Assessing Geographic Variation on Resubmergence Time to Reduce the Vibrio Risk Associated with Routine Oyster Aquaculture Handling

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Introduction

Off-bottom oyster aquaculture is the culture of oysters, usually held in some type of mesh container (basket, bag, cage, etc.) that is held above the seafloor (suspended or floating) in food-rich coastal waters. Farmers will routinely handle their oysters for grading, splitting, tumbling, control of biofouling through desiccation, and many other reasons in order to improve oyster quality (Walton *et al.* 2013). During these handling practices, the oysters are removed from the water for extended periods of time and exposed to elevated air temperatures, permitting the increased growth of *Vibrio* bacteria within the oysters (Cook 1994, Gooch *et al.* 2002). After handling, the oysters are resubmersed at the farm site and resume filter feeding, allowing the elevated levels of *Vibrio* within the oysters to be purged back to ambient levels that would be expected if the exposure had not occurred (Kinsey *et al.* 2015, Grodeska *et al.* 2017).

Previous research in Alabama has shown that a 7-day resubmersion period was appropriate for oysters raised on the adjustable longline system and subjected to a 24 hour desiccation period (Grodeska *et al.* 2017). The data from this study was able to achieve a reduction in the resubmersion time, from 14 days to 7 days, but only for Alabama farmers using that gear system and performing a 24-hour desiccation (Byron Webb, personal communication, January 30, 2020). Alabama farmers who were using other gear types or handling types were excluded from this reduction. Additionally, other states were uncertain if these findings applied to their waters as well. In response, more research has been conducted to examine the effects of different handling types and gear types on the resubmersion times required in Alabama waters, but the effects of geography have not been tested (Pruente *et al. in prep*).

To expand and complement ongoing research, our group proposed to focus on the question of geographic variability in resubmersion times. More specifically, we wanted to see if there were significant differences in resubmersion times after routine handling of oysters at several sites along the Gulf Coast. Several research and commercial oyster farms were chosen in Mississippi, Alabama, and Florida to serve as the field sites in order to assess the geographic variability in resubmersion times.

Approach and Methodology

During the study, resubmersion trials were performed at four commercial or research farm sites along the Northern Gulf of Mexico (Fig. 1): 1) Portersville Bay, AL, 2) Mobile Bay, AL, 3) Dauphin Island, AL, and 4) Pensacola Bay, FL. A fifth site was proposed for this study in Deer Island, MS, but the trials were not completed as the site was subjected to multiple months of low salinity (>5 ppt) from unprecedented rainfall along the Mississippi River, resulting in high oyster mortality during the study period. The water temperature and salinity were recorded at each site using a HOBO Saltwater Conductivity Data Logger (Onset Computer Corporation, Bourne, Massachusetts). Prior to each trial, two OysterGro cages were deployed at each farm site and stocked with six bags of oysters each (150-200 per bag), then remained submerged for at least two weeks before being handled.

Figure 1. Map of the field sites used during the study.

A total of two trials per site were completed between May-August 2019, when the risk of *Vibrio* infection is the highest. During each trial, oysters were randomly assigned to either a submersed control treatment or a desiccated treatment. The submersed control oysters remained submerged at the farm site throughout the trial, in order to provide the ambient levels of *Vibrio* spp. within oysters. The desiccated oysters were flipped up into the desiccating, or air-drying, position in the OysterGro cages for 24 $\pm$ 2 hours, then returned to the water. Triplicate samples (15 oysters/sample) were collected from the submersed control oysters prior to desiccation (pre-treatment). Then, triplicate samples were collected from each treatment type immediately after desiccation and prior to resubmersion (post-treatment), and 7 and 14 days after resubmersion. Oysters were collected at each farm site, placed into a cooler with gel ice packs, and transported to the FDA Gulf Coast Seafood Laboratory for microbiological processing.

