FINAL REPORT -- Saltonstall-Kennedy Program

TITLE: HARVEST MANAGEMENT TOOLS TO CONTROL THE LEVELS OF *VIBRIO PARAHAEMOLYTICUS* IN OYSTERS AND OTHER BIVALVE SHELLFISH

PRINCIPAL INVESTIGATORS AND AFFILIATION
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1 ABSTRACT

This research project addressed a significant food safety issue affecting the production, marketing, and consumption of bivalve shellfish. The primary focus was *Vibrio parahaemolyticus (Vp),* a common bacterial contaminant of bivalve shellfish, primarily oysters, and a major source of seafood-related food poisoning. Numerous outbreaks of *Vp* illnesses associated with consumption of raw or poorly cooked shellfish have occurred between 1997 and 2013, both in the United States, and elsewhere where shellfish are consumed. On the U.S. West Coast, elevated *Vp* levels in growing waters and associated illnesses, have resulted in extensive seasonal harvest closures among many growing areas, and product recalls, particularly in Washington. Furthermore, *Vp* levels in shellfish in Washington, and other areas, appear to be increasing, and there is concern that outbreaks can be expected to occur with higher frequency and severity across a greater geographic range in the future.

Currently, only a few viable management tools are available to the shellfish producer to control *Vp* in shellfish, aside from closure of growing areas for harvest and restrictions on post harvest times to refrigeration. The primary goal of this project was to examine methods to allow the shellfish farmer and harvester to produce and sell shellfish for raw or fresh consumption during warm summer to fall months when *Vp* levels are typically elevated. All research took place in Washington State, where *Vp*-related food poisoning was often implicated. During the 2010 through 2013 seasons we completed a series of experiments, which focused on methods to relay oyster to locations with lower ambient *Vp* levels and/or different temperature and salinity conditions, addressed shellfish handling and storage procedures, and applied alternative depuration methods. Key project findings indicated when total *Vp* levels in oysters were moderate "prior" to intertidal exposure, placing the oysters in open seawater for 24+ hours "typically" resulted in a 1 to 2 log average reduction in *Vp*. The open seawater treatment was either open or flowing seawater, or relaying to another location having lower ambient *Vp* levels.

2 INTRODUCTION

This research project was conducted from early-2010 through mid-2013 to address a significant food safety issue affecting the production, marketing, and consumption of bivalve shellfish associated with elevated levels of *Vibrio parahaemolyticus* (*Vp*), a marine bacteria found naturally in many coastal regions of the U.S. and elsewhere. Harvest of shellfish, primarily oysters, containing moderate, but not closure-inducing levels of *Vp* can, after some duration of air exposure, lead to possible illness inducing numbers in the shellfish meats which are consumed raw.

Numerous outbreaks of *Vp* illnesses associated with consumption of raw or poorly cooked shellfish have occurred between 1997 and 2013, both in the United States, and elsewhere where shellfish are consumed. On the U.S. West Coast, elevated *Vp* levels in growing waters and associated illnesses, have resulted in extensive seasonal harvest closures among many growing areas, and product recalls, particularly in Washington. Substantial costs are associated with reduced farm and harvester revenue and payrolls, and lost opportunity for tribal and recreational harvest. Furthermore, *Vp* levels in shellfish in Washington, and other areas, appear to be increasing, and there is concern that outbreaks can be expected to occur with higher frequency and severity across a greater geographic range in the future.

The 4 to 8 hours when the intertidal oysters are exposed to ambient air and solar heating allows for additional *Vp* growth that cannot be effectively inhibited by refrigeration during the period of intertidal exposure. Therefore, under these conditions, and for raw and under-cooked shellfish, alternative mitigation strategies are necessary.

The USFDA reviewed the efficacy of depuration and relaying (USFDA 2005). In the United States, depuration is conducted exclusively in closed systems with UV light disinfection. While proven effective for reduction of most human enteric bacteria, depuration was generally reported to have no significant effect on decreasing the level of *Vibrio* spp. in naturally infected oysters or clams, and these microbes may even multiply in depurating shellfish, tank water, and plumbing systems. Relaying was used most commonly with shellfish harvested from water having marginal bacteriological quality. USFDA reported little available information on this approach in relation to reducing the levels of *Vp*. Relaying was viewed as not likely to have a significant impact since *Vp* is ubiquitous in estuarine environments. Nevertheless, several short-term experiments have been conducted in Washington and British Columbia, and the east coast U.S. which suggested both on-site transfers and relaying indicated potential for *Vibrio* reduction in shellfish subject to conditions resulting in a rapid increase of these bacteria in shellfish tissues (Audemard, et al., 2011; Buenaventura, et al., 2002; Herwig and Cheney, 2001; and Nordstrom, et al. 2004).

