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Research Article

RESEARCH ARTICLE

Vibrio parahaemolyticus risk assessment in the Pacific Northwest: it's not what's in the water

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One sentence summary: Although present in high concentrations in the overlying water column, *tdh*+ strains of *Vibrio parahaemolyticus* do not readily accumulate in oysters in Washington State waters.

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ABSTRACT

The Gram-negative bacterium *Vibrio parahaemolyticus* (Vp) is a major cause of illness associated with the consumption of raw or undercooked seafood, primarily oysters. This species is a natural member of the bacterial community in brackish waters and is bioaccumulated by oysters through filter feeding. Only a subset of strains is thought to be pathogenic. Currently known virulence markers include the gene for the thermostable direct hemolysin (*tdh*). In this work we analyzed water and oysters for total Vp and strains encoding *tdh* from 26 oyster-growing areas of the Puget Sound and Pacific coast of Washington state in 2007 and 2008. In addition, possible plankton-associated Vp were assessed from net tow samples. The density of both total and *tdh*+ Vp in the water column were considerably higher in 2008 than 2007. However, the concentrations of both total and *tdh*+ Vp in the oyster tissue was similar for both years. A high proportion of Vp strains in the water column was found to be *tdh*+ in both 2007 and 2008; however, *tdh*+ strains were detected at much lower levels in oysters. The data show that analysis of Vp density in the oysters is a better risk assessment tool than density in the overlying water column.

Keywords: *Vibrio parahaemolyticus*; ecology; oysters; water; marine pathogen

INTRODUCTION

The Washington state shellfish industry is one of the most productive in the United States, accounting for 25% of domestic production by weight, with an estimated farm gate value of over 100 million dollars (Pacific Shellfish Institute 2019). This industry is an important part of the economy of the counties adjacent to the Puget Sound and the Washington coast. Shellfish aquaculture provides over 3000 jobs, and a 2012 study estimated the industry to contribute some 270 million dollars to the economy of these regions (NOAA Fisheries Northwest Region 2019). Oysters constitute a major part of the total production of shellfish in the state, the primary species being the Pacific oyster, *Magallana gigas* (formerly *Crassostrea gigas*).

In recent years, oyster growers in Washington state and British Columbia have experienced forced closures due to either algal biotoxins (Trainer *et al.* 2013) or outbreaks of illness due to

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vibriosis (Centers for Disease Control and Prevention 1998; Taylor *et al*. 2018). These closures are costly to the shellfish industry and, in addition, close beaches to recreational harvest further impacting the economy of coastal areas. Vibriosis is a disease caused by a number of bacterial species of the genus *Vibrio*, which are a natural part of the bacterial communities in marine and estuarine waters around the globe (Thompson, Iida and Swings 2004). These include *Vibrio cholerae*, *Vibrio vulnificus* and *Vibrio parahaemolyticus*, the latter of greatest concern in waters of the Pacific Northwest since the majority of vibriosis cases in the region are attributed to this species. *V. parahaemolyticus* (Vp) is a Gram-negative, rod-shaped bacterium.

Commonly, infections are acquired by ingestion of raw or undercooked seafood, usually oysters, that naturally bioaccumulate Vp through filter feeding. Disease caused by ingestion of raw or undercooked oysters harboring Vp usually manifests as mild or moderate gastroenteritis that is generally self-limiting, although severe cases involving sepsis can require hospitalization and in rare cases have led to death of severely immune-compromised individuals (Klontz 1990). This bacterium can also cause skin infections through exposure of open wounds to seawater containing Vp (Daniels *et al*. 2000; Kelly and Stroh 1988).

It is thought that only a small subset of Vp strains can cause disease. The identification of reliable virulence markers has been an active area of research (Hiyoshi *et al*. 2010; Broberg, Calder and Orth 2011). The majority of strains isolated from clinical cases, usually by culture from stool samples, are found to encode for either or both of two hemolysins: the thermostable direct hemolysin (TDH) and the TDH-related hemolysin (TRH) (Raghunath 2015). The genomes of Vp strains that are *tdh*+ can contain either one or two copies of this gene (Nishibuchi and Kaper 1990). The first strain to be sequenced, RIMD2210633, was shown to contain two copies of *tdh* but did not encode for *trh* (Makino *et al*. 2003). This strain also was shown to have gene clusters encoding for Type III secretion systems designated T3SS1 and T3SS2 (Park *et al*. 2004). The T3SS1 appears to be present in all strains of Vp (Broberg, Calder and Orth 2011); therefore, while T3SS1 may have some role in pathogenesis once *in vivo*, it is of no value as a virulence marker. On the other hand, T3SS2 has been shown to be involved in enterotoxicity (Hiyoshi *et al*. 2010). After characterization of T3SS2 in RIMD2210633, a related but distinct T3SS2 was characterized in a *trh*+ strain (TH3996) (Okada *et al*. 2009). To distinguish the two systems, the original type-3 system associated with *tdh* was designated T3SS-2α, while that associated with *trh* was given the designation T3SS-2β. Not all clinical isolates contain either of these putative virulence markers. A number of reports have identified clinical strains lacking *tdh* and *trh* (Bhoopong *et al*. 2007; Jones *et al*. 2012; Banerjee *et al*. 2018) and recent comparative genomic analyses of a number of *tdh*-, *trh*- clinical isolates (Ronholm *et al*. 2015) suggest a number of other candidate virulence markers and illustrate that our understanding of the pathogenicity of this organism remains incomplete.