The samples were processed following the NSSP methods and analyzed using a three-tube Most-Probable-Number (MPN) as described in the FDA's *Bacteriological Analytical Manual* (Kaysner *et al.* 2004, Blodgett 2010, NSSP 2017). Oysters were washed under cold tap water with a sterile brush, aseptically shucked into a sterile blender, and blended for 90 seconds. Then, oyster homogenate was serially diluted 10-fold to 1:100,000 in sterile phosphate buffered saline and inoculated in triplicate tubes of alkaline peptone water. Three tubes containing 10 mL of APW were inoculated with 1 gram of oyster homogenate each to allow for a limit of detection of 0.3 MPN/gram. MPN tubes were incubated for 18-24 hours at 35ºCm then examined for turbidity. DNA extracts were prepared for each turbid tube by heating a 1 mL aliquot at 95ºC for 10 minutes, then stored at -20ºC. Extracts were thawed and centrifuged at 12,500 x g for 2 minutes, then tested for total *V. vulnificus*, total *V. parahaemolyticus,* and pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+) using the real time PCR assays described in Kinsey *et al*. (2015, NSSP 2017). The number of MPN tubes positive for each target was used to determine the levels of each *Vibrio* spp. using a standard MPN table (Blodgett 2010).

The environmental data (water temperature and salinity) were reported as average daily means, minimums, and maximums. Prior to analysis, the *Vibrio* spp. data (reported as MPN/gram of oyster homogenate) were log transformed for normalization. When the *Vibrio* spp. were not detected (<0.3 MPN/gram), half of the limit of detection (0.15 MPN/gram) was

substituted prior to log transformation. First, the post-treatment *Vibrio* spp. levels in the desiccated oysters were compared to the pre-treatment levels in the control oysters to determine if the desiccation elevated the *Vibrio* spp. levels (>0.5 log MPN/gram). Then, the mean *Vibrio* spp. levels in the desiccated oysters were compared to the mean levels in the submersed control to determine when the levels were similar between treatments. The *Vibrio* spp. levels were considered similar when they had returned within 0.5 log MPN/gram of the control oyster levels on that sampling day.

Results

The environmental conditions varied at each site between the two trials (Table 1). The average water temperature during the entire study period ranged from 28.5 to 31.3°C and was similar at all sites. Conversely, the average salinities had more variation, as the salinity was higher in Trial II than in Trial I across all sites. Dauphin Island was the only site that had similar average salinities during both trials, but a wider range of salinity was seen during Trial I than in Trial II.

Table 1. Environmental conditions at the farm sites, reported as a mean with range in parentheses.

Site	Trial	Water Temperature (°C)	Salinity (ppt)
Portersville Bay, AL	I (May 13-May 28)	28.5 (24.4-33.0)	7.7 (4.3-13.7)
	II (Jul 8-Jul 23)	30.4 (26.6-34.2)	13.7 (5.8-16.1)
Dauphin Island, AL	I (May 20-Jun 4)	29.3 (26.0-33.1)	18.9 (2.3-36.8)
	II (Jul 16-Jul 31)	29.8 (26.6-33.1)	19.2 (15.5-20.9)
Mobile Bay, AL	I (Jun 18-Jul 3)	30.2 (27.4-34.4)	7.6 (4.8-12.0)
	II (Aug 6-Aug 21)	31.3 (28.8-34.6)	12.5 (6.1-14.8)
Pensacola Bay, FL	I (Jun 3-Jun 18)	29.7 (27.7-32.8)	10.9 (4.1-14.3)
	II (Jul 22-Aug 6)	30.5 (28.4-32.8)	16.9 (12.4-24.3)

The variation in salinity at each site resulted in variation in the average *Vibrio* spp. levels in the submersed control oysters during each trial. The sites that experienced greater variation in salinity between trials (Portersville Bay, Mobile Bay, Pensacola Bay) had variations in *Vibrio* spp. levels in control oysters between the trials (Figure 2). Dauphin Island had similar average salinities and water temperatures as well as similar *Vibrio* spp. levels in the control oysters between the two trials.

