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Minimizing Risks of Vibrio Bacteria in Farm-Raised Oysters Grown in Intertidal Environments of the Delaware Bay

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Summary

New Jersey's oyster farms are concentrated on the extensive intertidal sand flats of the lower Delaware Bay where they are exposed twice daily during low tide. Previous studies from the Pacific Northwest indicate that intertidal exposure accelerates the proliferation of vibrios, increasing the risk to human health. We conducted a preliminary study to test whether this result applies to mid-Atlantic intertidal environments. Oysters were collected from subtidal and intertidal rack and bag grow-out systems monthly from June through August 2014. Samples were collected at the initial exposure of intertidal oysters on the receding tide, and then at three and 24 hours following this initial exposure. Total and pathogenic Vp levels were enumerated using a most-probable number quantitative PCR assay with probes targeting the thermolabile direct hemolysin (*tlh*) and thermostable direct hemolysin (*tdh*) genes associated with pathogenicity. Observed Vp densities (± 95% CI) ranged from 19 (5 - 68) to 1,100 (260 – 4,700) CFU/g for total Vp, 0 to 11 (3 – 43) CFU/g for *trh,* and 0 to 459 (100 - 2,100) CFU/g for *tdh*. We did not see a significant difference between levels of total and pathogenic Vp between subtidal and intertidal oysters, nor was there a significant increase in vibrio burdens over the time course of low-tide exposure. This initial result suggests that the relationship between grow-out conditions and vibrio levels in oysters is not as straightforward as previously thought, and highlights the need for locally relevant aquaculture practices to minimize the risk of vibrio illness. Based on these preliminary results, oysters grown in intertidal areas in the lower Delaware Bay do not appear to have elevated levels of Vp. Observed Vp levels are well within acceptable levels with respect to human health risks.

Objectives

1. Determine if levels of Vp increase in oyster tissues when intertidal oysters are exposed during low tides.
2. Determine if Vp levels rapidly decline to pre-low tide levels upon immersion with the incoming tide.
3. Determine whether virulent strains of Vp are present at the intertidal Delaware Bay area oyster farms.

Introduction

Illnesses associated with the consumption of raw and undercooked shellfish have increased over the past decade, presenting serious concerns to shellfish farmers, resource managers, and public health officials. The majority of seafood-associated illnesses, both in the mid-Atlantic and worldwide, are caused by infection with *Vibrio parahaemolyticus* (Vp), bacteria that occur naturally throughout estuaries and marine environments. Filter-feeding bivalves such as oysters accumulate these naturally occurring bacteria in their tissues through regular feeding processes, posing health risks to individuals consuming these shellfish raw. Both regulator and consumer concerns over such illnesses threaten the sustainability and growth of the shellfish aquaculture industry. Oyster aquaculture has emerged as a significant industry in New Jersey with an anticipated harvest of 2 million oysters in 2014. The majority of New Jersey oyster farms are located on the intertidal shores of the lower Delaware Bay where growing conditions are ideal. However, some data suggest that the intertidal environment may provide ideal conditions for Vp to proliferate in oyster tissues (Nordstrom et al. 2004). The ability to predict where, when and under what conditions Vp presents a health risk is limited by a poor understanding of its basic ecology and relationship with farm practices. The objective of this work is to answer three basic questions: 1) do levels of Vp in oyster tissues increase when intertidal oysters are exposed during low tides; and if so, 2) do Vp levels rapidly decline to pre-low tide levels upon immersion with the next tide; and 3) are virulent strains of Vp present at the intertidal Delaware Bay area oyster farms? Answers to these fundamental questions will be used to develop best management practices for the safe harvest of oysters, ensuring consumer health and a sustainable and profitable future for oyster farms in New Jersey and from Maine to Florida.

