 | 7 |

[a] Number of days after resubmersion when *Vibrio* spp. levels in treatment oysters were not significantly different from control oysters ($P > 0.05$), as determined by the mixed-effects model. ALS, adjustable longline system; TR, tumbled and refrigerated; des, desiccated; OG, OysterGro system.

[b] Cases where statistical significance does not agree with biological relevance (i.e., *Vibrio* spp. levels in the treatment oysters were still over 0.5 log MPN/g higher than levels in control oysters).

Journal of Food Protection, 2021

# Effects of farm location on *Vibrio parahaemolyticus* and *Vibrio vulnificus* levels in oysters after desiccation and resubmersion in the Northern Gulf of Mexico

M.D. McGough, V.L. Pruente, W.C. Walton, J.L. Jones

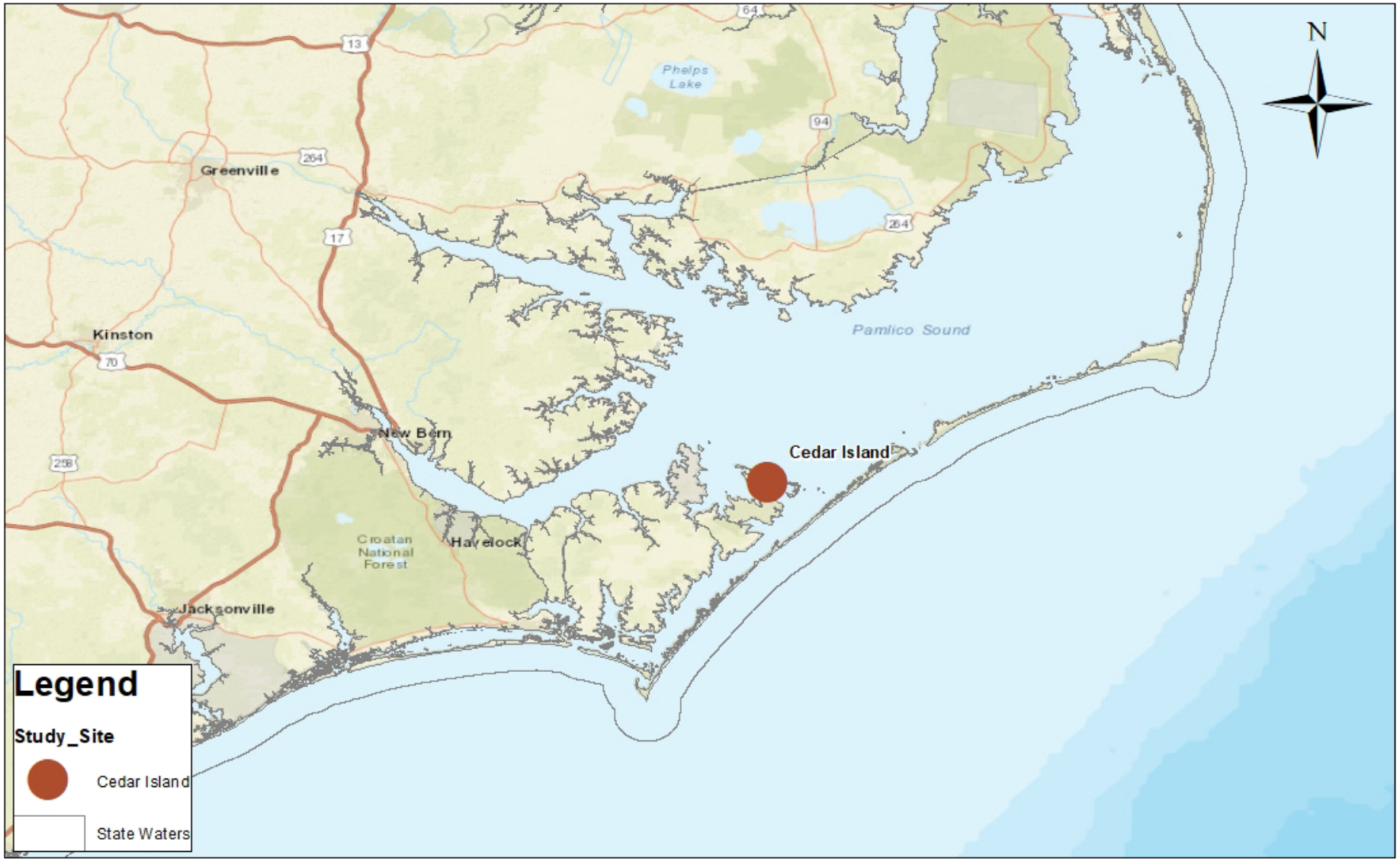
TABLE 2. *Number of days for vibrio levels in desiccated oysters to return to ambient levels*

| | Day[a] | | | |
|---|---|---|---|---|
| Location | Total Vp | Pathogenic Vp (*tdh*+) | Pathogenic Vp (*trh*+) | *V. vulnificus* |
| Mississippi Sound I | 7 | 14[b] | 7 | 7[b] |
| Mississippi Sound II | 7 | 7 | 7 | 7 |
| Mobile Bay I | 14[b] | NC[c] | NC | 7 |
| Mobile Bay II | 7 | NC | 7[b] | 7 |
| Bayou Sullivan I | NC | NC | NC | 7 |
| Bayou Sullivan II | 14 | 14 | 7 | 7 |
| East Bay I | 14[b] | 7[b] | 7[b] | 7[b] |
| East Bay II | 7 | NC | NC | 7 |