There have been many attempts to characterize the ecology of Vp in coastal regions all over the globe with the objective of understanding how abiotic variables (e.g. temperature, salinity, turbidity) and biotic variables (e.g. phytoplankton, chlorophyll *a*, copepods) correlate with the density of Vp in oysters and the water column. An excellent review of these studies has recently been published (Takemura, Chien and Polz 2014). Water temperature is most consistently found to positively correlate with higher density of Vp in the water column. Salinity has also been found to affect Vp density although depending on the location and range of salinity, Vp density is reportedly either positively or negatively correlated with this variable. In our previous study of the Puget Sound from 2008–2009 (Paranjpye *et al*. 2015), we looked at Vp density in the water column and its dependence upon abiotic (e.g. water temperature, salinity and a range of nutrients) and biotic factors (chlorophyll *a* and phytoplankton). We found that the Vp density in the water column did not directly correlate with temperature in that peak densities occurred prior to maximum water temperature, but rather displayed a strong seasonal trend with maximum Vp density occurring in late June around the summer solstice. In agreement with our previous study (Johnson *et al*. 2012), Vp density did not correlate with salinity. However, density was found to be negatively correlated with silicate. A mesocosm study (Frischkorn, Stojanovski and Paranjpye 2013) found that the diatom *Thalassiosira weissflogii* increases chitin production when starved of silicic acid and since Vp, like most *Vibrios* have the ability to utilize chitin as a nutrient, higher chitin availability served to explain the inverse relationship between Vp density and silicate.

In this study, we also looked at Vp density in oysters as well as in the overlying water column of 26 distinct oyster growing locations distributed among 6 areas of the Puget Sound basin and Pacific coast of Washington state in 2007 and 2008. The level of total Vp was measured by targeting the thermolabile hemolysin gene (*tl*), a single copy of which is found in the genome of all known strains of Vp. We also measured the level of potentially virulent (*tdh*+) Vp. The aim of this study was to improve our understanding how Vp density in oysters and overlying water vary with environmental parameters and geographic locations and how potentially virulent Vp partition between the water and oyster matrices.

MATERIALS AND METHODS

Sampling sites

Since 1998, the Washington State Department of Health (WA DOH) has been monitoring oysters from a number of shellfish growing areas of the state waters for the concentrations of Vp in oysters. The locations of the growing sites for the years 2007–2008 (n = 26) are shown in Fig. 1, and their precise locations are given in Table S1 (Supporting Information). For the purposes of this study, these 26 sites were grouped into 6 geographic areas indicated in Fig. 1.

Collection of samples

Oysters were sampled from the intertidal regions of 26 growing areas indicated in Fig. 1 and listed in Table S1 (Supporting Information). Samples were collected weekly from May 21 until October 10 in 2007 and from June 2 through October 29 in 2008. When possible, collection was timed to coincide with the maximum extent of low tide before re-submergence when organisms experienced maximal exposure to air and sunlight to simulate a 'worst case' scenario. Approximately 15–20 oysters were collected at each site and placed in a sterile plastic bag. An additional oyster was opened on site and a thermometer was inserted into the meat to measure tissue temperature. This oyster was then discarded. The remaining oysters were transported on ice in a cooler, generally within 12 hr, to the Washington State Public Health Laboratory (PHL) in Shoreline, WA for analysis within 24 hr.

