Pacific Shellfish Institute Progress Report to ISSC
"Techniques and Practices for Vibrio Reduction"

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This report covers the contracted period of October 1, 2014 through August 31, 2015. Additional studies are planned to begin in the spring of 2016 using remaining funds, and ideally from additional 2016 funding from Interstate Shellfish Sanitation Conference (ISSC). The Pacific Shellfish Institute (PSI) has been engaged in laboratory and field experiments focused on *Vibrio parahaemolyticus* (*Vp*) for over a decade, and we believe existing research provides evidence that elevated *Vp* levels in intertidal cultivated shellfish can be mitigated by on site exposure to ambient water conditions. Although still preliminary, results detailed here support on site *Vp* mitigation methods. These study results will assist in advancing farm based *Vp* reduction and project partners are enthusiastic in continuing the study in 2016.

Hama Hama Oyster Company Study:
This ISSC study was based at the Hama Hama Oyster Company, near the town of Lilliwaup, Washington. The study site is located at 47.535° N latitude and -123.039° W longitude in central Hood Canal (Figure 1).

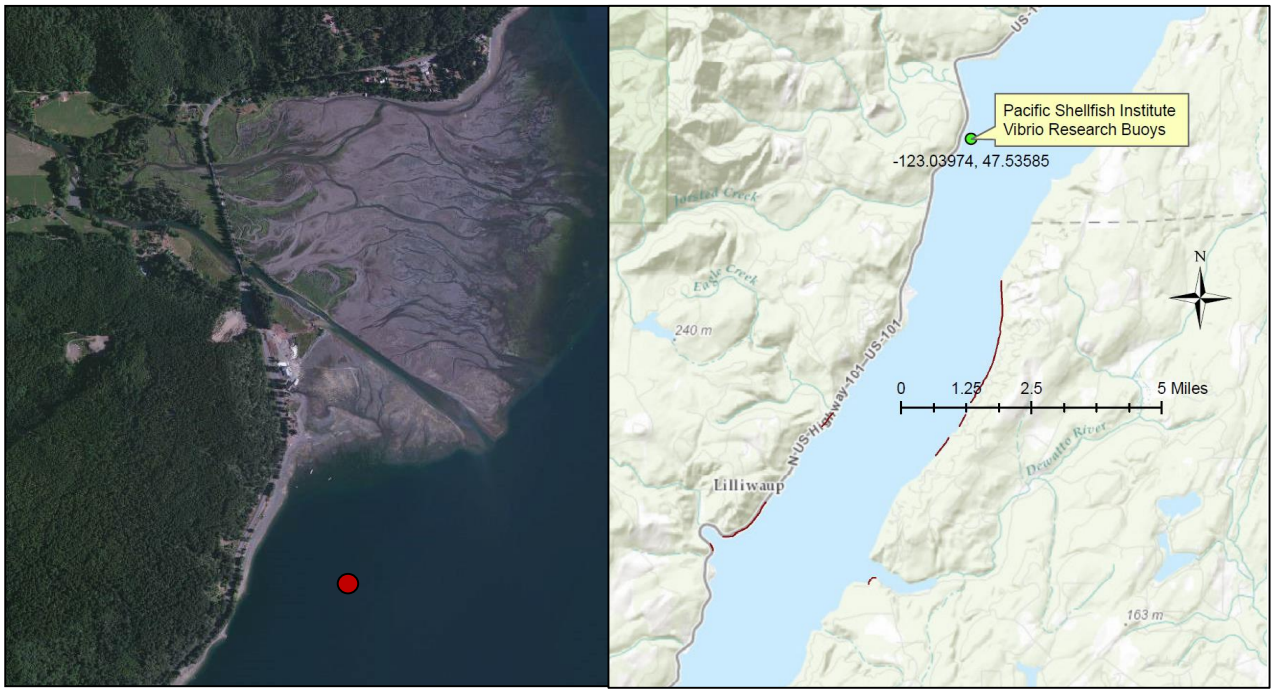

Figure 1. Hama Hama deep water holding area (red dot on left, aerial photograph) in relation to the farm/delta, and the broader region of central Hood Canal (green dot on right, at 47.53N -123.03W).

