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Development of a Matrix Tool for the Prediction of *Vibrio* Species in Oysters Harvested from North Carolina

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The United States has federal regulations in place to reduce the risk of seafood-related infection caused by the estuarine bacteria *Vibrio vulnificus* and *Vibrio parahaemolyticus*. However, data to support the development of regulations have been generated in a very few specific regions of the nation. More regionally specific data are needed to further understand the dynamics of human infection relating to shellfish-harvesting conditions in other areas. In this study, oysters and water were collected from four oyster harvest sites in North Carolina over an 11-month period. Samples were analyzed for the abundances of total *Vibrio* spp., *V. vulnificus*, and *V. parahaemolyticus*; environmental parameters, including salinity, water temperature, wind velocity, and precipitation, were also measured simultaneously. By utilizing these data, preliminary predictive management tools for estimating the abundance of *V. vulnificus* bacteria in shellfish were developed. This work highlights the need for further research to elucidate the full suite of factors that drive *V. parahaemolyticus* abundance.

In the United States, it is estimated that as many as 84,000 people annually contract food-borne infections caused by *Vibrio* bacteria (1). These aquatic bacteria are found in coastal or estuarine environments as part of the natural flora but can become highly concentrated in filter-feeding sea life, including shellfish such as oysters (2, 3). Because oysters are often consumed raw or undercooked, vibrios concentrated within the oysters remain viable and infectious. Reported infections from food-borne *Vibrio* spp. are on the rise and are currently at the highest level since tracking began (4). While no fewer than 12 species of *Vibrio* are capable of infection, the 2 most common in the United States are *Vibrio parahaemolyticus* and *Vibrio vulnificus*, which cause the most infections and the most deaths, respectively (5–7). Symptoms associated with infections caused by these two species range from gastroenteritis to grievous wound infections or primary septicemia, with case fatality rates as high as 50% (2, 7–11).

Both of these important bacterial species have been reported to exhibit seasonality, with warmer water temperatures resulting in increased *Vibrio* occurrence and concentrations in oysters (12–15). As a consequence, more than 75% of the infections caused by *Vibrio* spp. in the United States are observed between May and October (14). While no maximum environmental temperature has been reported, the minimum water temperature needed for the isolation of culturable *V. vulnificus* from oysters differs among studies but is most often reported in the range of 12 to 17°C; however, lower temperatures have also been documented in individual studies (3, 12, 13, 15–20). Similarly, *V. parahaemolyticus* can grow in culture at a minimum temperature of approximately 10°C (21). The typical minimum water temperatures associated with oyster-related human disease reported for *V. vulnificus* and *V. parahaemolyticus* are ca. 20°C and 15°C, respectively (20–22). In addition to water temperature, the warmer air temperatures from June through September in the United States can also contribute to the increased rate of infections. Empirical data and predictive modeling have both shown that the growth of *Vibrio* spp. in shellfish after harvest poses considerable risk to consumers. This bacterial growth is a result of the internal warming that oysters undergo after being removed from the water. The inability of oysters to expel these growing bacteria also contributes to their increased numbers. Currently, federal regulations are in place that limit the time oysters can be exposed to warm air temperatures during harvest (23–26).

Salinity is also a factor in *Vibrio* abundance. *V. vulnificus* and *V. parahaemolyticus* concentrations in oysters appear to have a nonlinear relationship with water column salinity, although this is confounded by conflicting reports of positive, negative, and noncorrelating data (3, 13, 15, 16, 27–35). The optimal salinity ranges for *V. vulnificus* and *V. parahaemolyticus* in oysters have been reported to be 5 to 25‰ and 10 to 34‰, respectively (3, 20). Not surprisingly, given their close relationship, these salinity values overlap the optimal salinity ranges for oyster growth, survival, and recruitment. Salinity ranges of 10 to 28‰ have been found permissive for growth and reproduction, and salinity ranges of 18 to 22‰ have been found permissive for the settlement of oyster larvae (36, 37). Confounding these findings are the combined effects of salinity and temperature on the concentrations of *Vibrio* spp. in oysters. Most research finds that as water temperatures increase, so do the survivability and abundance of *V. vulnificus* at greater salinities. This indicates that the salinity and water temperature