[a] Number of days after resubmersion when vibrio levels were not significantly higher than ambient levels. Vp, *V. parahaemolyticus*.
[b] Recovery times based on the biologically relevant mean 0.50 log MPN/g.
[c] NC indicates levels were not significantly higher than ambient levels after desiccation and the difference in means was <0.50 log MPN/g, so no recovery time could be determined.

Journal of Food Protection, 2021

# Resubmersion Studies - North Carolina



# Effects of tumbling, refrigeration, and resubmersion on *Vibrio parahaemolyticus* and *V. vulnificus* levels in North Carolina cultured oysters (*C. virginica*)

V.L. Pruente, J.L. Jones, M.D. McGough, W.C. Walton

**Table 3**

Number of days for vibrio levels to recover to control levels.

| Day[a] | | | | |
|---|---|---|---|---|
| *Vibrio* spp. | TR[b] | TNR[c] | NTR[d] | NTNR[e] |
| *V. vulnificus* | 1 | 1 | 1 | 1 |
| Total *V. parahaemolyticus* | 14 | 7 | 7 | 7 |
| Pathogenic *V. parahaemolyticus* (*tdh*+) | 3 | 7 | 1 | 3 |
| Pathogenic *V. parahaemolyticus* (*trh*+) | 3 | 7 | 7 | 3 |

[a] Number of days after resubmersion when *Vibrio* spp. levels were not significantly higher than control levels ($p \geq 0.05$), determined by the mixed effects model.

[b] Tumbled, refrigerated treatment.

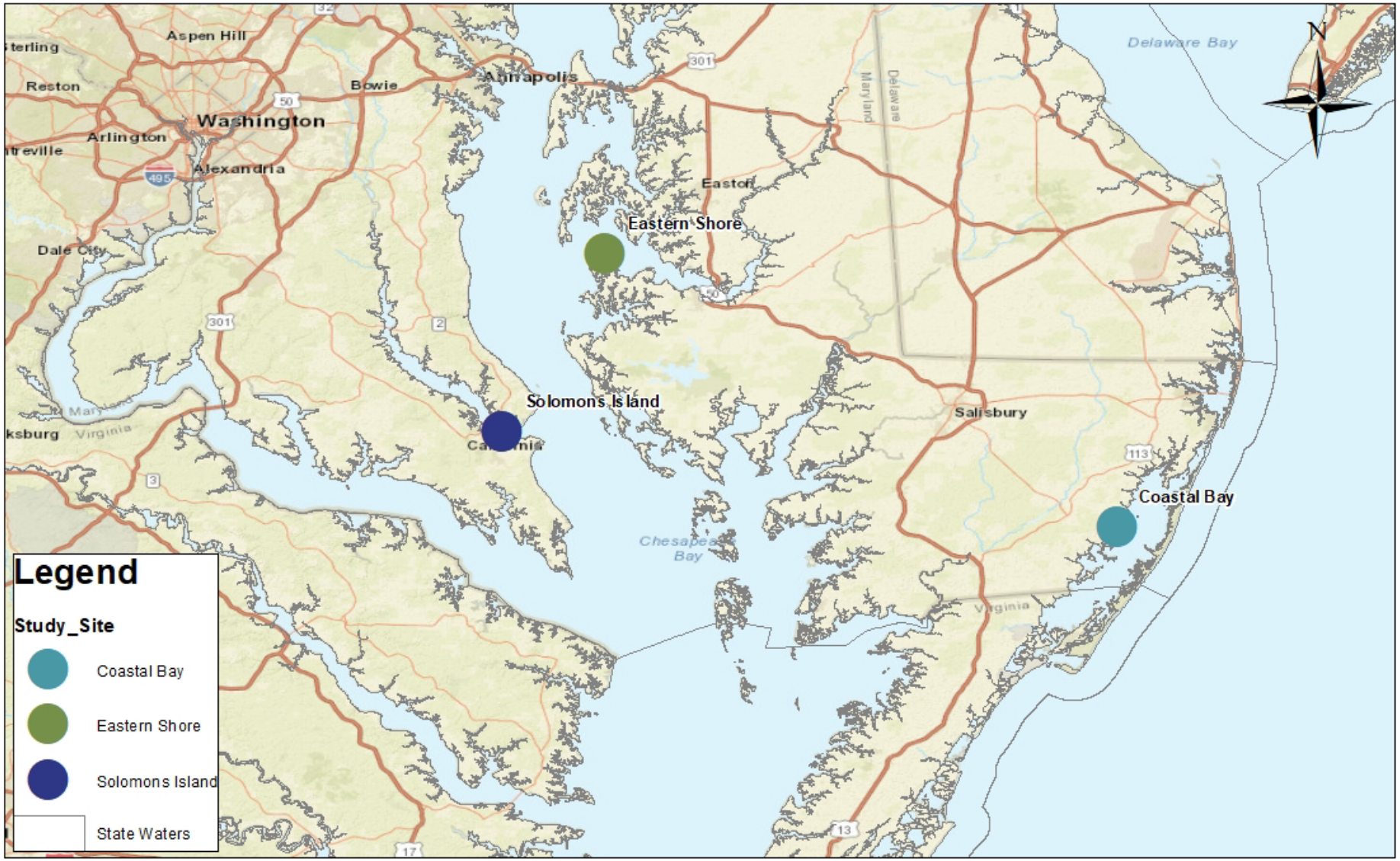
[c] Tumbled, desiccated treatment.