Methods

We conducted field studies during the summer 2014 to address these objectives, specifically testing the three following hypotheses: 1) total Vp burdens increase during intertidal aerial exposure, 2) total Vp burdens decrease following submersion after aerial exposure, and 3) markers associated with Vp pathogenicity represent a small fraction of total Vp in intertidal oysters. The study was conducted in collaboration with oyster farmer Elizabeth Haskin, owner of Betsy's Cape Shore Salts. Ms. Haskin employs rack and bag grow-out systems in the intertidal on the Cape Shore flats in the lower Delaware Bay. We placed near-

market to market-size oysters into six mesh bags at typical grow-out densities (150 per bag) in early June 2014. Additional bags containing surplus oysters were deployed at the same time. These oysters were used for replacement of oysters sampled and lost to mortality. Three bags were randomly assigned to a location on the farm in an area having the longest air exposure during low tide (intertidal rack), and the remaining three bags were assigned to an adjacent offshore area, where oysters remained submerged at low tide (subtidal rack).

We continuously monitored water temperatures at each rack system using HOBO® data loggers (Onset Computers Corp., Bourne, MA USA) to assure homogeneity in the environmental conditions among the intertidal and subtidal grow-out systems. Oysters were collected across a tidal cycle, once monthly in June, July, and August. Specific collection dates each month targeted the Spring tide. On each sample date, 12 oysters were randomly sampled from each bag along a time course spanning from 1) first emergence on the ebbing tide, 2) three hours after low tide air exposure, and 3) approximately 24 hours later just prior to the following day's low tide aerial exposure. Samples were placed in coolers on icepacks and immediately transported to the laboratory where they were processed for Vp analysis. *Vibrio parahaemolyticus* densities were enumerated by the most probable number (MPN) technique on a pooled sample of 12 oysters incorporating a multiplex real-time PCR assay to enable the detection of the Vp species-specific thermolabile hemolysin (*tlh*) gene, and the thermostable direct hemolysin (*tdh*) and thermostable-related hemolysin (*trh*) genes which have been identified as key determinants of Vp pathogenicity (Nordstrom et al. 2007, Cox and Gomez-Chiarri 2012, Jones et al. 2013). Briefly, oysters were cleaned and shucked, and entire shell contents (animal and liquor) were emptied into sterile vessels and homogenized. We performed a three-tube MPN, as described in the FDA Bacteriological Analytical Manual (DePaola and Kaysner, 2004), on triplicate samples taken from each homogenate. After incubation, a 1 ml sample from each tube was removed and boiled for 10 min for real-time qPCR. The real-time qPCR analysis incorporated the internal amplification control (IAC) from Nordstrom et al. (2007) to ensure PCR integrity and eliminate false-negative reporting. Quantities of total Vp, *trh* Vp, and *tdh* Vp were estimated as the most probable number (MPN) from the three-tube assay and log10 transformed to be analyzed by ANOVA. We also tested for differences in the presence of the *trh* and *tdh* markers over the tidal cycle and over the time course of sampling using logistic regression.

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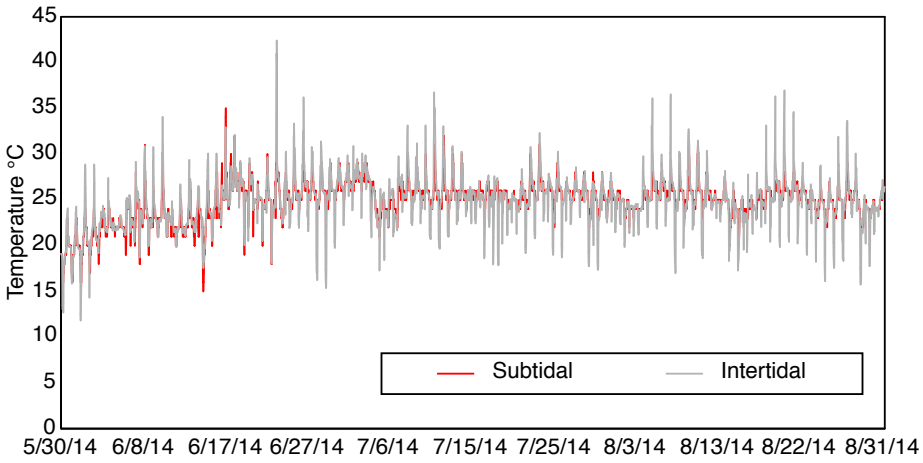

Figure 1. Temperatures recorded in intertidal and subtidal bags holding oysters in our experimental system. Temperatures were recorded using HOBO® data loggers programmed to dowload data at 15 minute intervals.