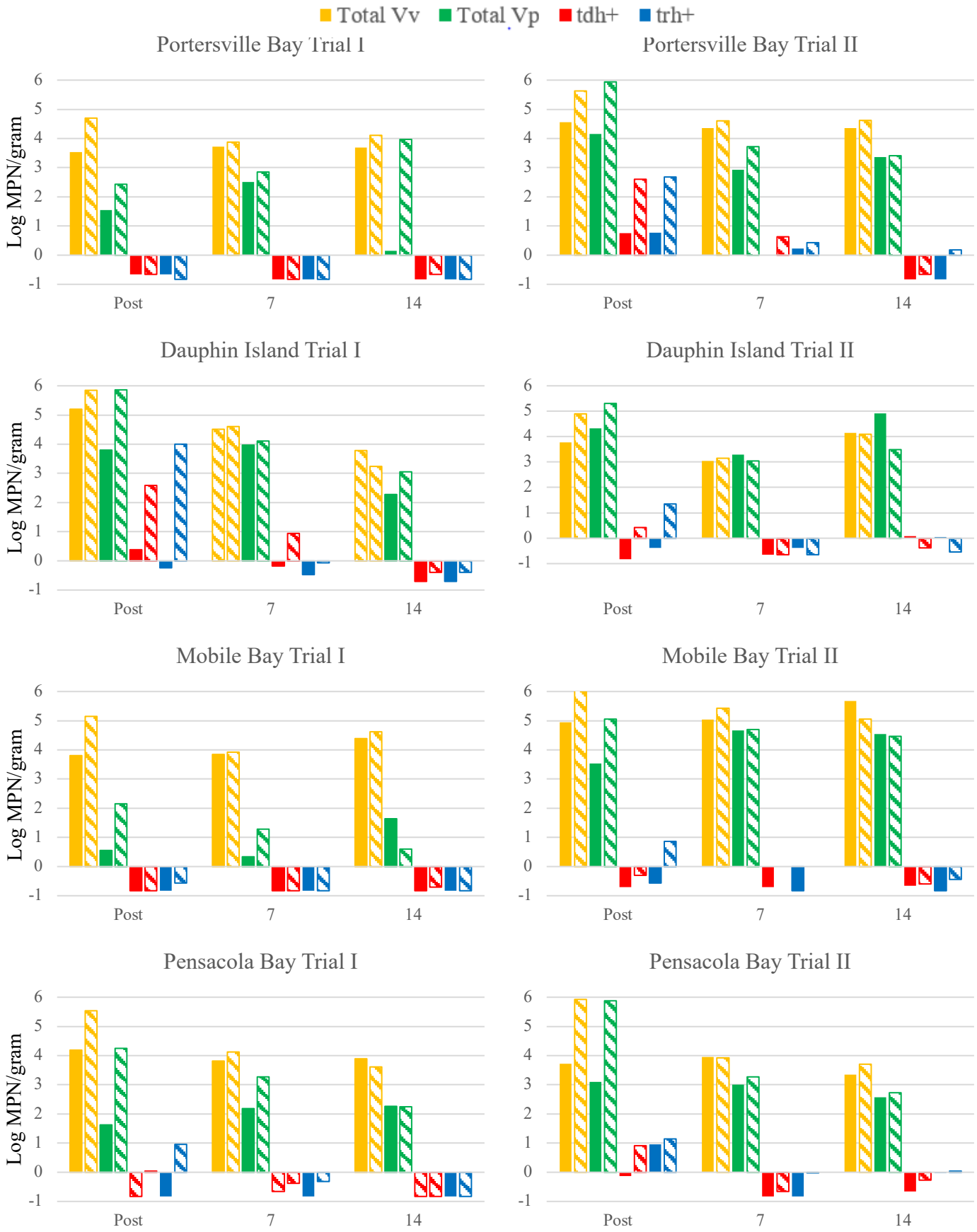

Figure 2. Levels of *Vibrio* spp. in submersed control (solid bars) and desiccated (striped bars) oysters after desiccation and resubmersion.