During the summer of 2015, a total of 5 separate trials were conducted in efforts to evaluate the effectiveness of deep water holding. For Trial 1, oysters were transferred on June 17, 2015 from Hama Hama Oyster Company bed S2 to 35' and 55' depths just south of the farm. The purpose of this study was to evaluate whether oysters at these depths would accumulate *Vp* at a lower rate than those at the intertidal zone. Results from this study are promising, showing on average a 1.5 to 1.9 log reduction of *Vp* at both depths when compared with intertidal oysters (Figure 2). *Vp* levels reported here are most probable number (MPN) total Vp, following BAM chapter 9 methodologies performed by AmTest Laboratories in Kirkland, Washington.[1]

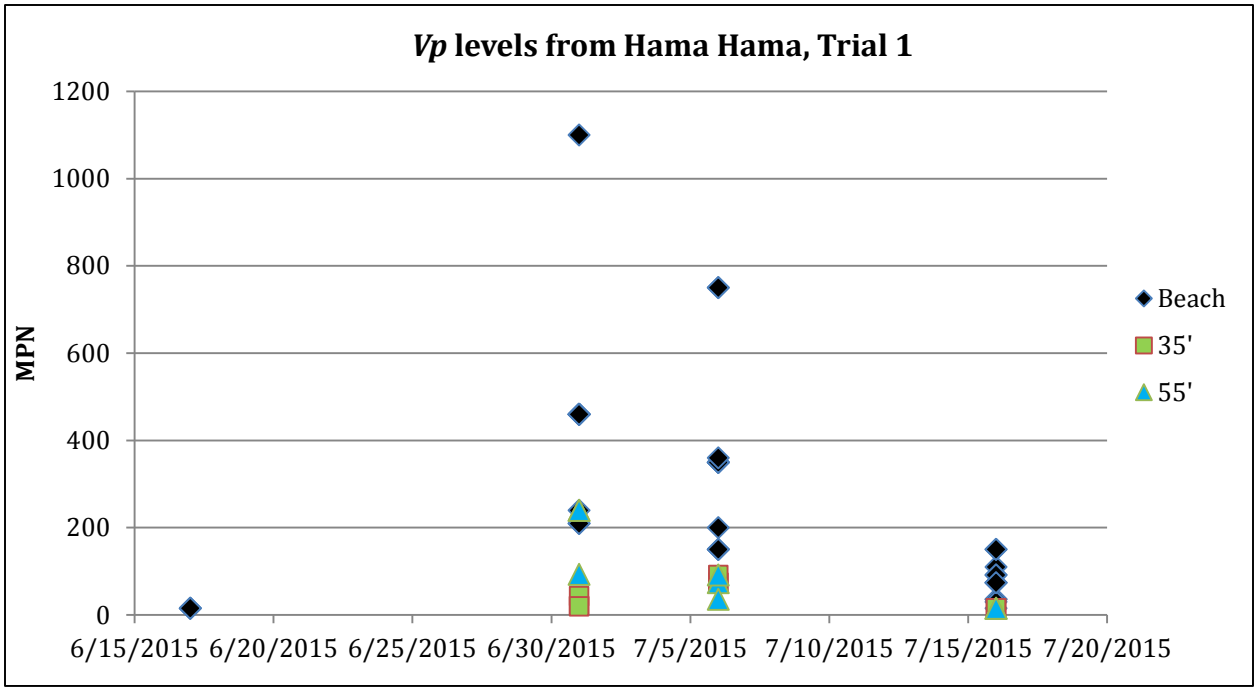

Figure 2. *Vibrio parahaemolyticus* levels of oysters harvested from suspended depths (35' and 55') and the intertidal zone (Beach). Levels are most probable number (MPN) total *Vp*, following BAM chapter 9 methodologies performed by AmTest Laboratories in Kirkland, Washington.