Results and Discussion

Recorded water temperatures ranged from 14.33°C to 41.34°C in subtidal bags and 11.82°C to 42.40°C in intertidal bags (Fig. 1). We did not see a significant difference in mean temperatures between the subtidal and intertidal bags (ANOVA, $F_{1,10654} = 3.14$, $P = 0.08$), but we did see a greater daily range of temperatures recorded between the subtidal and intertidal bags (ANOVA, $F_{1,220} = 57.95$, $P < 0.001$). The daily temperature range, measured as the difference between the minimum and maximum temperatures recorded daily, varied from 0.96°C to 23.11°C in subtidal bags and 1.14°C to 24.36°C in intertidal bags. Levels of total Vp (95%CI) ranged from 19 (5 - 68) to 1,100 (260 – 4,700) CFU/g (Fig. 2). We did not find a significant effect of intertidal exposure on levels of total Vp (ANOVA, $F_{1,40} = 0.07$, $P = 0.79$), nor did we observes significant differences between Vp densities in oysters tissues along the tidal cycle (ANOVA, $F_{2,40} = 0.60$, $P = 0.55$). This result does not lend evidence for our hypotheses that total Vp burdens increase during intertidal aerial exposure. Densities of total Vp in oyster tissues after re-immersion with the incoming tide were similar to the densities we observed before aerial exposure. This pattern can result from either or both declining densities of Vp after re-immersion or limited, if any, increases in Vp density with aerial exposure. We also did not observe any significant difference in densities of total Vp within oyster tissues over the months sampled (ANOVA, $F_{2,40} = 0.25$, $P = 0.78$). These preliminary results suggest that that the aerial exposure experienced by oysters grown in the intertidal has little effect on densities of Vp in oyster tissues.

Patterns of the *tdh* and *trh* markers associated with Vp pathogenicity mirrored the patterns we saw in total Vp, however the levels of these markers observed in oyster tissues were much lower then the levels observed for total Vp (Fig. 2). We did not observe significant impacts of intertidal exposure on densities of *tdh* (ANOVA, $F_{1,40} = 1.05$, $P = 0.31$) and *trh* (ANOVA, $F_{1,40} = 0.28$, $P = 0.59$), nor did we see differences in *tdh* (ANOVA, $F_{2,40} = 0.37$, $P = 0.69$) and *trh* (ANOVA, $F_{2,40} = 1.03$, $P = 0.37$) over the tidal cycle. Densities of these markers were low in most of our samples and we did not observe *tdh* in any samples in July. This latter observation led to a significant effect of sampling month on the presence (Fig. 3; Analysis of Deviance, $\chi^2 = 18.47$, df = 2, $P < 0.001$) and density (ANOVA, $F_{2,40} = 4.91$, $P = 0.01$) of *tdh*. It remains open whether this result is due to limited sampling of a rare phenomenon (i.e. the presence of *tdh* genes in vibrios associated with Delaware Bay oysters) or presence of markers that have been associated with Vp pathogenicity, suggesting that virulent strains of Vp are likely present in Delaware Bay. Levels of these markers were approximately two orders of magnitude lower than the levels observed for total Vp, implying that virulent strains of Vp are likely to be only a small fraction of the total Vp present.