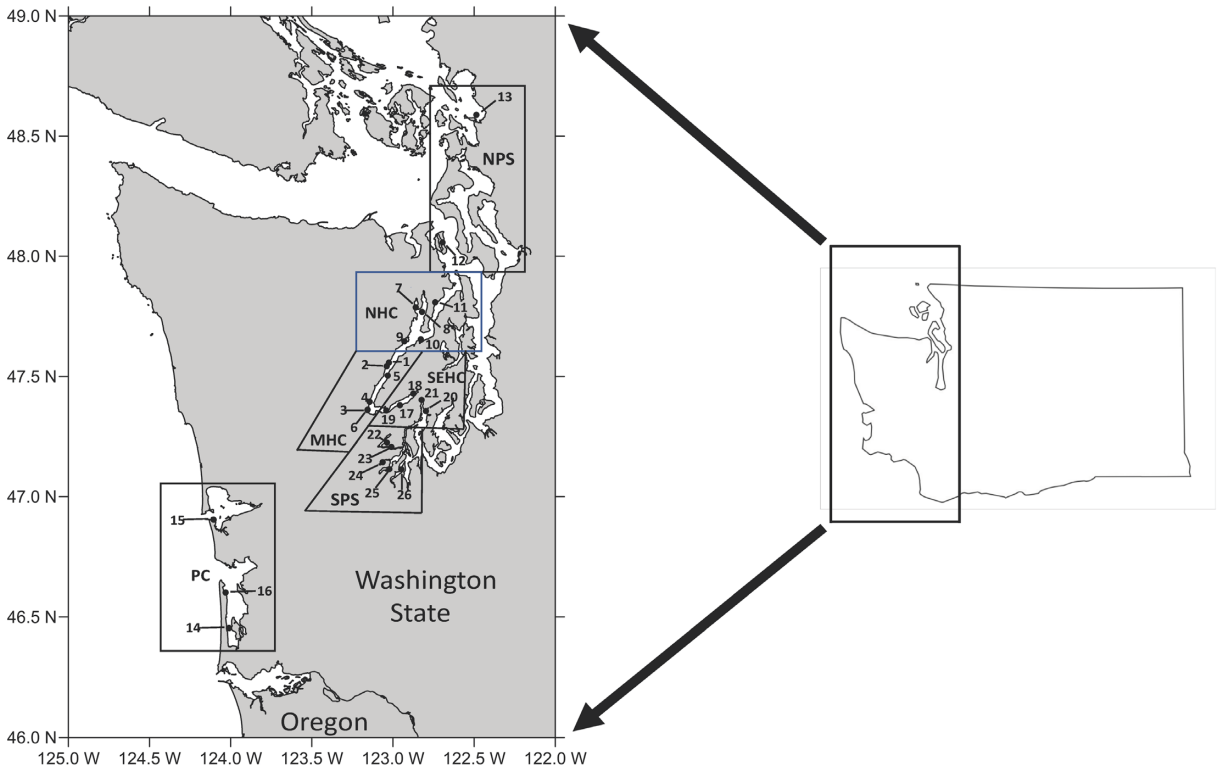

Figure 1. Location of the 26 oyster growing areas sampled in this work. The names and precise coordinates of each site are given in Table S1 (Supporting Information). For ease of interpretation, the 26 sites were grouped by area. NPS and SPS = North and South Puget Sound. NHC, MHC and SEHC = North, Mid- and Southeast Hood Canal, respectively. PC = Pacific Coast.

Approximately 900 ml of water from the same growing areas was collected in sterile 1 L polypropylene bottles at the same time as the oysters. Additionally, plankton samples were collected using 20 μm nets towed for approximately 20 feet. The sample from the net tow (ca. 400 ml) was then poured into a sterile 500 ml polypropylene bottle. The temperature of the water was measured and recorded at the surface and at a depth of 3 feet. Water salinity was also measured using a refractometer. Finally, an additional water sample (ca. 400 ml) was collected in a 500 ml brown bottle for analysis of chlorophyll *a* content. Water, net tow and chlorophyll *a* samples were placed on ice in a separate cooler, and transported to the Northwest Fisheries Science Center (NWFSC), generally on collection day, when they were prepared for extraction and analysis by methodology discussed below. Water samples alone were collected at select sites from late October through May of 2008.

Most probable number analysis of oyster tissue

Oyster samples were enriched following protocols outlined in the USA. Food and Drug, Bacterial Analytical Manual (FDA 2004). After enrichment, total DNA was extracted from each of these samples using a MagNA Pure 96 System (Roche Diagnostics), according to the preset DNA III bacteria protocol.

Extracted products were analyzed for total (*tl*+) and *tdh*+ Vp using a GeneAmp 7700 Real Time system (Applied Biosystems, Foster City, CA). All reagents, unless otherwise stated, were purchased from Applied Biosystems. Primer and probe sequences are given in Table 1. Each 25 μl reaction contained (final concentration): $MgCl_2$ (6.5 mM), the four primers (1 μM each), both probes (0.1 μM each), dNTPs (100 μM each), 1X TaqMan buffer A and 0.625 U AmpliTaq Gold. PCR cycling conditions were as follows: an initiation step at 50°C (2 min) and initial denaturation at 94°C (10 min) were followed by 45 cycles each consisting of a 15 s, 94°C denaturation step and a 1 min 65°C annealing step. To avoid false positives due to nonspecific amplification of homologues in closely-related *Vibrio* species (e.g. *V. alginolyticus*), most probable number (MPN) templates with cycle thresholds greater than 32 for *tl* and 35 for *tdh* were considered negative for those targets. Each unknown contained 3 μl of template. Every plate also included a no template control with 3 μl of water and a three point standard curve using 3 μl of a serial dilution of genomic DNA from Vp strain NY477 (*tl*+, *tdh*+), providing information about amplification efficiency.

Total DNA extraction of water and net tow samples

A known weight (ca. 500 g) of water from each site was filtered through a 0.45 μm, 47 mm diameter polyethersulfone membrane filter (Steriltech Corp., Kent WA, USA) placed on the base of a reservoir on a vacuum manifold. Following filtration, the filter was then transferred to a Kapak Pouch (Kapak Corp. St. Paul MN, USA) using flame-sterilized forceps. The pouch was then heat-sealed and stored at -70°C until extraction of total DNA. Net tow samples were prepared in the same manner, but due to the generally higher turbidity of these samples, a lower (but known) mass was filtered. Total DNA was extracted from both water and net tow samples using a method adapted from Boström *et al.* (2004). Details of this protocol have been given in a previous report (Paranjpye *et al.* 2015).