In some cases, the *Vibrio* spp. levels did not elevate in comparison to the pre-treatment control levels after the 24-hour desiccation. For example, in Trial I at both Portersville Bay and Mobile Bay, the pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+) levels did not elevate from the control levels. The *tdh*+ and *trh*+ levels in the desiccated oysters ranged from 0.16 log MPN/gram lower than the control levels to 0.26 log MPN/gram greater. While the pathogenic *V. parahaemolyticus* levels did not elevate in those trials, the total *V. vulnificus* and total *V. parahaemolyticus* levels did elevate above 0.5 log MPN/gram. Therefore, the lack of elevation in pathogenic *V. parahaemolyticus* levels was most likely due to the relatively low levels in the oysters prior to desiccation (-0.74 to -0.82 log MPN/gram).

The *Vibrio* spp. levels in the control and desiccated oysters during each of the resubmersion trials can be found in Figure 2. Across all sites and trials, the *Vibrio* spp. levels in the desiccated oysters returned to levels similar to the control oysters (within 0.5 log MPN/gram) within 7 to 14 days of resubmersion. The resubmersion periods differed based on *Vibrio* spp., site, and trial, as summarized in Table 2. The total *V. vulnificus* levels in the desiccated oysters at all sites recovered after 7 days of resubmersion, while the total and pathogenic *V. parahaemolyticus* levels required 7 to 14 days of resubmersion.

Table 2. Number of days of resubmersion required for *Vibrio* spp. levels in desiccated oysters to return to levels similar to the control oysters[a]

Site	Trial	Total *V. vulnificus*[a]	Total *V. parahaemolyticus*[a]	Pathogenic *V. parahaemolyticus*[a] *tdh*+	*trh*+
Portersville Bay, AL	I	7	NA	NA	NA
	II	7	14	14	7
Dauphin Island, AL	I	7	7	14	7
	II	7	7	7	7
Mobile Bay, AL	I	7	14	NA	NA
	II	7	7	14	7
Pensacola Bay, FL	I	7	14	7	14
	II	7	7	7	14

[a]NA: *Vibrio* spp. levels never elevated from pre-treatment control levels after desiccation, so resubmersion period was not determined for that *Vibrio* spp.

Conclusions

Based on the data, the resubmersion times following routine desiccation vary more based on *Vibrio* spp. than geographic location. The total *V. vulnificus* levels required 7 days of resubmersion to return to ambient levels at all four sites, while the total and pathogenic *V. parahaemolyticus* levels required 7-14 days of resubmersion when considered across all sites. These results also show that the floating OysterGro cage system may require a longer resubmersion time than what was found for the adjustable longline system in Grodeska *et al.* (2017) and Pruente *et al.* (*in prep*), unless more locally appropriate data suggest otherwise.

There are a few caveats to note about this study. First, the study consisted of only two trials at each site, with only three replicate samples per treatment at each time point. Compared to other resubmersion studies, which performed five replicate trials over a two-year period, this study is limited in power due to the lack of replicate trials. Based on this, the difference in mean *Vibrio* spp. levels between the desiccated and control oysters was used in place of summary statistics. Second, not all *Vibrio* spp. levels elevated during desiccation, especially the pathogenic *V. parahaemolyticus* strains. While the levels were very low and, in some cases, close to the limit of detection to start, we cannot extrapolate the resubmersion times found here to instances when the pathogenic *V. parahaemolyticus* levels are higher to start and would significantly increase during desiccation. Overall, the data suggest that the resubmersion times across a broader geographical range than previous work are 14 days for oysters that were desiccated in OysterGro cages

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