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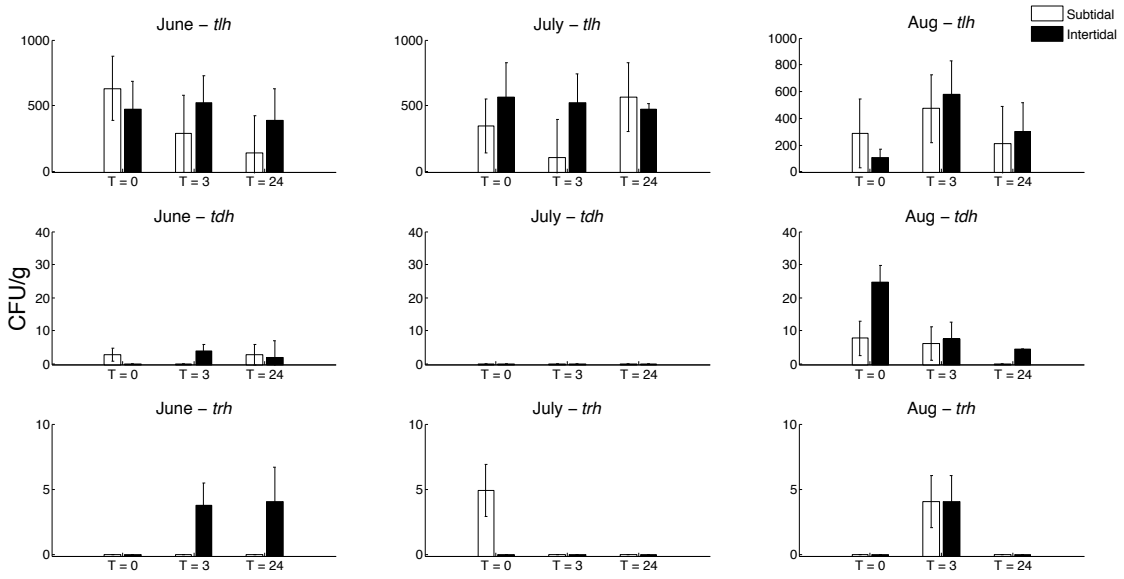

Figure 2. *Vibrio parahaemolyticus* density in intertidal and subtidal oysters sampled in June, July, and August (*tlh*, *tdh*, and *trh* levels are shown, respectively top, middle, and bottom charts for each sample month).

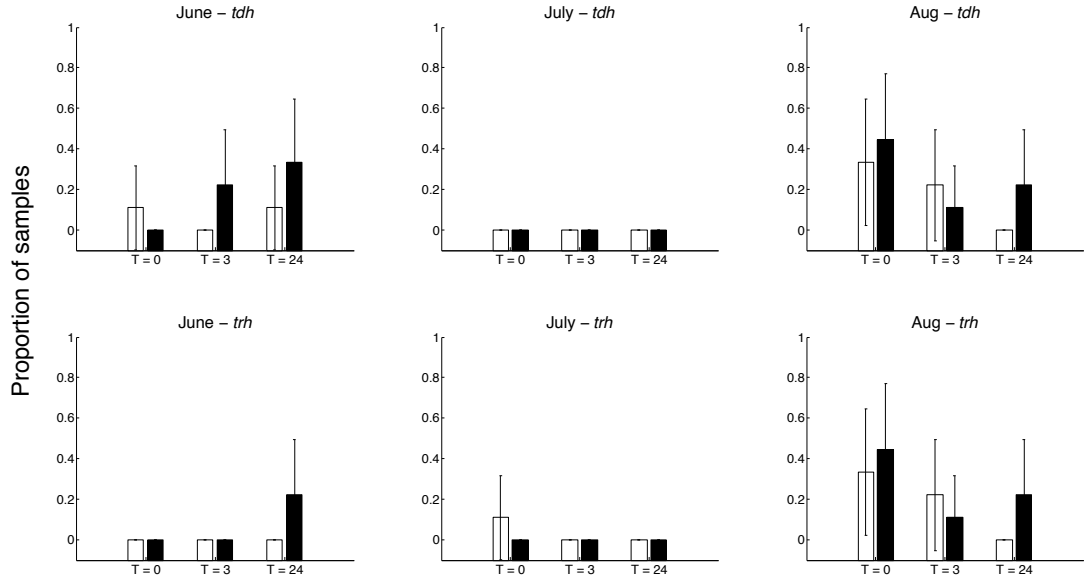

Figure 3. Proportion of samples containing the *tdh* (top row) and *trh* (bottom row) markers associated with *V. parahaemolyticus* pathogenicity in intertidal and subtidal oyster samples collected in June, July, and August.

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