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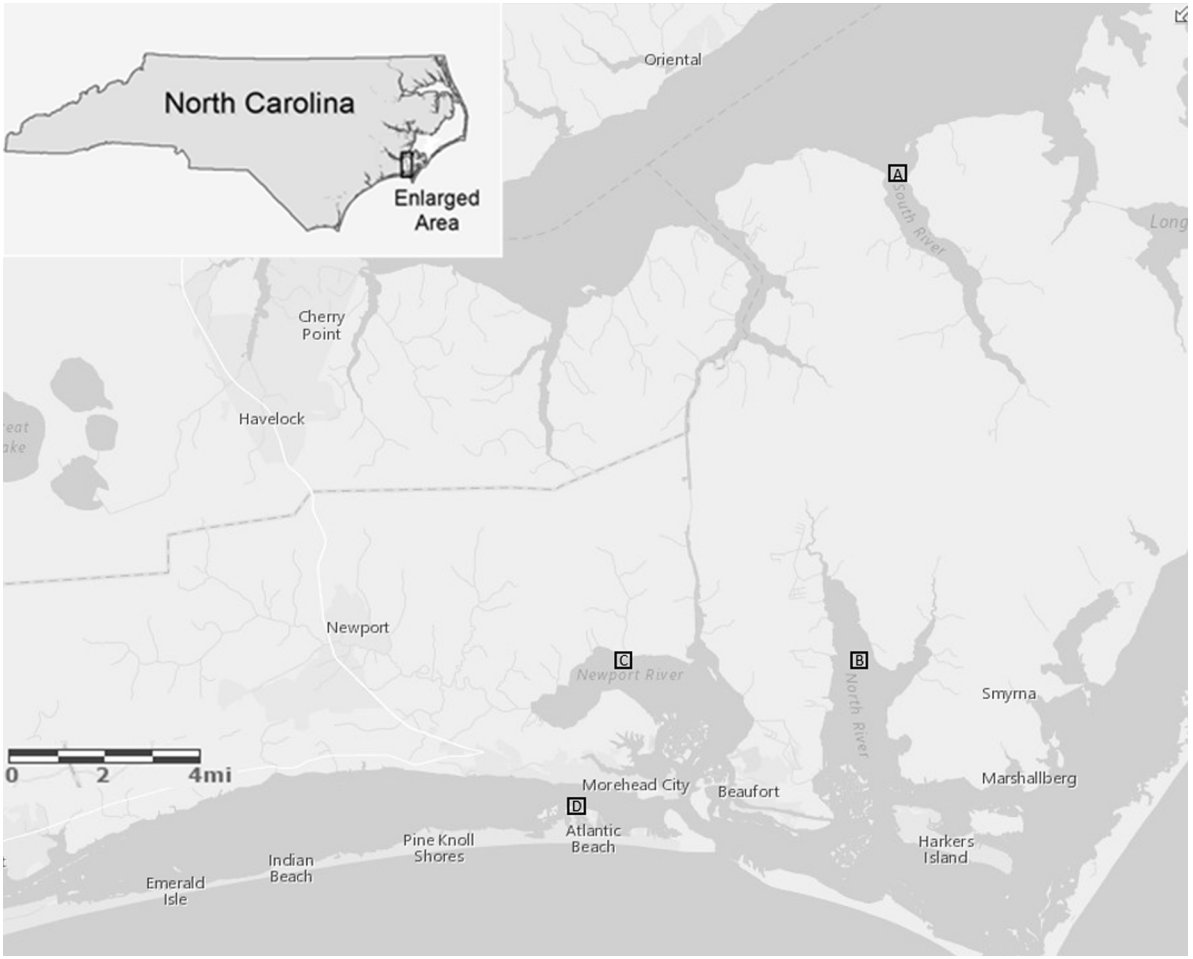

FIG 1 Map of eastern North Carolina. The oyster and water collection sites, South River (A), North River (B), Harlowe Creek (C), and Hoop Pole Creek (D), are indicated. Image from the North Carolina Department of Transportation (NCDOT).

should be viewed in conjunction and the individual effect of each should be observed with caution (3, 12, 19).

V. vulnificus strains are not equal in potential infectivity. Typically, the strains of *V. vulnificus* that most often cause disease via ingestion are those containing the C allele of *vcg* (the virulence-correlated gene). Those with the E allele of *vcg* are typically less likely to cause seafood-related disease (38, 39). Strains with the C allele of the *vcg* gene are referred to as C-genotype strains, and those with the E allele are termed E-genotype strains. Understanding the relative abundances of these two types could play a role in determining the risk to an oyster consumer.

In this study, oysters and water were collected from four actively utilized oyster harvest sites in North Carolina over an 11-month period. Samples were analyzed for the abundances of total *Vibrio* spp., *V. vulnificus*, and *V. parahaemolyticus*; environmental parameters, including salinity, water temperature, wind velocity, and precipitation, were also measured simultaneously. By utilizing these data, preliminary predictive management tools for estimating the abundances of these *Vibrio* spp. in shellfish were developed.