[d] Refrigerated only treatment.

[e] Desiccated only treatment.

Aquaculture, 2022

# Resubmersion Studies - Maryland



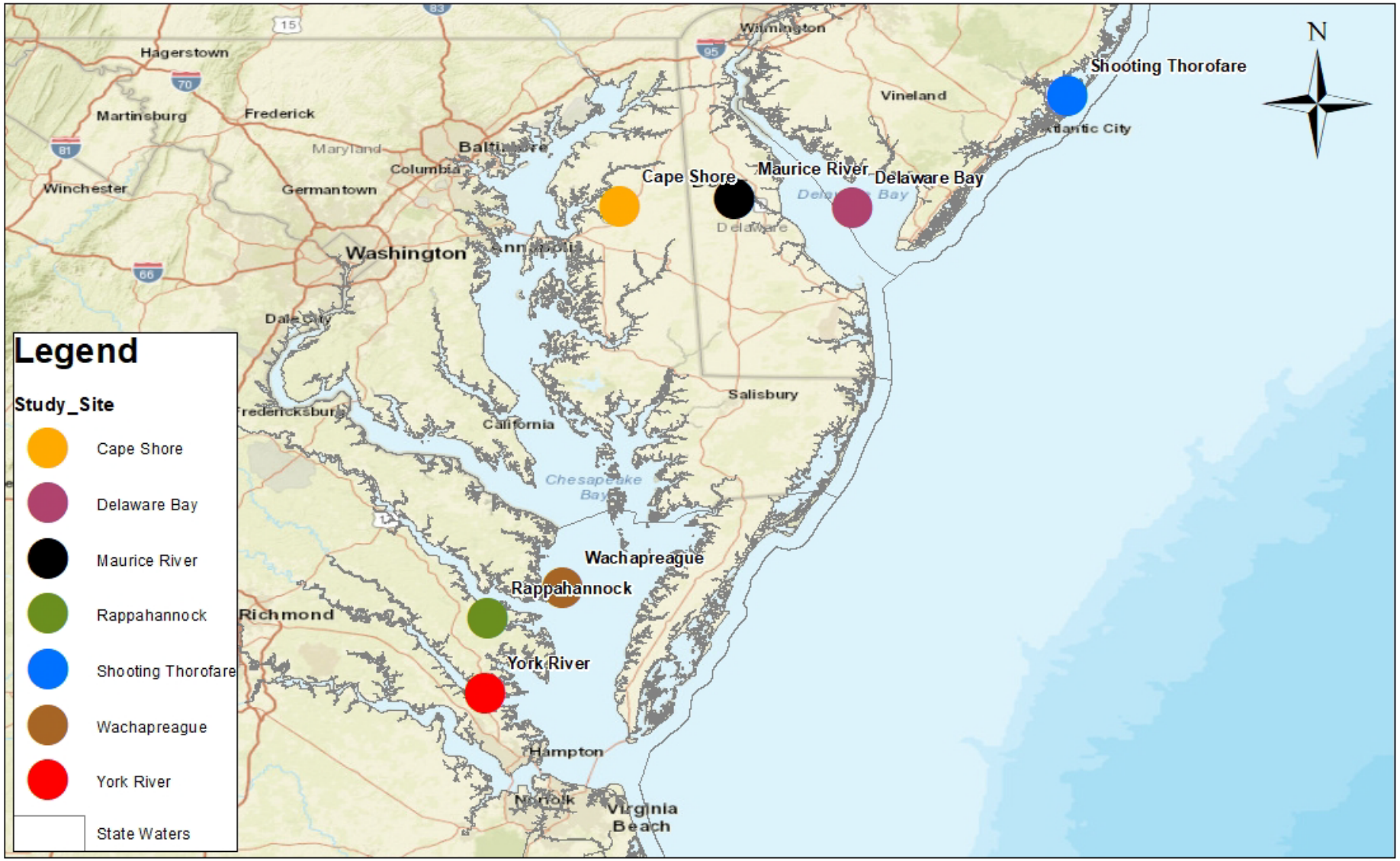
# Effectiveness of resubmergence of oysters as a method for *Vibrio* bacteria depuration in Maryland waters