Table 1. Probe and primers used for QPCR analyses of oyster MPN samples.

Primer or Probe	Sequence, 5′ to 3′
tl probe	6-FAM-TTCGTGCGAAAGTGCTTGAGATGA-TAMRA
tl-forward	AAAGCGGATTATGCAGAAGCACTG
tl-reverse	TTGATGACACTGCCAGATGCG
tdh probe	VIC-TTAATACCAATGCACCGGTCAATGT- TAMRA
tdh-forward	CCATCTGTCCCTTTTCCTGCC
tdh-reverse	GTTTGTCCAAAAGTCAGAGACC

QPCR analysis of water and net tow samples

Water and net tow samples were analyzed for total (*tl*+) and *tdh*+ Vp using the Mx3005P Real-time PCR System (Agilent Technologies, Santa Clara, CA) using a previously published assay (Nordstrom *et al.* 2007). Full details are also given elsewhere (Paranjpye *et al.* 2015).

Chlorophyll a analysis

A known mass of water was filtered through a 25 mm GF/F glass fiber filter (Whatman, Int. Maidstone UK). Filters were placed in an aluminum foil pouch to protect them from sunlight and stored at -20°C until analyses. Chlorophyll *a* levels were determined using a Turner Designs AU fluorometer by the non-acidification method developed by Welschmeyer (1994).

Statistical analysis

Analyses were performed using R 3.32 (R Core Team 2016). Given the skewed distribution of concentrations of total and *tdh*+ Vp, concentrations were $\log_{10}$-transformed (concentration +0.1), with 0.1 chosen as the additive constant to match the scale of the lowest observed non-zero concentrations. Model covariates considered including salinity, chlorophyll *a* levels, Julian Date and temperature. A polynomial of degree 2 (or quadratic) in Julian Date consistently explained more of the variance than any other combination of two variables. Temperature alone was also significant but explained far less of the variance and temperature was highly correlated with Julian Date. Adding salinity or chlorophyll *a* to the models added very little explanatory power. Total and *tdh*+ concentrations were, therefore, modeled as two-degree polynomial functions of Julian Date. For water Vp total concentrations in 2008, the third and fourth-degree terms were statistically significant; however, the curve looked generally similar to the 2-degree polynomial. For oyster *tdh*+ Vp concentrations in 2007, the square term was not statistically significant at the $\alpha = 0.05$ level. Two-degree polynomials are plotted in all cases for consistency and reflecting the generally observed increasing and then decreasing densities of Vp over the calendar year (Paranjpye *et al.* 2015). Differences in densities across sites for 2007 and 2008 were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparisons test. These analyses were conducted separately for Vp data from oysters, water and plankton.

RESULTS AND DISCUSSION

(Results—The raw data for the entire study is available at the following link: https://www.webapps.nwfsc.noaa.gov/apex/parrdata/inventory/tables/table/vibrio_parahaemolyticus_survey_washington_20072008)

Vp levels in the water column

Fig. 2a–d summarize the results of QPCR analyses of total (*tl*+) Vp density in water samples for 2007 and 2008 for all sites. The temporal trend for the 2 years is shown in Fig. 2a and d, while Fig. 2b and c are box plots for 2007 and 2008 that provide a summary of data for each year. Levels of Vp were highly significantly different between the 2 years. The density of Vp in the water column in 2008 is generally orders of magnitude higher than that of 2007. For example, while the mean number of cells in 2007 was 209 g.e. (genome equivalents) $(100g)^{-1}$ of water, the mean in 2008 was 39,300 g.e. $(100g)^{-1}$ water. Our previous study (Paranjpye *et al.* 2015), covering different sampling sites for 2008–2009, confirmed that levels of Vp in the water column were notably high in 2008. As with that study, we are unable to identify any difference in abiotic (e.g. higher water temperature) or biotic (e.g. chlorophyll *a*) factors that might explain why 2008 so markedly differs from 2007. For example, the mean chlorophyll *a* concentration was somewhat smaller in 2008 (4.43 $\mu g\ L^{-1}$) than 2007 (5.47 $\mu g\ L^{-1}$). The data for 2008 includes Vp density for the water column starting in early March prior to the onset of oyster sampling in the first week in June. It is notable that the density was already relatively high (100–10,000 g.e. $(100g)^{-1}$) in water (Fig. 2d) in early spring and remained elevated throughout the remainder of the study period. Fig. 2e-h show the same data for *tdh* for both years. These data display the same patterns as observed for total Vp. Levels of *tdh* were also considerably higher in 2008 (mean density 21,900 g.e. $(100g)^{-1}$ water) than the previous year (mean 309 g.e. $(100g)^{-1}$ water), and they also started off high in spring of that year (Fig. 2h). Levels of *tdh*+ Vp were also highly significantly different between the 2 years. For all samples that were positive for *tl* in 2007 (n = 280) and 2008 (n = 275), the average ratio of the number of genome equivalents of *tdh* to *tl* was 0.73 for 2007 and 0.51 for 2008. The seasonality and density of both *tl* and *tdh* for the net tow samples were found to be essentially identical to those observed for water (data not shown). We also examined whether the six different areas (see Fig. 1) showed any variation in density of total or *tdh*+ Vp in the water column. We found no statistically significant differences among the areas (Figs S1 and S2, Supporting Information).