3 PURPOSE

The emphasis of this project was on experimental analyses of factors affecting *Vp* levels and pre-harvest methods to minimize the risk of *Vp*-induced food poisoning. This was a collaborative effort between the research team (Pacific Shellfish Institute, University of Washington, and NOAA), public health agencies (Washington Department of Health - WDOH), and shellfish growers (Hama Hama Oyster Co., and others). During this period WDOH also addressed the same subject with complementary experiments. And, on the east coast researchers at VIMS were performing comparable relaying studies related to *Vibrio vulnificus*.

This project combined information gained from previous short-term studies with a field-based sampling and research program. All proposed research was directed to studies of *Vp* in oysters from Washington State, the most common species implicated in shellfish-related food poisoning. However, the research findings are applicable to other bivalve shellfish species consumed raw or uncooked and to other

regions in the U.S.

This project addressed FY-2009 NOAA Saltonstall-Kennedy program priorities: Aquaculture and the Optimum Utilization of Harvested Resources under Federal and State Management. The research also addressed the NOAA Marine Aquaculture goals to enhance regulatory programs and to promote the development of commercial marine aquaculture.

4 OBJECTIVES

1. Examine the effects of different holding practices on *Vp* clearance under natural conditions during tidal inundation.
2. Evaluate the rate and variability of the *Vp* growth and clearance responses at multiple sites under natural conditions before and during tidal inundation.
3. Assess key water quality and environmental parameters for possible correlations and interactions with *Vp* growth and clearance.
4. Produce a post-harvest management report complementing the existing USFDA national *Vp* risk assessment report, and the National Shellfish Sanitation Program (NSSP) *Vp* control plan.

5 APPROACH

5.1 METHODOLOGY AND FINDINGS

5.1.1 Hypotheses Tested

The primary hypotheses tested were:

- When oysters exhibit elevated *Vp* levels following intertidal exposure or unrefrigerated holding following harvest, a reduction of *Vp* to acceptable levels can be achieved by placing the animals in ambient seawater at the harvest site.
- Reductions in *Vp* levels will be significantly greater than have been achieved by placing oysters in controlled-temperature sterilized seawater depuration systems.
- Ambient seawater *Vp* reduction rates and levels are independent of the culture method, culture location, post-harvest holding practice, and oyster size or age.
- Other than water and air temperature, other environmental factors are not correlated with the ambient seawater *Vp* reduction rates and levels.
- Observations can be replicated to comply with public health guidance across a range of sampled conditions.

5.1.2 Experimental Design and Methods

Initial plans included an ambitious array of experiments over a two year period beginning in the summer season 2010 that depended on an assay for *Vp* proposed by our University of Washington partner that featured quantitative Polymerase Chain Reaction (qPCR). The qPCR technique would have allowed analysis of a large suite of replicated samples, initially from Hama Hama oyster farm site that was experiencing elevated *Vp* levels. The experiments and associated sampling program were to be repeated at one or more sites with similar *Vp* problems.

Unfortunately, the qPCR technique proved to be neither as consistent nor as sensitive as an updated MPN/PCR (probe-based) method used by WDOH. WDOH and NOAA also reported similar difficulties

using qPCR. The updated probe-based assay was unfortunately far more time consuming but provided consistent, sensitive results. Twelve samples per week (3 replicates of 4 composited samples of 12 oysters each) could be processed per week, at best, which markedly constrained the extent and complexity of the field studies.

Accordingly, we adapted in collaboration with WDOH, a more modest set of experiments at two farm sites in Hood Canal, and a relay site in northwest Puget Sound, near the town of Port Townsend, executed in late summer seasons of moderate (2011) to more extreme (2012, 2013) *Vp* levels. (Figure 1).