A. Ellett, P. Vidal, R. Adelizzi, J. Jacobs, S. Parveen, J. Meredith, K. Brohawn, K. Perkins, R. Tower, R. Myers

| Month | Site | Total Vp | Vv |
|---|---|---|---|
| August 2019 | Tred Avon | 10 | 7 |
| | Solomon's Island | Undetermined | Undetermined |
| | Coastal Bay | 10-14 | 2 |
| September 2019 | Tred Avon | 7 | 10 |
| | Solomon's Island | 7 | 7 |
| | Coastal Bay | 14 | Undetermined |

Pub in prep (NOAA)

# Resubmersion Studies - New Jersey & Virginia



# Effects of intertidal harvest practices on levels of *Vibrio parahaemolyticus* and *Vibrio vulnificus* bacteria in oysters

J.L. Jones, T.P. Kinsey, L.W. Johnson, R. Porso, B. Friedman, M. Curtis, P. Wesighan, R. Schuster, J.C. Bowers

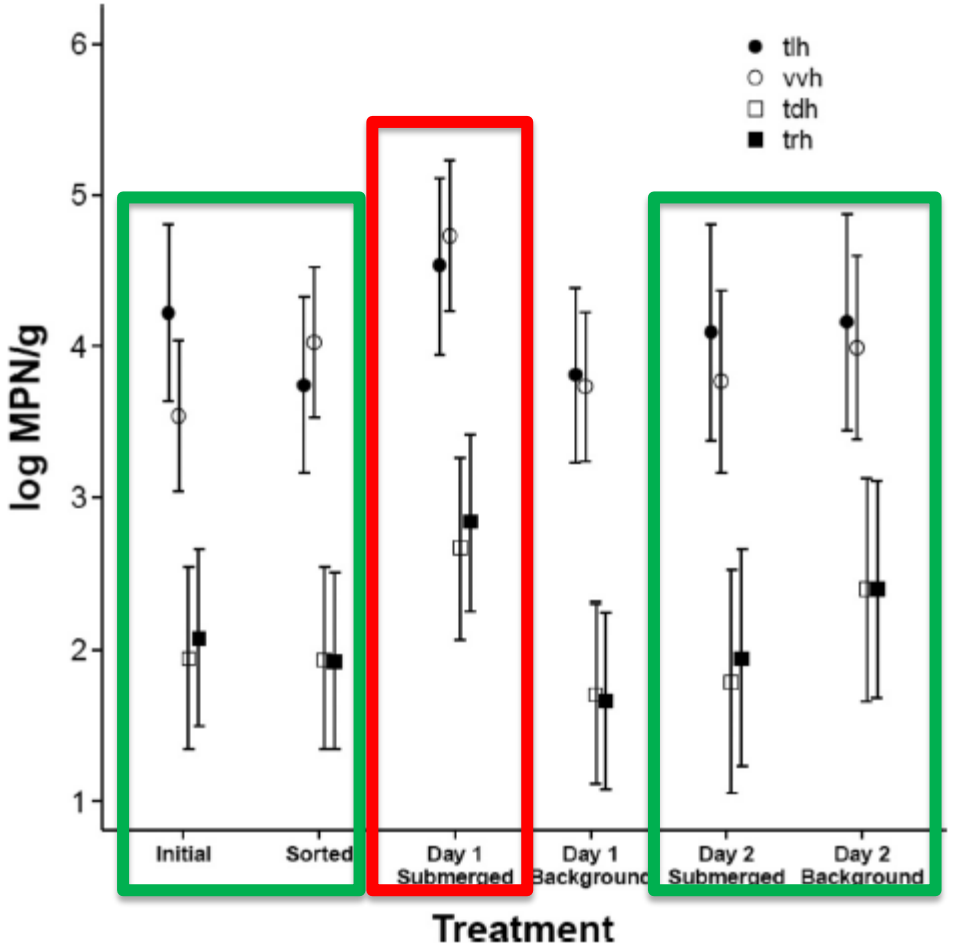
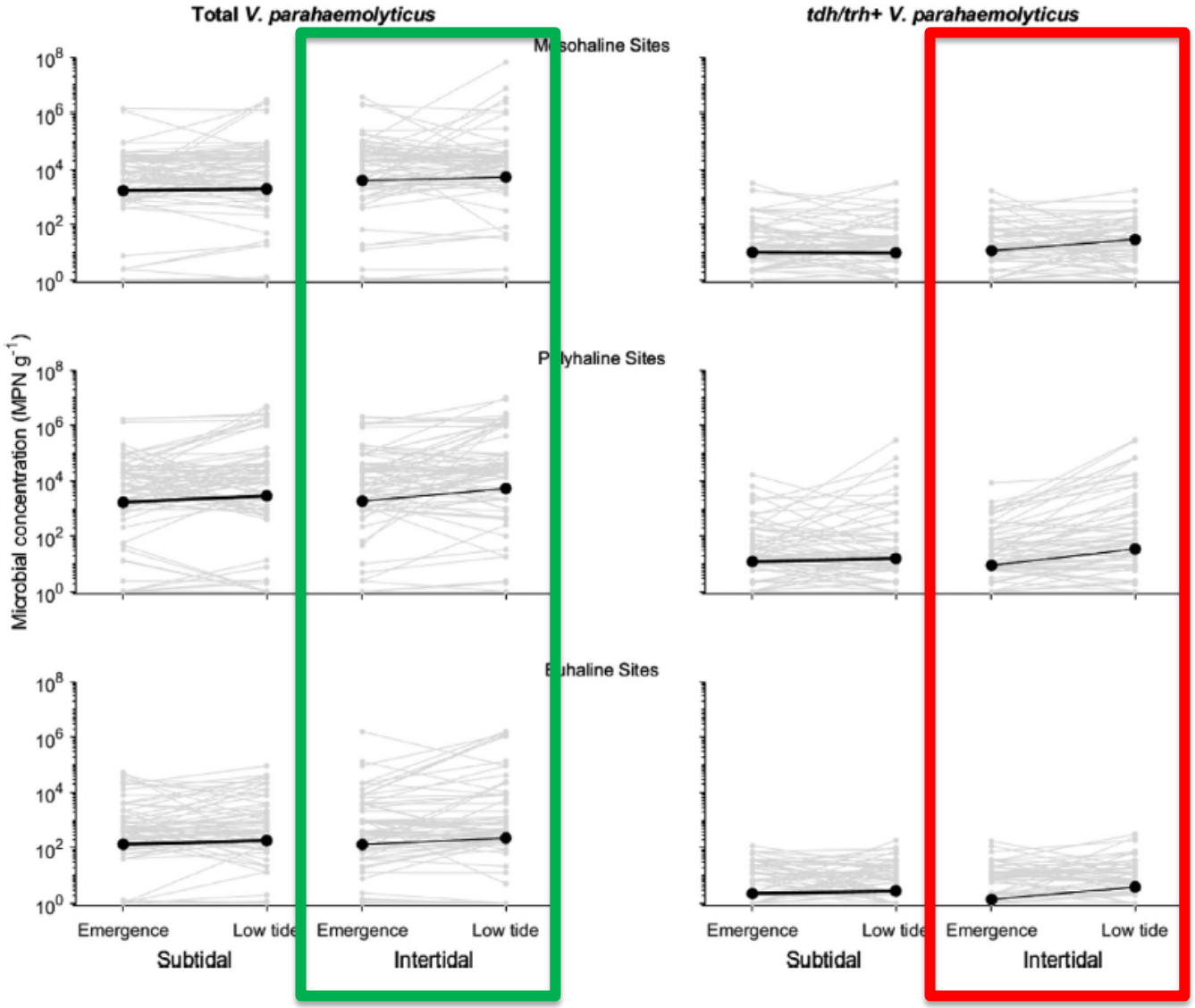



**FIG 4** Mean levels of total (*tlh*) and pathogenic (*tdh* and *trh*) *V. parahaemolyticus* and *V. vulnificus* in oysters collected from each treatment in New Jersey. The error bars represent 95% confidence intervals (2 standard errors estimated by ANOVA).