As in previous reports, (Johnson *et al.* 2012; Paranjpye *et al.* 2015) we found that strains encoding for *tdh* are present in waters of the Pacific Northwest at significantly higher densities than those reported elsewhere (Islam *et al.* 2004; Haley *et al.* 2014; Chen *et al.* 2017; Martinez-Urtaza *et al.* 2008; Greenfield *et al.* 2017), although it has recently been reported that strains encoding *tdh* have been isolated with increasing frequency on the Atlantic coast of the Northeast US (Xu *et al.* 2017) and Canada (Banerjee *et al.* 2018) as well as one study from South Carolina (Gutierrez West, Klein and Lovell 2013). In samples from the Puget Sound and Washington state coast, it was not uncommon in our QPCR analyses of water or net tow samples for the

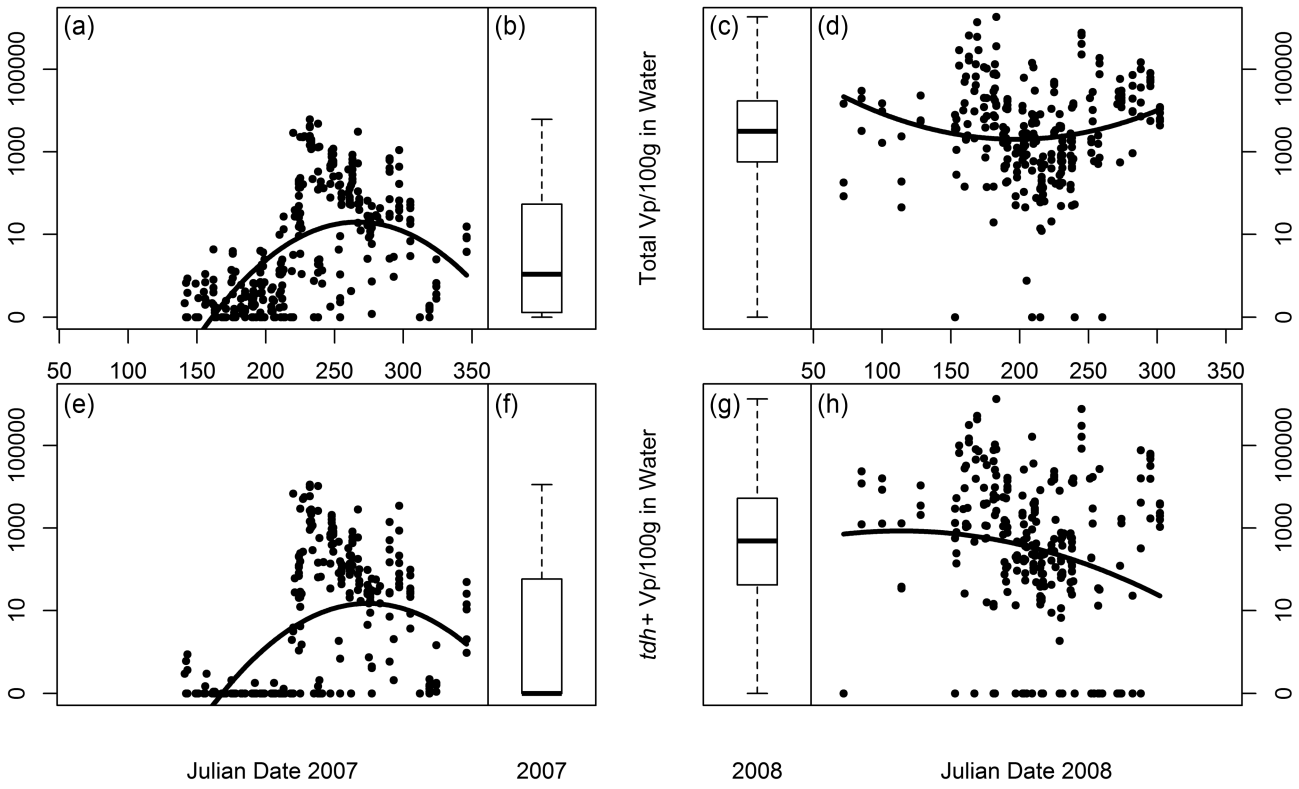

Figure 2. Total and *tdh*+ Vp in seawater (transformed via $\log_{10}(x+0.1)$) by year and Julian Date across all sampled sites. Curves are fitted 2-degree polynomials in Julian Date. For plot (h), the quadratic term in Julian Date was not significant, but the quadratic form was plotted for consistency. Levels of both total and *tdh*+ Vp were significantly different between the 2 years.