Temperatures at these depths ranged from 51-52°F at 55' and 52-54°F at 35' (Figure 3). These temperatures are optimal for *Vp* purging according to lab-based studies conducted by Dr. Su at the Oregon State University Seafood Laboratory.[2] Specifically, Dr. Su's studies found that depurations worked best at 7°C /45°F to 15°C/59°F, while 22°C/68°F worked, but with less *Vp* reduction and 5°C was too cold for active feeding.

[1] As previously planned, and reported to ISSC in June 2015, PSI utilized the services of AmTest Laboratories to be consistent with ancillary *Vp* studies being conducted in the region. During the summer of 2015 there was consensus among PSI's collaborators, including the Washington Department of Health, that measure of total *Vp* was desirable, as opposed to thermolabile hemolysin (tlh+) and thermostable-direct hemolysin positive (tdh+) *Vp*. FDA staff were also consulted on the issue and agreed.

[2] Phuvasate, Sureerat; Chen, Ming-Hui and Yi-Cheng Su. 2012. Reductions of *Vibrio parahaemolyticus* in Pacific oysters (*Crassostrea gigas*) by depuration at various temperatures. Food Microbiology 31, pp 51-56.

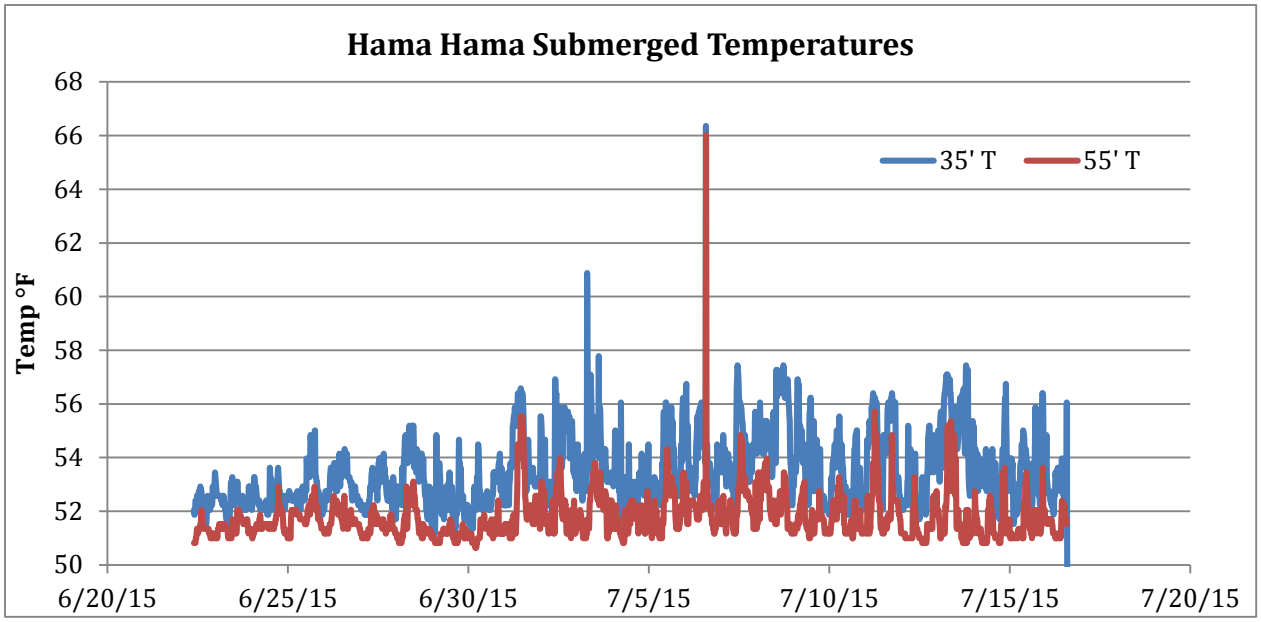

Figure 3. Temperatures at 35' and 55' depths during Trial 1. Data were collected with Onset HOBO Pendant Temperature loggers.