Appl Environ Microbiol, 2016

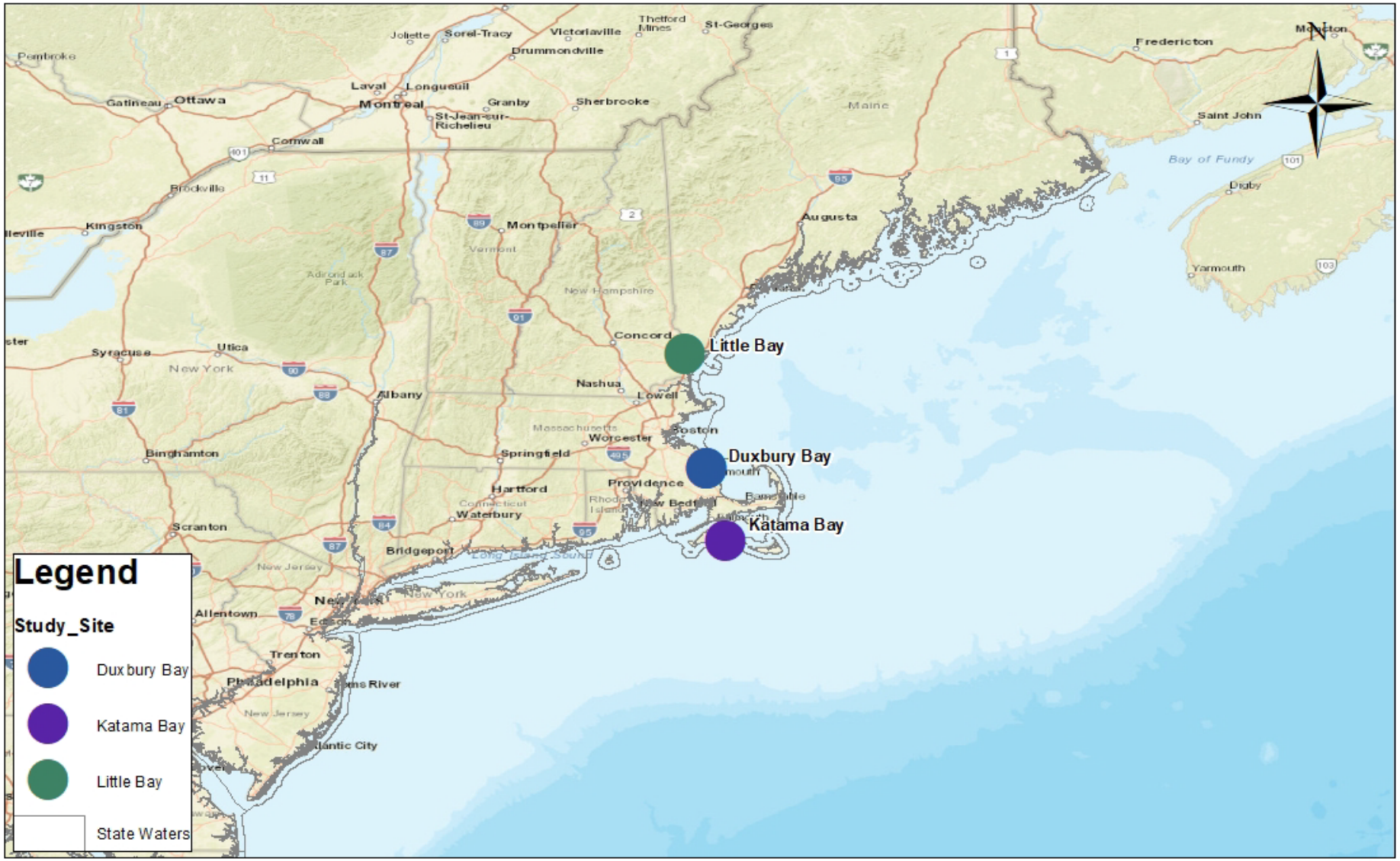
# Pathogenic *Vibrio parahaemolyticus* increase in intertidal-farmed oysters in the Mid-Atlantic region, but only at low tide