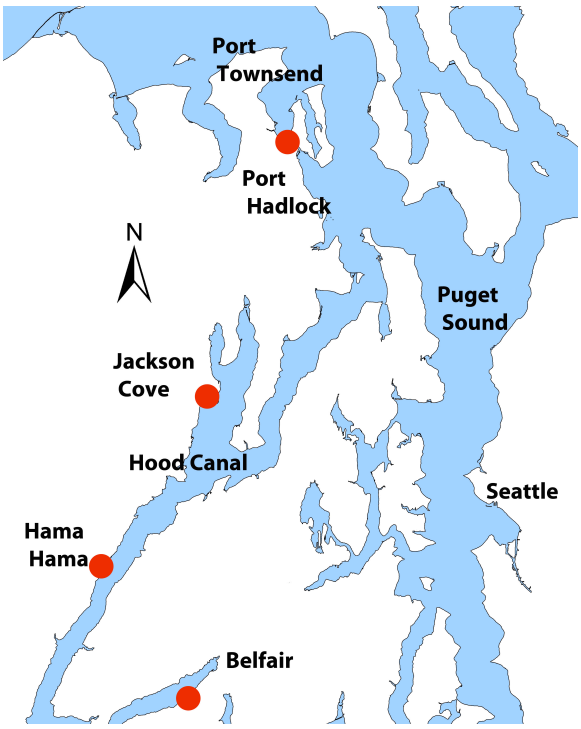

Figure 1. Puget Sound and Hood Canal, Washington showing locations for field experiments.

5.1.2.1 Field trials completed in 2011 and 2012

Small and medium Pacific oysters were collected from farm locations at Hama Hama (Figures 2 and 3) and Belfair in central and south Hood Canal prior to air exposure. They were split into control, pre-treatment and post-treatment or heat abuse groups. Post-treatment time periods ranged from 0 to 72 hrs. Control and post-treatment oysters were suspended in mesh bags at open water locations or, in a few cases, a flow through holding tank. Once all sample groups were removed from the water they were immediately transferred to a chiller or cooler, temp ~ 4 C or 40 F and hand-carried on-ice to the UW laboratory.

Heat abuse treatment was initially held on the beach to expose the animals to ambient air temperatures of up to ~40 C or 100 F. This proved to be a very imprecise process, created highly variable heating conditions. Most subsequent heat abuse treatments were in a temperature controlled incubator with the temperature maintained at ~30 to 35 C, or 85 to 95 C. Temperature loggers were used for all control and treatment groups, and ambient water quality data were collected from available instrumented buoys in Hood Canal.

5.1.2.2 Field trials completed in 2013

Experimental treatments involving oyster relays were completed in August 2013. Oysters were relayed or transferred from Jackson Cove in Hood Canal to Port Hadlock in Port Townsend when total *Vp* and high virulence factor tlh+ numbers increased to levels approaching the closure limit (as determined in consultation with WDOH. Initially two background samples were taken at each location, and in harvest and growout bags immediately prior to relay at Port Hadlock. At 2, 7, and 14 days post relay two background samples were collected from each site and two each in harvest and growout bags at Port Hadlock. Temperature loggers were deployed at each location. These experiments were repeated two weeks later, with similar sampling (background and growout bags only) prior to relay, and 2 and 7 days post relay. A total of 42 samples were collected in 2013. All samples were processed by Dr. Rohinee

Paranjpye at the NOAA Northwest Fisheries Science Center using WDOH qPCR *Vp* analysis protocols.

Figure 2. Aerial photograph of a project study site in central Hood Canal, on the Hama Hama River delta.

Figure 3. Hama Hama oyster farm with the Olympic Mountains in the background. This is primarily a bottom culture farm with a large tub or basket used to hold oysters picked from the beds. Inset, commercial-scale mesh backs in a "long-line" ready to be deployed for time-series sampling; a temperature logger was installed on the bag with red flagging.

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5.1.3 Objectives 1 and 2 – Summary of Results, *Vp* Reduction Experiments

5.1.3.1 2009

Activities originally proposed to begin in the early summer of 2009 were delayed due to the late contract start date coupled with an absence of a strong naturally occurring Vp outbreak in Puget Sound. The proposed first year field activities were conducted during the summer 2010 period.