MATERIALS AND METHODS

Sampling sites. Samples were collected from four oyster harvest sites in eastern North Carolina, including North River, South River, Hoop Pole Creek, and Harlowe Creek (Fig. 1). These sites were selected because they represent areas that include high and low salinities, that experience either wide fluctuations in water column salinity or very small exchanges in salinity, and that are accessible (within 10 km) to the laboratory, allowing for rapid processing after oyster harvest and water collection.

Oyster sample collection and processing. Oyster samples were collected from 4 February 2013 to 18 December 2013. There were 56 separate sampling events each for water and oyster samples. Ten market-sized oysters were collected from each sampling site on each of the days of sampling. Sites were typically sampled every 2 weeks, and alternate sites were sampled weekly. Shellfish were collected by dredge, rake, tongs, or hand, and were then placed in plastic bags, which were kept in coolers on ice during transport to the laboratory. In all cases, samples were transported and processed within 5 h of collection. Shellfish were cleaned of mud with a brush, rinsed with 70% ethanol, and dried with paper towels. Oysters were aseptically shucked with ethanol-sterilized instruments, and oyster meat was rinsed gently with sterile phosphate-buffered saline (PBS; Amresco, Solon, OH) to remove sediment. The 10 oysters from each site were separated into two groups of 5 oysters each. The meats from each group were pooled, drained of mantle fluid and hemolymph, and weighed, and an equal amount of PBS (wt/vol) was added to each batch of oyster tissues. The tissues were homogenized in a Waring (Stamford, CT) blender with three cycles of blending for 15 s, followed by 5 s of rest. Oyster homogenates were diluted 1:10 with PBS, and 100 μl of the original ho-

mogenate and 100 μl the diluted sample were each used for bacterial culture, as described below.

Water sample collection and processing. Water samples for each site were collected simultaneously with oyster samples. Sterile clear plastic 1-liter Nalgene (Rochester, NY) bottles were rinsed three times with water immediately surrounding the oyster sample collection area; then they were filled, capped, and placed on ice. Salinity was measured with an HI 96822 digital refractometer (Hanna Instruments, Carrollton, TX). Water temperature was measured at the time of collection, and statistics for wind speed and cumulative 24-h precipitation around the sample area were collected from local weather stations. Water samples of 1 to 10 ml were vacuum filtered through a 47-mm-diameter, 0.45-μm-pore-size mixed cellulose ester filter (Pall, Port Washington, NY) and were placed on selective media as described below.

Media and growth conditions. CHROMagar *Vibrio* medium (CHROMagar, Paris, France) was prepared as per the manufacturer's instructions and was used to select for presumptive *V. vulnificus* (dark blue colonies) and *V. parahaemolyticus* (dark purple colonies) isolates from water and oysters. Thiosulfate-citrate-bile salts-sucrose (TCBS) agar and heart infusion broth (HI) were prepared according to the manufacturer's instructions (Becton, Dickinson and Company [BD], Franklin Lakes, NJ). TCBS was used to estimate total *Vibrio* sp. abundance, with green and yellow colonies summed. Heart infusion broth was used to grow pure cultures of individual isolates, as detailed below. All media were incubated at 37°C for 24 h. After incubation, the colonies growing on these plates were counted, and the data were transformed to CFU per gram of oyster or CFU per milliliter of water. This presumed number was then multiplied by the percentage of isolates that were molecularly confirmed to be *V. vulnificus* or *V. parahaemolyticus* (via the method described below) in order to obtain an assumed value at each point.

Molecular confirmation of isolates. After incubation and enumeration, 10 presumptive *V. vulnificus* colonies and 10 presumptive *V. parahaemolyticus* colonies from both water and oyster samples were isolated from each site at each sampling point. These were grown in pure culture in HI broth, boiled for 10 min, and centrifuged at 10,000 × *g* for 10 min, and the pellet was discarded. The remaining supernatants were stored at −20°C until they were used as templates for PCR confirmation. Molecular identification of *V. parahaemolyticus* was confirmed by targeting the *flaE* gene, using primers flaE F and flaE R as described by Tarr et al. (40). *V. vulnificus* was confirmed based on the presence of the hemolysin/cytolysin gene *vvhA* and was further genotyped using a multiplex PCR based on the identification of one of two alleles of the virulence-correlated gene, *vcgC* or *vcgE*, by using primers and protocols previously published by Warner and Oliver (41), with slight modifications. The master mix comprised 1× GoTaq buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (dNTP), 0.4 μM *vvhA*, *vcgC*, and *vcgE* primers, and 1.25 U of GoTaq DNA polymerase. Molecular-grade water (9.25 μl/reaction) and dimethyl sulfoxide (DMSO; 1 μl/reaction) were added. All PCRs were performed in a Techne TC-5000 thermal cycler (Bibby Scientific US, Burlington, NJ).