T. Ben-Horin, C. Audemard, L. Calvo, K.S. Reece, D. Bushek



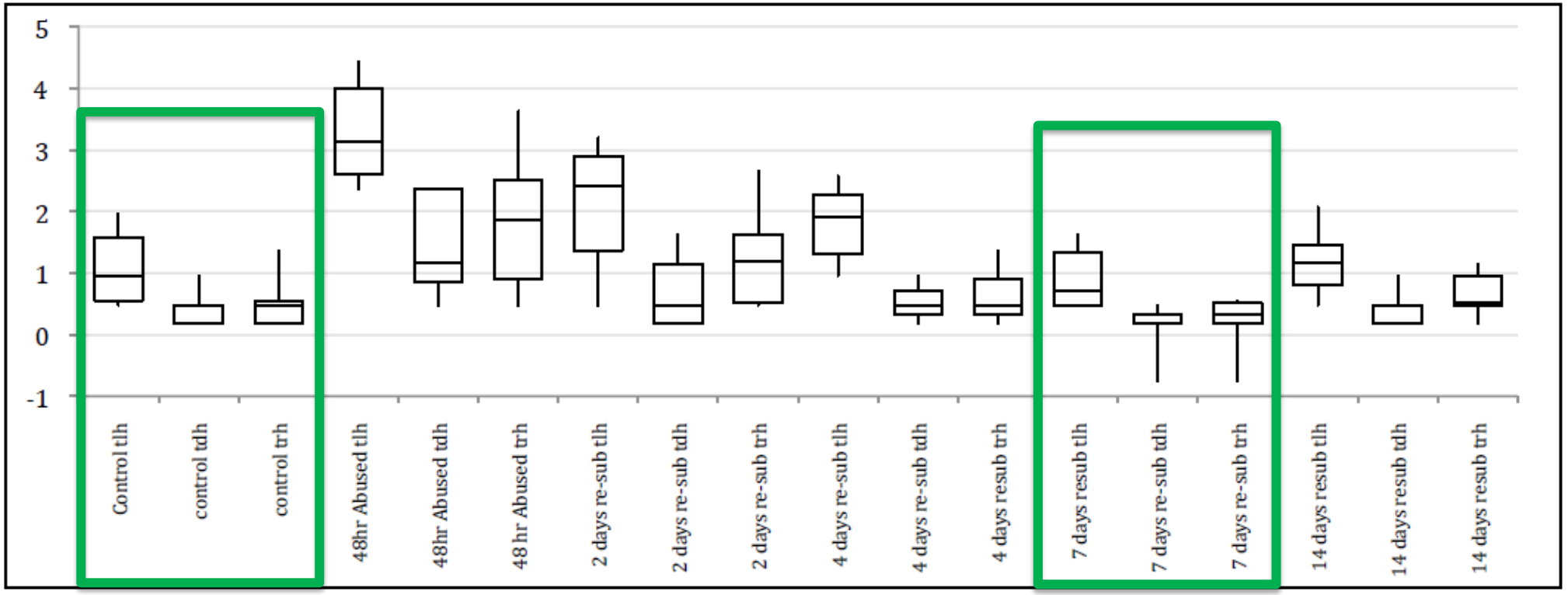

N American Journal of Aquaculture, 2021

# Resubmersion Studies - New Hampshire and Massachusetts



# Techniques and practices for *Vibrio parahaemolyticus* reduction in Massachusetts

Final Report to the Interstate Shellfish Sanitation Conference
Submitted August 31, 2017




ISSC Research Report, 2017

# Oyster culture and harvest practices to reduce pathogenic *Vibrio parahaemolyticus* concentrations in the Northeast US

Final Report to the Interstate Shellfish Sanitation Conference
Submitted June 16, 2020

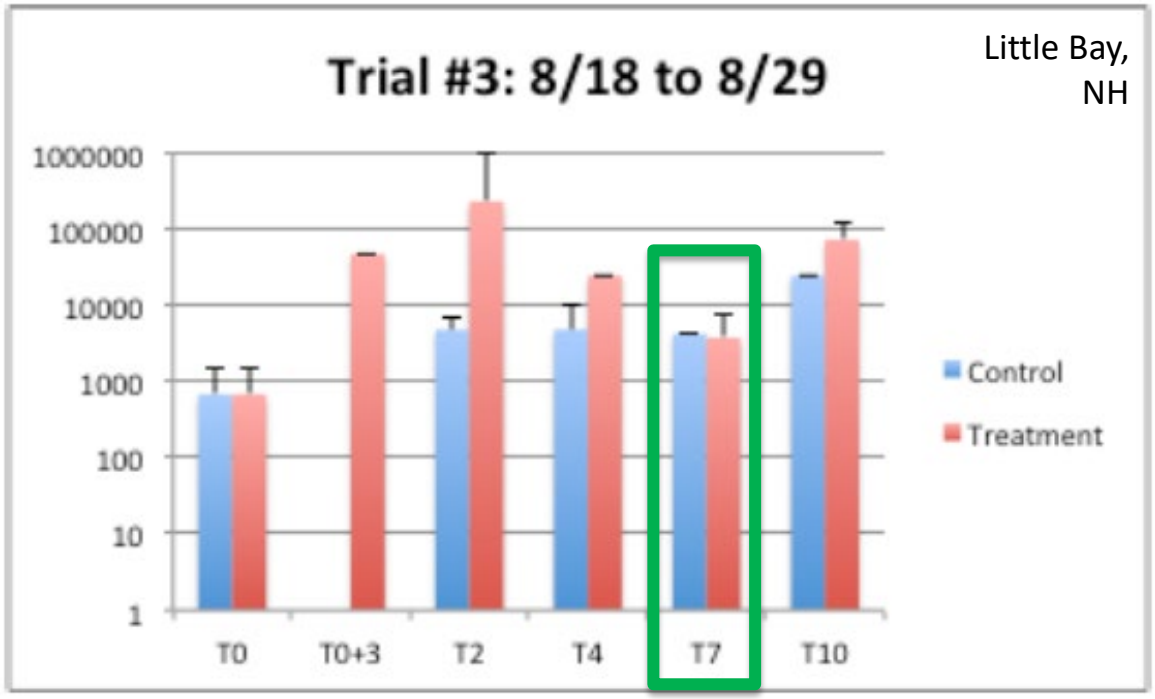



**Figure 4. Geometric mean *V. parahaemolyticus* concentrations in air-exposed and control oysters during the 10-day Trial 3.**