5.1.3.2 2010

The study site was instrumented with submersible temperature loggers (at tide exposure and immersion locations) to record air and water temperatures at approximately 15 minute intervals through the sample period. Additional water quality data were obtained from a Washington Department of Ecology buoy deployed in central Hood Canal. Examples of our 2010 temperature observations are shown in Figure 7.

5.1.3.3 2011

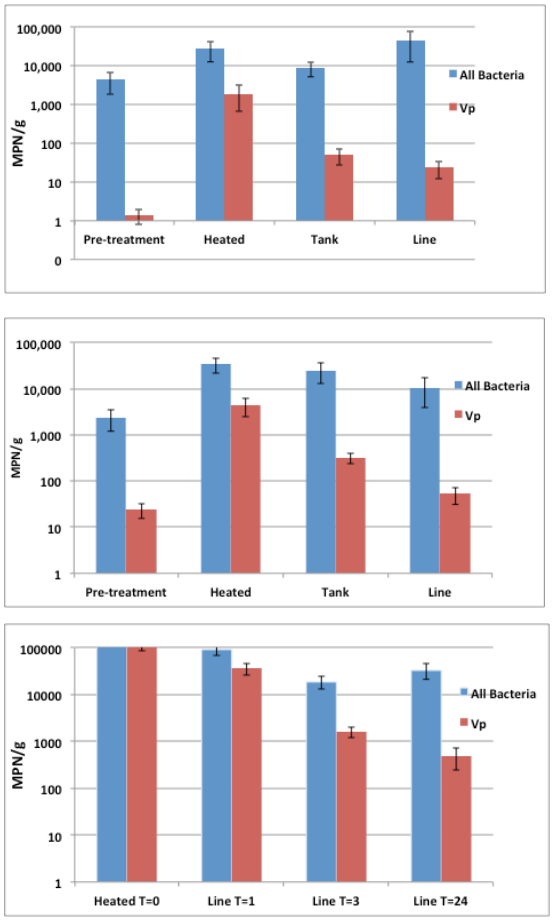


Figure 4. Total bacteria and total *Vp* per gram oyster tissue, 2011. Clockwise from top left: early August; mid-August, early September.

The findings from selected experiments completed during August-September 2011 are illustrated in Figure 4. Oysters exposed to moderate natural (sunlight) heating conditions exhibited a 1 to 3 log increase in *Vp* levels, and variable modest to good depuration. These experiments were conducted at a time when central and south Hood Canal had been closed due to confirmed *Vp* illnesses. *Vp* in oyster tissue reported by WDOH and measured during our experiments in both oyster tissue and sea water were frequently elevated. Total bacterial counts were extremely high in the heat treated samples and neared the upper limit of detection. However, there were wide variations in total bacteria and *Vp* between replicates within each sample. Experiments conducted by WDOH at the same Hama Hama site also resulted in highly variable *Vp* counts between and within sample groups. For example, when WDOH sampled heat abused oysters in flow through holding tanks and open water every 12 hours for 5 days, *Vp* counts did not decline at a steady rate, but instead exhibited considerable variation between sample periods. Also levels of virulent (tdh) *Vp*, showed no downward trends. It appeared *Vp* uptake and replication rates differed widely between individual oysters, which then had a marked affect on the average *Vp* counts in the composited samples.

5.1.3.4 2012

Experimental treatments were conducted at Hama Hama and Belfair, Washington study sites from early July through August. The following tests were completed:

- Hama Hama – July 17 and 24, August 27 to September 5 – medium oysters, incubator heat abused 6 hr, with 24 hr and 7 day open-water depurations.
- Belfair – August 7, 14 and 21 – small and medium oysters, incubator heat abuse for 6 hrs, and 24 hr in open-water.

The results from one of the experiments completed during this reporting period are illustrated in Figure 5. In this experiment two control groups were set aside to match heat treated oysters held in open or flowing seawater for 24 hours and 7 days. The artificial (incubator) heat process resulted in moderately high bacterial loads. *Vp* levels declined 3 log units by 24 hours, but were elevated somewhat at 7 days to about 1.5 log units below the heated levels.