Statistics. Mean values were compared using one-way analysis of variance (ANOVA) with a Holm-Sidak posttest for multiple comparisons. Multiple linear regression analysis included all recorded variables, with backward and forward stepwise regression performed using F-tests to ascertain the variables that best explained the output variable. The significant variables were then used to create multiple linear regression equations. Segmented regression analysis used the Levenberg-Marquardt algorithm with either two or three points, and iterations were performed until convergence was achieved and a chi-square tolerance of 1E−9 was reached. A two-tailed test was used to calculate the Spearman rank correlation coefficients. Statistical significance for all tests was measured at an α value of 0.05.

RESULTS

Environmental conditions measured. The minimum, maximum, and mean values for salinity, water temperature, and wind

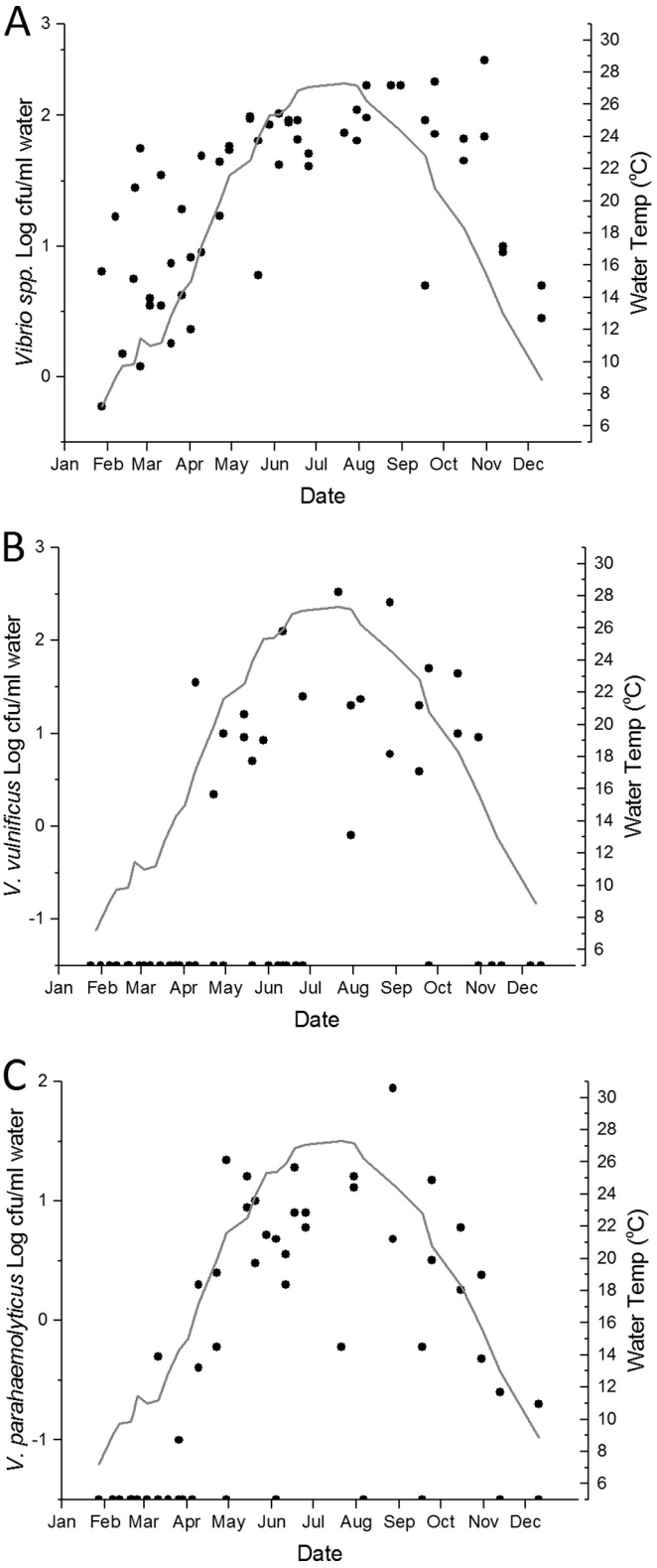

FIG 2 Log_{10} CFU of total *Vibrio* spp. (A), *V. vulnificus* (B), or *V. parahaemolyticus* (C) per milliliter of water sample by collection date (black dots). Results below the limit of detection were assigned the value of −1.5 log CFU/ml. The gray line represents the monthly moving average of the water temperature at the time of sample collection.