Trials 2 through 5 examined the possibility of reducing *Vp* in temperature abused oysters collected from intertidal zones by deploying them at depths of 35' and 55'. Trials were conducted with temperature abused oysters from Jackson Cove in Dabob Bay, northern Hood Canal (47.740° N -122.867° W), Pickering Passage in southern Puget Sound (47.264° N -122.923° W) and Hama Hama (47.535° N -123.039° W) in an effort to find elevated *Vp*. Background levels of *Vp* in temperature abused oysters did not achieve the desired level of >10,000 MPN, but we did observe a significant reduction of *Vp* as early as Day 1 in oysters held at 55' (Figure 4) and at both depths by Day 3.

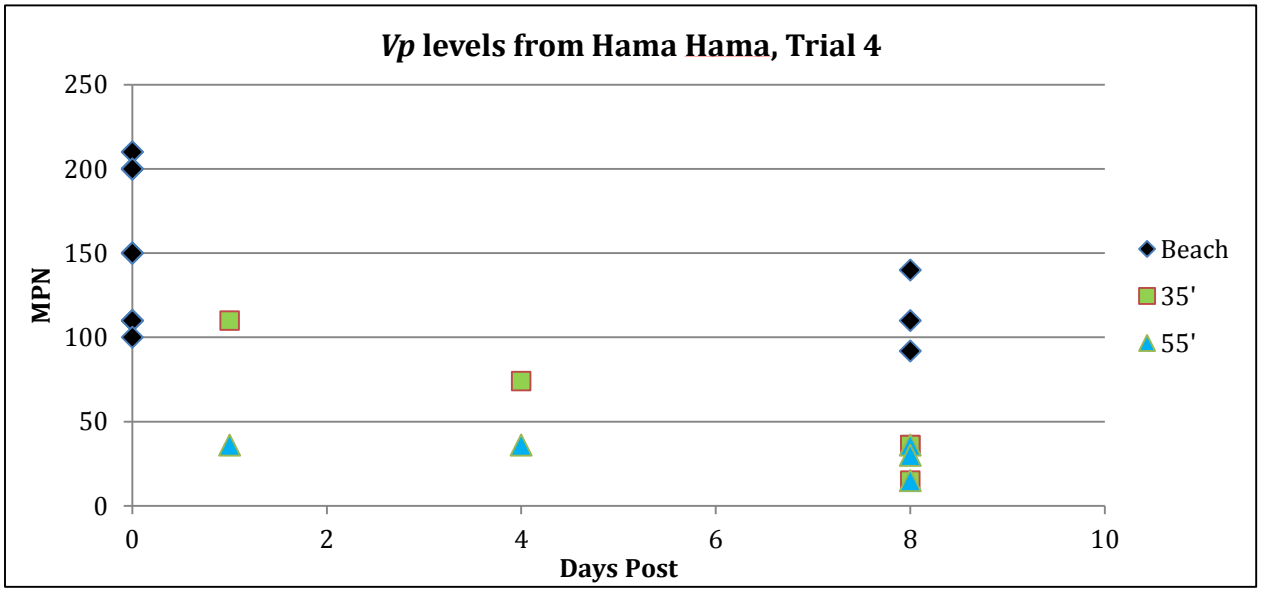

Figure 4. *Vp* levels of temperature abused oysters (Day 0) held at 35' and 55' compared with intertidal oysters. Levels are most probable number (MPN) total *Vp*, following BAM chapter 9 methodologies performed by AmTest Laboratories in Kirkland, Washington.

In tandem with the above trials, the effectiveness of icing prior to shipment verses direct gel packed shipment was evaluated. Three dozen oysters from each intertidal relay were subjected to an ice slurry to reduce their internal temperatures to below 4°C prior to shipment in gel packs. The results were compared with oysters handled in ambient temperature prior gel pack shipment. The *Vp* levels of the iced oysters were elevated compared to the direct shipped oysters in the first 2 samples by a log of 1.64 and 1.12, but showed slightly reduced levels in the last 2 samples by a log of 0.35 and 0.52. The direct shipped oyster samples were replicated up to 12 times to gather information on variance and standard deviation. There was significant deviation in these samples to question results that displaced minimal or low reduction or increase. Accordingly, the slight reduction seen in the last two iced samples may not be significant.