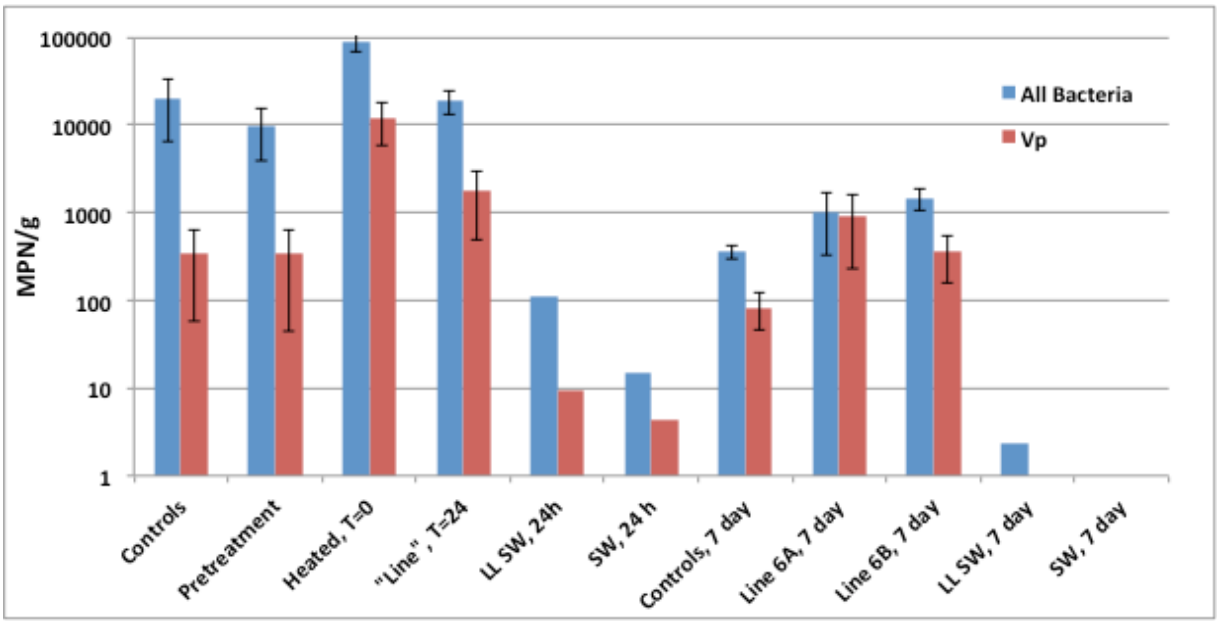


Figure 5. Total bacteria and total *Vp* per gram oyster tissue in control and treatment groups held for up to 7 days in a holding tank ("line") and open water at the Hama Hama site, late August to early September 2012.

5.1.3.5 2013

The results from the second experiment completed are illustrated in Figure 6. In this experiment *Vp* levels in the "0" hour and growout bag relay samples were highly elevated, and reflected the results from WDOH sampling during the same period. Two days post relay, when the oysters had been transferred to Port Hadlock, *Vp* levels in the relay growout bags fell 2 to 3 log units, whereas the Jackson Cove levels remained high. At seven days both Jackson Cove and Port Hadlock bacteria levels declined, likely reflecting a response to a marked drop in water temperatures at both sites. In general, these findings were consistent through 7 days for the first experiment of growout and harvest bags. However,

in that test, *Vp* levels in all 14 day samples increased by over one log unit over the 7 day levels. This inconsistency and sample variation paralleled observations made both by WDOH and the USFDA (Andy DePaola, Gulf Coast Seafood Laboratory, Dauphin Is., AL, personal communication).