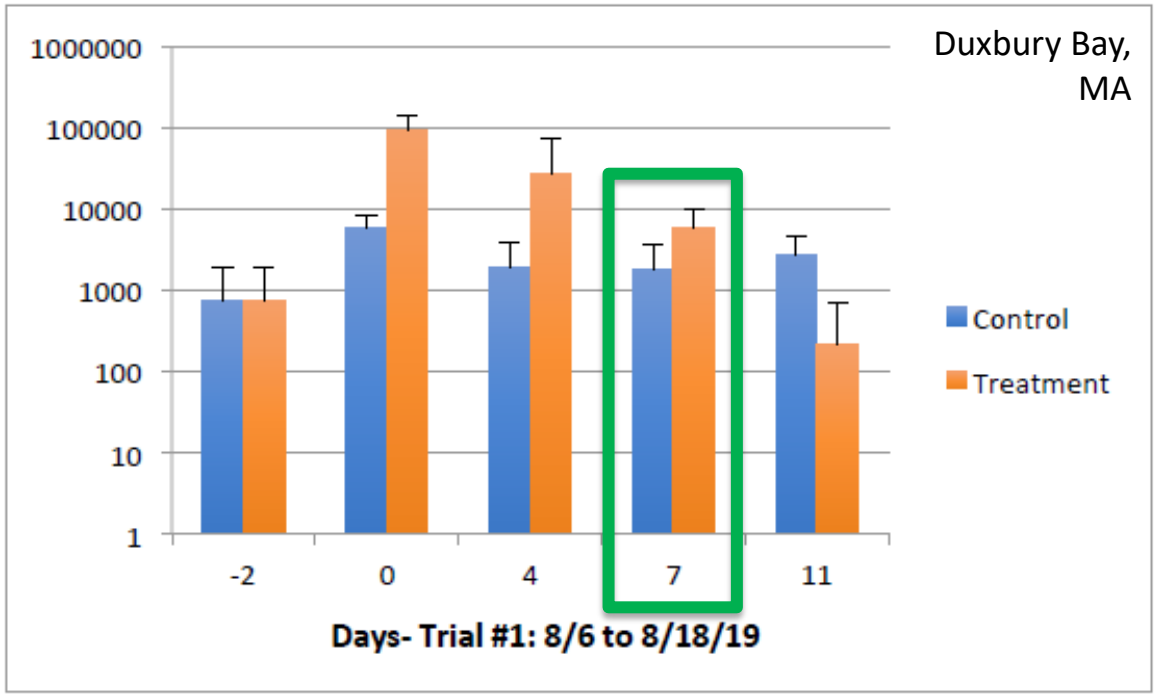



**Figure 6. *V. parahaemolyticus* concentrations in abused and control oysters in Trials #1 (top) and #2 (bottom) in Duxbury Bay, MA.**

ISSC Research Report, 2020

# Research Conclusions

- Handling practices and/or intertidal air exposure have the potential to increase vibrio levels
- Resubmersion has the potential to be an effective mitigation strategy
- How long should the resubmersion period be? **It depends…**

# Geographic Variability

- Different vibrio targets recover in different amounts of time in different locations
  - In general, *Vv* < Total *Vp* < Pathogenic *Vp*
- Oyster behavior might have an effect:
  - Pacific NW and NE intertidal oysters purge elevated vibrios in a tidal cycle
  - Gulf and East Coast subtidal oysters purge in 7-14 days

# Handling Type

- No added benefit of refrigerating oysters while desiccating
  - Gulf Coast: made recovery times longer
  - NJ: no effect on recovery time
- Rough handling (tumbling) had variable effects
  - AL/NC: didn't affect recovery times
  - WA: tumbling in flip bags seemed to affect recovery times (and vibrio levels)

# Gear Type

- Variable recovery times between gear types:
  - AL: no differences in floating vs. suspended gear in one study, but another study showed differences with bottom cages
  - WA: differences in recovery times between flip bag and beach cultured oysters

# Temporal Variability

- Majority of research performed in peak vibrio season (June – Sept)
  - Produced consistent results
- Shoulder season:
  - Pruente et al. 2021: May required longer resubmersion times than July

# Study Design and Statistical Analysis

- Replicate trials, with duplicate/triplicate samples
- Vibrio levels are not statistically significantly different, but differences are sometimes >1 log
  - More frequent when analyzing trials individually
  - Increased Type II errors (false negative)
- Need to consider biological significance with statistical significance (Pruente et al. 2020):
  - >0.5 log MPN/g difference in means = significant for public health
  - <0.5 log MPN/g = natural variability in vibrio levels and method variability

# Conclusion

How long should the resubmersion period be?

– More data is needed!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# Questions?