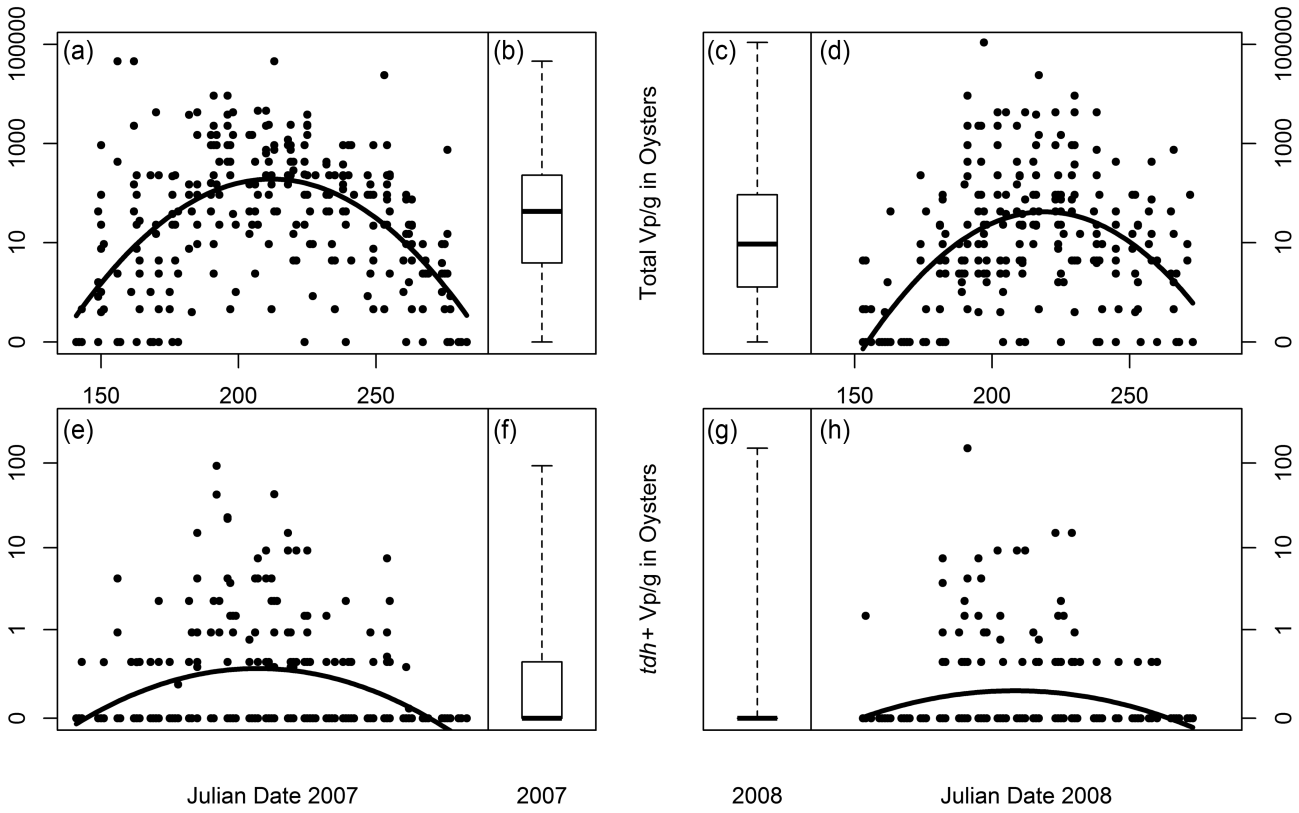

Figure 3. Total and *tdh*+ Vp in oysters (transformed via $\log_{10}(x+0.1)$) by year and Julian Date across all sampled sites. Curves are fitted 2-degree polynomials in Julian Date. Levels of both total and *tdh*+ Vp were significantly different between the 2 years.