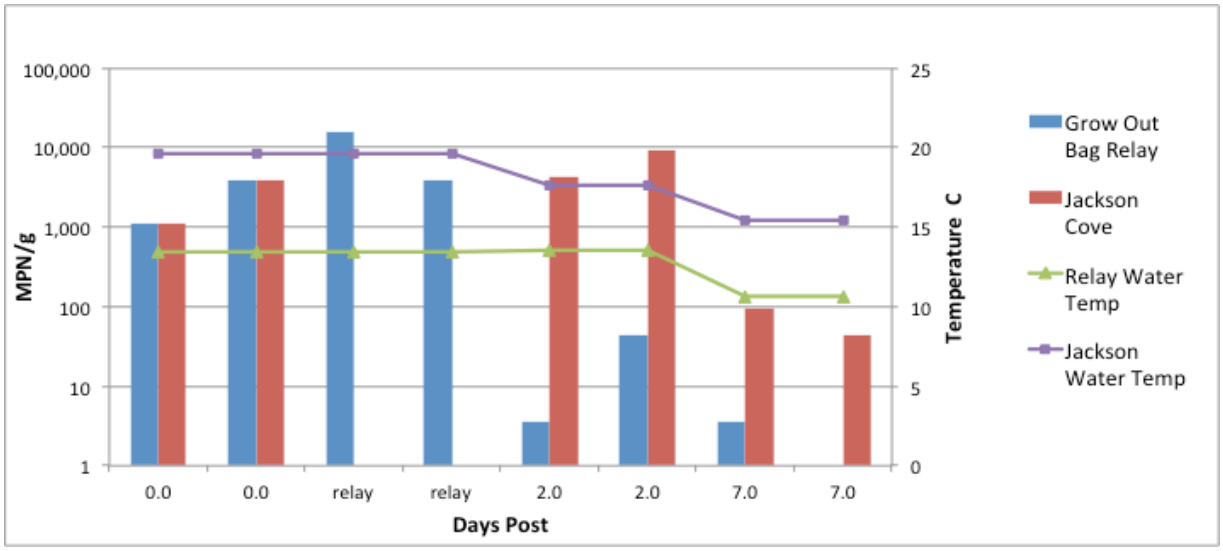


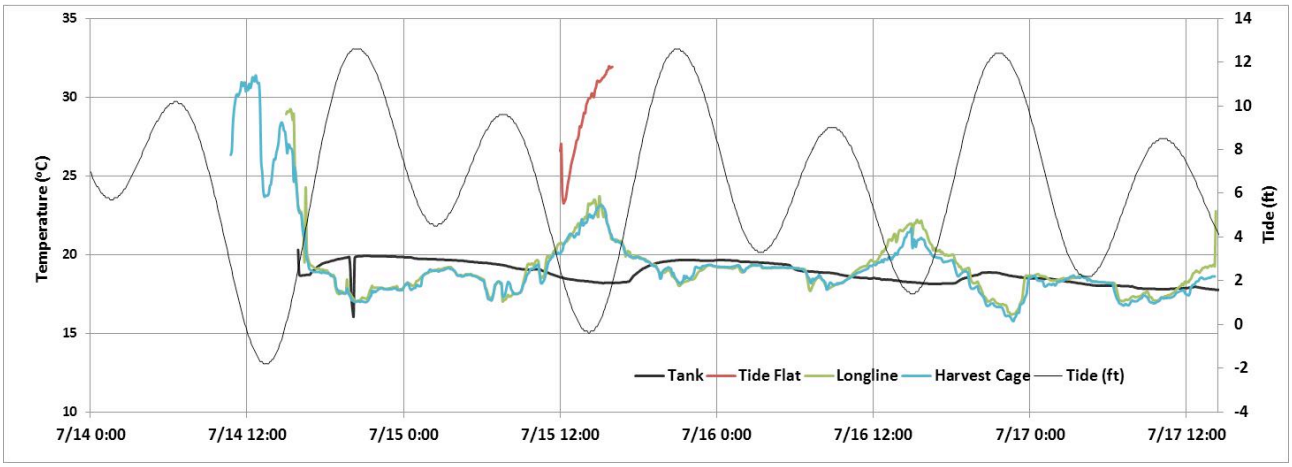
Figure 6. Total *Vp* per gram oyster tissue in control and growout bag treatment groups held for up to 7 days in open water sites at Jackson Cove, Hood Canal, and Port Hadlock, Port Townsend, August 2013.

5.1.4 Objective 3 – Summary of Results, Environmental Monitoring

Environmental data were collected in conjunction with this project, and WDOH and other studies in Hood Canal. These data included water and air temperatures logged during field experiments, WDOE water quality buoy data (http://nvs.nanoos.org/Explorer), shellfish hatchery and nursery system records, and plankton sampling and ocean acidification observations made by PSI and University of Washington personnel and Hood Canal volunteers. This information was used during to assess possible correlations between *Vp* levels in shellfish and specific water quality parameters. An example of the temperature logs for treatment and control groups sampled in July 2010 is shown in Figure 7.