Paralytic Shellfish Poisoning (PSP), caused by the dinoflagellate *Alexandrium catenella* was also prevalent in Hood Canal during 2015. Growing areas Hood Canal 1, 2, 5 and 6, as well as Quilcene and Dabob Bay (in northern Hood Canal) each had closures during summer 2015. Partnering with the Washington Department of Health, the PSI and Hama Hama study team decided to see if PSP levels also differed among the oysters held at depth. In contrast to the *Vp* studies, oysters held at 30' contained the most PSP toxin, an order of magnitude more than the intertidal oyster (Table 1). Oysters held at 50' did not contain a detectable amount of the toxin, which may serve as a side benefit of deep water holding. The results makes sense, due to the increased access on phytoplankton at the 30' depth and decreased access at 50'. This small experiment would need to be replicated and validated before being used to prevent PSP.

Table 1. Saxitoxin (PSP toxin) levels in oysters from the intertidal zone and at depth (35' and 55').

	Intertidal	35'	55'
6/25/2015	146		
7/6/2015	77	735	<38
7/16/2015	<38	130	<38

~ug/100g Saxitoxin

In summary, this ISSC funded study found that deep water holding can reduce and hold down *Vp* levels. Additionally, hot weather and temperature abuse alone cannot elevate *Vp* levels. During summer 2015 the background levels of *Vp* were low in Washington Department of Health samples, Taylor Shellfish Farms studies and the PSI studies detailed here. The lack of rain in the summer of 2015 may be of consideration. PSI's planned 2016 studies will use a wider range of oyster sources for targeting *Vp* hot spots. The deep water depuration/holding technique will also be pursued further. Approximately $10K remains in the existing ISSC allocation to PSI for this research. The 2015 budget remained reasonably low due to the cost of the lab [lower cost for total *Vp*, as opposed to to thermolabile hemolysin (tlh+) and thermostable-direct hemolysin positive (tdh+) *Vp*] and significant assistance from Hama Hama Oyster Company staff. Additional funding is also being pursued from federal and state agencies and shellfish farmers, due to a growing need for this research. Finally, the companion study analyzing PSP levels found that PSP can accumulate at higher levels in the submerged oysters if suspended in the phytoplankton zone. This finding may warrant consideration when new shellfish farm sites are developed.

Pacific Shellfish Institute Ancillary Data Summary to ISSC
"Techniques and Practices for Vibrio Reduction"
Andrew Suhrbier
4-11-16

The below summary explains 2015 Pacific Shellfish Institute field research design changes and includes more information on experiments comparing ice slurry cooled oysters with those cooled only via gel packs inside Styrofoam shipping boxes.

- **Compare levels of *V. parahaemolyticus* clearance in oysters from areas with consistently high levels of *V. parahaemolyticus* after holding in deep water intake for various time intervals.**

  Our study design changed from using deep water intakes to submergence, but we monitored performance of Taylor Shellfish experiments utilizing deep water intake at their Quilcene hatchery in northern Hood Canal. However, as Taylor Shellfish experimented with holding oysters in tanks filled with pumped cool deep water, the company realized the practice was not suitable on the farm scale. Water temperatures were not reliable and most oyster harvest occurred hours away from the deep water intake facility, therefore adding possible heat exposure time and increasing costs. Taylor Shellfish subsequently began pursuing deep water submergence, similar to PSI experiments with the Hama Hama Shellfish Company. These promising results are discussed in depth in PSI's December 2015 progress report.

- **Compare levels of *V. parahaemolyticus* clearance in oysters from areas with consistently high levels of *V. parahaemolyticus* to oysters resubmerged in nearby deeper cooler waters using sink floats.**

  The farmer who provided a verbal agreement to study sink float effectiveness with PSI decided he was too busy to add additional research on his farm. Areas with sink floats that reside in cool water were not found.

- **Compare levels of *V. parahaemolyticus* in oysters placed in ice-slurry prior to shipment with ice-gel packs to shipment no ice-slurry treatment and shipment with ice-gel packs only.**

  The effectiveness of icing prior to shipment verses direct gel pack shipment was evaluated while conducting deep water holding trials. Three dozen oysters from each intertidal sampling were subjected to an ice slurry, after temperature abuse, to reduce their internal temperatures to below 4°C prior to shipment in gel packs. These oysters were compared with temperature abused oysters handled in ambient temperature prior to gel pack shipment. When time permitted, additional oysters were collected prior to low tide exposure and were either immediately iced or put in a cooler with gel ice. A total of 4 trials were completed on separate days.