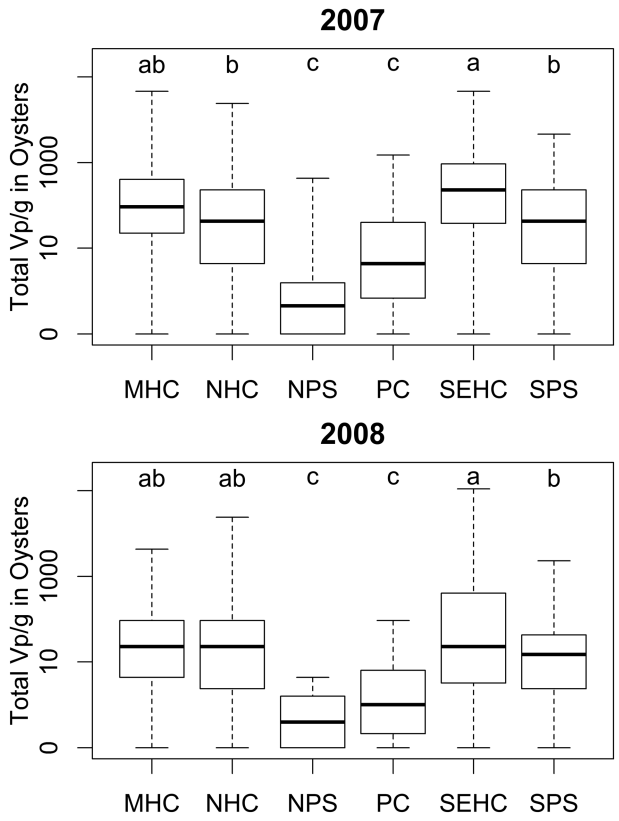

Figure 4. Total Vp in oysters by area and year. Letters indicate significant differences among sites within a year. If the same letter occurs above two sites, they do not have significantly different (at the $\alpha = 0.05$ level) transformed values of total Vp within that year. Letters are ordered from highest (a) to lowest (c) concentration within a year. Multiple comparisons were made using the Tukey test.

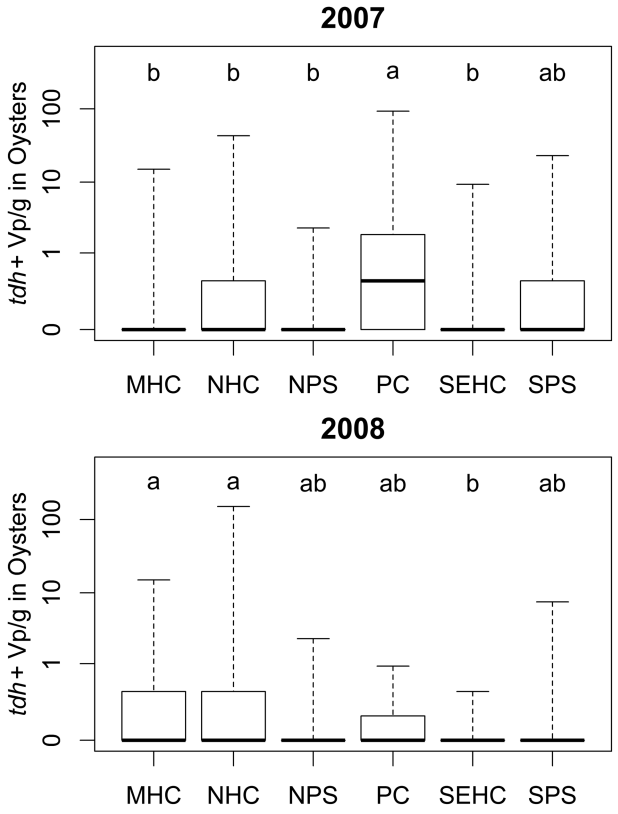

Figure 5. *tdh*+ Vp in oysters by area for 2007 and 2008. Letters indicate significant differences among sites within a year. If the same letter occurs above two sites, they do not have significantly different (at the $\alpha = 0.05$ level) transformed values of total Vp within that year. Letters are ordered from highest (a) to lowest (b) concentration within a year. Multiple comparisons were made using the Tukey test.

number of copies of *tdh* to exceed the number of copies of *tl*. As mentioned in the introduction, this is a feasible result since *tdh*+ strains of Vp can contain up to two copies of *tdh* while in all known strains, *tl* is a single copy gene. This finding suggests that strains encoding for *tdh* may have some sort of selective advantage in the waters of the Pacific Northwest of the USA. It has been suggested elsewhere (McLaughlin *et al.* 2005) that lower water temperature tends to be associated with higher levels of *tdh*+ strains of Vp. Furthermore, an elegant mesocosm study (Matz *et al.* 2011) demonstrated that *tdh*+ Vp strains not only resisted grazing pressure from a suite of protist taxa that included representative flagellates, ciliates and amoebae, these strains are actually parasitic to the would-be predatory protists. Matz *et al.* (2011) tested the pandemic type strain of Vp (RIMD2210633, genotype *tdh*+, *trh*-) and its isogenic mutants defective in *tdh*, T3SS1 and T3SS2, respectively. Only the mutant defective in T3SS2 activity was susceptible to predation suggesting that T3SS2 expression is responsible for the apparent resistance of *tdh*+ strains to grazing pressure. A number of *trh*+ strains were also tested for grazing resistance and it was found that these strains were considerably more vulnerable to predation. Therefore, this work suggests that T3SS-2α specifically is associated with grazing resistance. However, as appealing as this hypothesis is, grazers tested in the mesocosm study are found in waters world-wide begging the question of why Vp encoding *tdh* (and T3SS-2α) are not more commonly found elsewhere and suggests that further work is needed to fully understand the apparent advantage afforded *tdh*+ strains in the Pacific Northwest.