5.1.5 Objective 4 – Summary of Results, Extension of Results / Education and Outreach

This task was facilitated with communications at various levels continued throughout this project between PSI and WDOH staff, NOAA and USFDA personnel, and shellfish growers. Experiments were coordinated with WDOH to ensure field trials coincided with their *Vp* observations from routine sampling. PSI staff presented the project results to shellfish growers at regional meetings held in 2012 and 2013. They participated in a panel discussion at the West Coast *Vibrio* Management workshop, held April 16-18 2013 reviewing existing and future strategies for *Vp* control, and gaps in our current understanding of environmental influences on these bacteria. WDOH staff also reviewed the project findings at a biennial ISSC/FDA meeting in mid-2013 to report on the progress of this research and related WDOH and NOAA *Vp* studies in the Pacific Northwest.



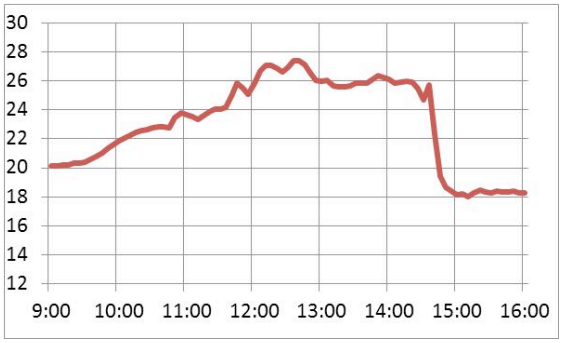


Figure 7. Top, time and temperature records from the July 2010 sampling event at the Hama Hama farm site; information is provided for each treatment during the full duration of the field experiment. Left, beach temperature during the August 2010 sampling on the Hama Hama. Beach temperatures were relatively moderate in both periods, reaching 32 C (89F) in July and 27 C (80 F) in August, but water temperatures were fairly high, often exceeding 20 C (68 F).

5.2 SUMMARY OF FINDINGS

Based on the late summer review of the last three summers of findings from experiments conducted by PSI and WDOH, we made the following preliminary project conclusions:

- When total *Vp* levels in small to medium diploid oysters are moderate (for example less than 100 / g) "prior" to intertidal exposure,
- when there are little to no *Vp* in the seawater,
- there are no reported illnesses in the harvest area,
- and when total *Vp* levels are elevated in oysters by sunlight, or other heat source,
- placing the oysters in open seawater for 24+ hours will "typically" result in a 1 to 2 log average reduction in *Vp* approaching the pre-heat abuse moderate or ambient condition.

However:

- when *Vp* levels are elevated in both seawater and oysters (for example greater than 100 / g) prior to intertidal exposure,
- with open water treatment there may be little to no reduction in *Vp* , or at best a reduction to the elevated pre-abuse condition.

In all cases *Vp* levels between individual oysters may be extremely variable and high counts may be masked in the composite samples. Also, no open water depuration would be allowed if an illness has been reported in product originating from the farm site. However, late summer 2013 experiments demonstrated relaying of oysters with moderate contamination to cooler waters is a promising approach to *Vp* reduction provided the transfers are not from closed harvest site. This option remains to be investigated with additional sampling in subsequent summer seasons.

5.3 EDUCATION OUTREACH

See Section 5.1.5.

5.4 PROBLEMS

The principal problem affecting completion of Obj 1 to 2 was associated with severe limitations in the qPCR methodology proposed by our University of Washington partner. This set the project back one year and required modifications to both the sampling scheme and timing to allow experiments and *Vp* analyses to be extended through the entire period of elevated *Vp* levels. Laboratory capacity and throughput critically limited the total number of samples, and the cost per sample remained high, at $200 to $250 per sample. Another issue, which could not be controlled, was the natural variability of *Vp* in both seawater and oyster tissues. We relied on WDOH to identify locations and dates of increasing *Vp* levels, and these events changed somewhat from year to year. Fortunately both the grower partners and *Vp* assay laboratories were very flexible and able to adapt to the new experimental approach and variable timing of sampling events. However, we were still limited in the total number of samples that could be processed per sampling event and during in any one season.