Again, elevated *Vp* levels were not found in temperature abused oysters collected at our 3 study locations. As displayed in Charts 1-4, no significant difference was found in oysters iced prior to shipment and those only placed in gel pack lined coolers. There was a significant reduction in *Vp* levels in oysters collected prior to low tide exposure during the 7-16-15 trial. No significant reduction was observed in the 8-2-15 trial, when the average for all study groups was below 200 MPN total *Vp*.

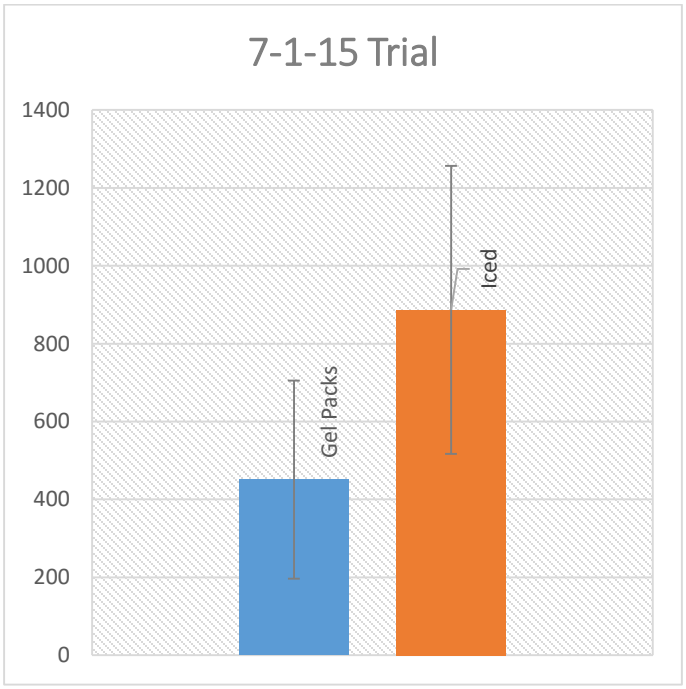

Chart 1: MPN total *Vp* in non-iced oysters and those subjected to ice slurry on 7-1-15.