Vp levels in oysters

In this study we also examined the density of Vp in filter-feeding oysters from these same waters. The results for total Vp for all areas are summarized in Fig. 3a–d. Fig. 3a and d show the temporal trend for 2007 and 2008, respectively, while Fig. 3b and c provide box plot summaries for each. The median total Vp density in oysters does not greatly differ between 2007 and 2008, this in spite of the much higher density of total Vp in the water column in 2008 relative to 2007 (Fig. 2b and c). In fact, the mean density of Vp was somewhat lower in 2008 (810 g.e. g^{-1}) than 2007 (977 g.e. g^{-1}). Despite the smaller difference in oysters than in water, mean transformed levels of *tl*+ Vp in oysters were statistically significantly different between the 2 years. There was a substantial overlap in values across the 2 years; however, such that this difference may not be biologically meaningful. Given the strikingly higher levels of total Vp in the water column in 2008 than 2007, the similarity of the level of total Vp in oysters for both years seems to suggest that Vp accumulation in oysters reaches a steady-state level at which point density in the overlying water column becomes essentially irrelevant.

Fig. 3e–h show analogous data for *tdh*+ Vp in oysters for all areas. As is the case for total Vp, data from the 2 years are quite similar, though still with significantly different log-scale mean values. As with total Vp, however, this difference is not likely to have biological significance. Surprisingly, *tl*+, *tdh*+ strains were detected less often and at much lower levels in oysters than strains of the *tl*+, *tdh*- genotype. Of those oysters in which Vp

could be detected, the average ratio of the MPN for *tdh* to that of *tl* was ca. 0.003 for both years. Recall that the QPCR findings of the water samples was that the ratio of *tdh* to *tl* averaged 0.73 and 0.51 in 2007 and 2008, respectively. We found that, while *tdh*+ strains comprised a high portion of the Vp community in the water column, these strains were considerably less likely to be retained by filter-feeding oysters in those same waters. Because *tdh* is still considered a good virulence marker, this finding may well explain why illness associated with the consumption of raw oysters is not more common than it is in the Pacific Northwest given the high level of this genotype in the water column.

We investigated whether there are discernible differences in oyster MPN data between the six areas (see Fig. 1) for both years. Oysters harvested in the SEHC area are found to have highest total Vp for both years, although not significantly higher than MHC in either year nor NHC in 2008 (Fig. 4). Lowest levels were seen in oysters for both years in PC and NPS. Fig. 5 shows comparable data for *tdh* in oysters of all six areas. In 2007, the PC samples exhibit significantly higher levels of *tdh* than all other areas with the exception of SPS, while in 2008 samples from the PC show no significant difference from any of the other five areas with SEHC having the lowest levels of *tdh*, quite opposite to concentrations of total Vp in that area. Consistent with the data of Fig. 3, the levels of *tdh* are quite low relative to total Vp in all areas for both years.

A similar disparity between the bacterial community in the water column and the community present in oysters has also been reported involving another *Vibrio* species, *V. vulnificus*. . A subset of strains of this species, referred to as biotype 1, is most often involved in human infections that can be serious and even deadly, especially in immune compromized individuals (Strom and Paranjpye 2000)). However, like Vp, strains belonging to the biotype 1 group are genetically heterogeneous and not all isolates are virulent. Nucleotide polymorphisms in a number of genes have been proposed to distinguish virulent strains from those less likely to cause disease. Targets include the genes encoding the 16S ribosomal subunit (Nilsson *et al.* 2003), the cytotoxin-hemolysin *vvh* (Senoh *et al.* 2005), *pil*F (Roig *et al.* 2010) and a gene of unknown function dubbed the virulence correlated gene, or *vcg* (Rosche, Yano and Oliver 2005). In the case of the latter target, illness-associated strains are most likely to assay as encoding the 'clinical' variant (*vcg*C) whereas strains less likely to cause illness more often encode for the 'environmental' variant (*vcg*E). In a study comparing the density of *V. vulnificus* between the oysters and the associated water column off the North Carolina coast (Warner and Oliver 2008), it was reported that while the abundances of *vcg*E and *vcg*C strains were essentially equal in the water column, nearly 85% of 880 strains isolated from oysters were of the *vcg*E genotype. It was suggested that *vcg*E strains have a selective advantage in oysters and may help explain the relative rarity of infections due to ingestions of raw or undercooked oysters, since *vcg*C strains are much more likely to cause illness. Later work (Froelich, Ayrapetyan and Oliver 2013) provided a feasible explanation for the apparent differential uptake of the two genotypes. This work involved the laboratory generation of marine snow, particulate matter that if of optimal size (ca 7 μm) is efficiently captured by filter feeding oysters. It was demonstrated that E-type strains incorporate more readily into marine snow of the optimal size than C-type strains, thus explaining the surprisingly low density of *vcg*C strains in oysters relative to the water column.

The rather high proportion of *tl*+, *tdh*+ strains of Vp in the waters of the Pacific Northwest appears to be unique. It must be said that the foregoing discussion regarding a selective advantage for these strains due to low temperature and/or grazing resistance is purely speculative at present. Similarly, the observation that though in high concentration in the water column, strains of this genotype do not accumulate efficiently in oysters may or may not have to do with a defect in binding to particulate matter accumulated by filter-feeding oysters. It is apparent that further study of the dynamics and ecology of this organism is necessary for a more complete picture. It is also apparent that analysis of Vp in the water column provides little useful information for risk assessment, at least in the waters of the Puget Sound and the Washington coast.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflicts of interest. None declared.

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