5.5 NEED FOR ADDITIONAL WORK

This research highlighted additional studies to continue collaboration and share results with emphasis on the following: 1) conduct additional field trials to evaluate longer duration open-water depuration; 2) evaluate the benefits of relaying prior to *Vp* closure; 3) examine effects of holding oysters in tubs, grow bags, etc.; 4) test the responses of triploid oysters vs diploids; 5) assess sample collection timing vs tidal exposure; and 6) evaluate alternate *Vp* assay methods. With regards to items 1 and 2, PSI and WDOH plan during the summer 2014 period to 1) repeat relay study with larger sample volume; 2) examine on ground and suspended culture differences; and 3) examine handling effects on test results. Also, PSI and WDOH were informed late fall 2013 that USFDA may have rapid assay methods available for comparison trials sometime in 2014.

6 EVALUATION

6.1 DESCRIBE THE EXTENT TO WHICH PROJECT GOALS AND OBJECTIVES WERE ATTAINED

6.1.1 Were goals and objectives attained?

Many of our goals and objectives were attained. Although we were unable to achieve the sampling intensity originally proposed, we were able to extend the proposed studies two additional seasons and complete additional and replicated experiments. We ascertained the influences of holding treatments typically employed by shellfish farmers at several sites (Obj. 1 and 2). Working in collaboration with WDOH and NOAA shellfish biologists and *Vibrio* specialists, we conducted pilot studies to determine the efficacy of relaying to reduce *Vp* in oyster tissues (an extension of Obj. 1). We examined the influences

of air and water temperatures on *Vp* under natural and controlled conditions, and gathered data on other relevant water quality parameters (Obj. 3 and 4). Finally, we coordinated presentations of the field observations and analyses with USFDA, NOAA and WDOH, and presented the project findings at meetings for growers and public health agencies (Obj. 4). However, as noted earlier, further work remains before the project information related to *Vp* reduction can be considered under the national *Vp* control plan.

6.1.2 Were modifications made to the goals and objectives?

We modified the objectives slightly to enable progress on the overall goals. For example, major limitations of the *Vp* assay discovered by the University of Washington during the first year of intensive sampling, necessitated repeated deviations from the planned schedule and sampling effort to meet our objectives. The natural variability in the timing and level of *Vp* contamination of oysters also required considerable flexibility in both sampling schedules and harvest sites. We also added, during the last season of the project and in collaboration with WDOH and USFDA, experiments to assess the benefits of post-harvest oyster relay for *Vp* reduction as a prospective tool for growers and public health agencies.

6.2 DISSEMINATION OF PROJECT RESULTS

See Section 5.5.5.

7 REFERENCES

Audemard, C., H. I. Kator, M. W. Rhodes, et al. 2011. High salinity relay as a postharvest processing strategy to reduce *Vibrio vulnificus* levels in Chesapeake Bay oysters (*Crassostrea virginica*). J.Food Prot. 74(11):1902-1907.

Buenaventura, E., K. Schallie, M. Green, D. Oakes, T. Borralho, K. Felton, A. Chong, A. Napper, E. Wong, J. Liu, Z. Jiwani, J. Lomas, J. Scott, C. Florek, K. Christensen, E. Roberts, and D. Graham. 2002. Vibrio parahaemolyticus levels in Pacific oysters (*Crassostrea gigas*) during a tide cycle, p. 76, Federal Food Safety and Nutrition Research Meeting, Guelph, Ontario, Canada

Herwig, R.P., and D.P. Cheney. 2001. Field and preliminary laboratory evaluations of the influences of water and air temperature, dissolved oxygen on concentrations of *Vibrio parahaemolyticus* in the Pacific oyster, *Crassostrea gigas*. Final Report submitted to the Interstate Shellfish Sanitation Conference. Columbia, South Carolina. University of Washington and Pacific Shellfish Institute.

Nordstrom, J.L., C.A. Kaysner, G.M. Blackstone, M.C.L. Vickery, J.C. Bowers, and A. DePaola. 2004. Effect of intertidal exposure on *Vibrio parahaemolyticus* levels in Pacific Northwest oysters. Journal of Food Protection 67:2178-2182.

U.S. Food and Drug Administration. 2005. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. Center for Food Safety & Applied Nutrition, FDA.