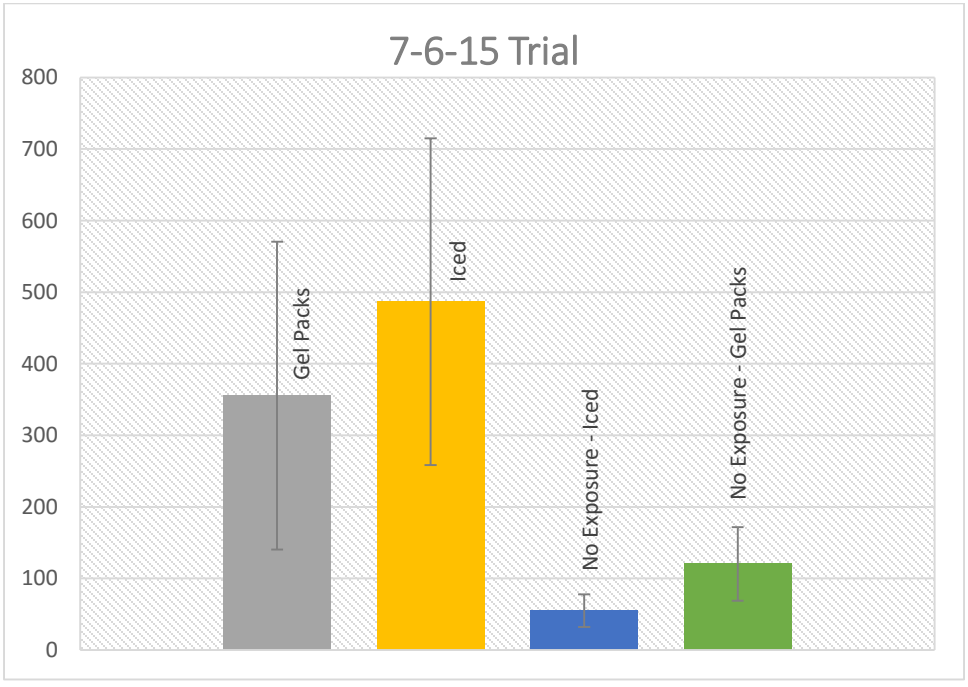

Chart 2: MPN total *Vp* in non-iced oysters, those subjected to ice slurry, those collected prior to exposure and iced and those collected prior to exposure and not-iced on 7-6-15.

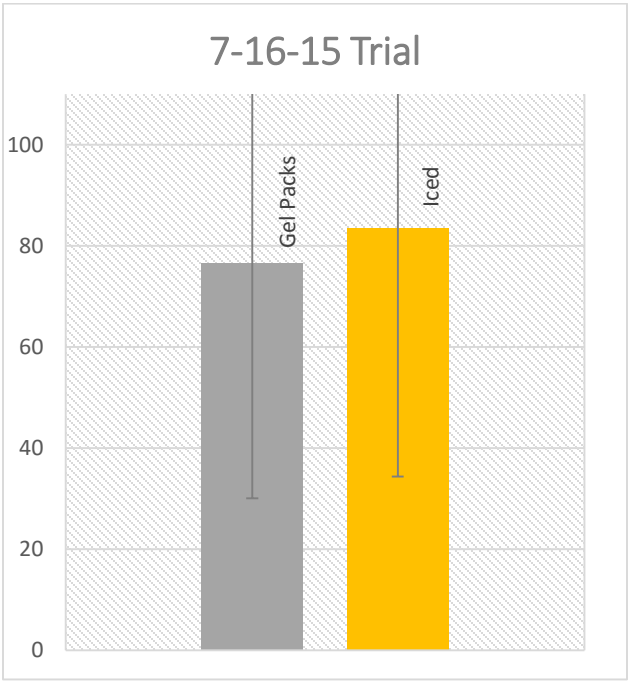

Chart 3: MPN total *Vp* in non-iced oysters and those subjected to ice slurry on 7-16-15.

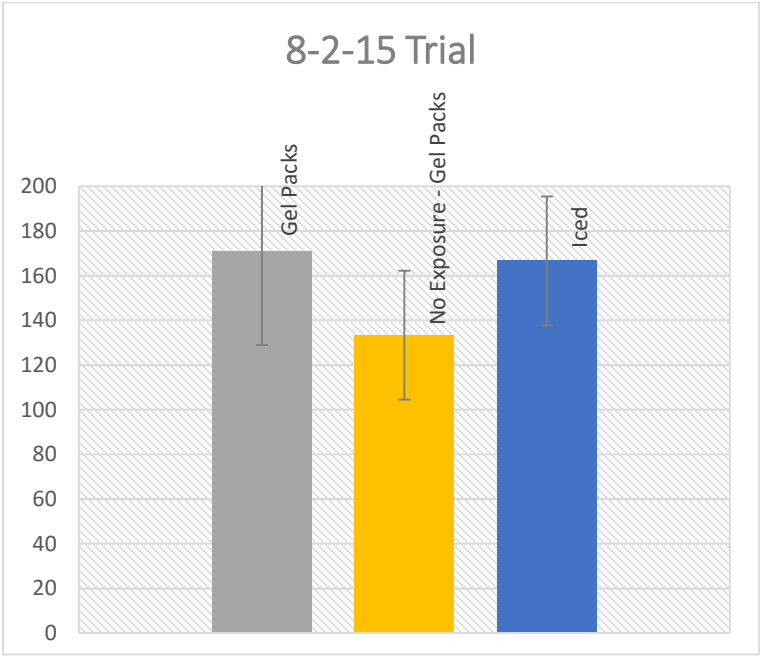

Chart 4: MPN total *Vp* in non-iced oysters, those subjected to ice slurry and those collected prior to exposure and iced 8-2-16.