TABLE 1 Individual statistics from each iteration of significant[a] linear regression of *Vibrio* abundance and water temperature organized by species

Vibrio abundance	Result of linear regression analysis with water temp		
	Intercept (SE)	Slope (SE)	Adjusted R^2
In water samples (log CFU/ml)			
Total *Vibrio* spp.	0.07735 (0.17455)	0.07051 (0.00881)	0.53413
V. vulnificus	−2.51143 (0.46534)	0.11205 (0.02341)	0.29652
V. parahaemolyticus	−2.47574 (0.34208)	0.11772 (0.01689)	0.48765
In oyster samples (log CFU/g)			
Total *Vibrio* spp.	1.72746 (0.3125)	0.08538 (0.01577)	0.33971
V. vulnificus	−0.44585 (0.4805)	0.08297 (0.02394)	0.17195
V. parahaemolyticus	−0.95556 (0.34248)	0.1221 (0.01729)	0.47062

[a] At a P value of <0.05.

velocity at the time of sample collection, the total-*Vibrio*, *V. vulnificus*, and *V. parahaemolyticus* abundances in both water and oyster samples, and the total precipitation 24 h prior to sample collection at each site are displayed in Table S1 in the supplemental material. Hoop Pole Creek had the highest maximum and mean salinities ($P < 0.01$), while South River had the lowest minimum and average salinities ($P < 0.001$). The Hoop Pole Creek and South River sites had the narrowest salinity ranges, while Harlowe Creek had the largest salinity range (see Table S1 and Fig. S1 in the supplemental material).

***Vibrio* spp. in North Carolina water and oysters.** Culturable *Vibrio* spp. were detected in all water samples, including samples in all temperature and salinity ranges (Fig. 2A; see also Table S1 and Fig. S1 in the supplemental material). Log total-*Vibrio* concentrations in the water column exhibited a strong, significant linear relationship with water temperature (Table 1), but not with salinity. Culturable *Vibrio* spp. were recovered from all but two oyster samplings (Fig. 3A), and there was a significant but weak linear relationship between log total-*Vibrio* concentrations in oyster meats and water temperature (Table 1). Water temperature was the only factor that exhibited a significant correlation with total *Vibrio* spp. in both oysters ($n = 56$; $r = 0.64$; $P < 0.0001$) and water ($n = 56$; $r = 0.72$; $P < 0.0001$). There was no significant difference in mean total-*Vibrio* concentrations in water or oyster samples among the sampling sites, although Hoop Pole Creek exhibited the highest variability of total-*Vibrio* concentrations (see Table S1 in the supplemental material).

Concentrations of culturable *V. vulnificus* bacteria in both water and oyster samples (n, 53 for each sample type) correlated positively with water temperature (for water samples, r was 0.53 and P was <0.0001; for oyster samples, r was 0.41 and P was 0.002) and negatively with salinity (for water samples, r was −0.37 and P was 0.007; for oyster samples, r was −0.47 and P was <0.001). A seasonal trend was observed with both water and oyster samples, but even when the waters were very warm (>22°C), numerous samples were below the limit of detection (Table 1; Fig. 2B and 3B). Multiple linear regression analysis revealed that the combined factors of salinity and temperature are best for predicting the abundance of *V. vulnificus* in water and oyster samples (Table 2).

Heat maps of the combined effects of water temperature and salinity on *V. vulnificus* in North Carolina water and oysters serve as a quick visual aid for assessing *V. vulnificus* concentrations and are intended for future use by water quality managers (Fig. 4A and B). Segmented regression of temperature and bacterial abundance revealed that the critical temperature for *V. vulnificus* cells to be culturable in the estuarine waters of central eastern North Carolina is ca. 16°C. If water temperature is <16.1°C, log [*V. vulnificus*] = −1.5 + (−4.03367E−9 × water temperature); if water temperature is >16.1°C and <16.8°C, log [*V. vulnificus*] = −1.5 + [2.20882 × (water temperature − 16.1)]; and if water temperature is >16.8°C, log [*V. vulnificus*] = 0.0462 + [0.02754 × (water temperature − 16.8)]; $R^2 = 0.31$.

Hoop Pole Creek had significantly lower *V. vulnificus* levels in water samples than South River, a finding that mirrors the differing salinity levels at the two sites (Fig. 5; see also Fig. S1 in the supplemental material) ($P < 0.001$). Nevertheless, there were no significant differences in mean *V. vulnificus* concentrations in oysters among the sites.

Culturable *V. parahaemolyticus* abundances in water and oysters (n, 51 for each sample type) correlated only with water temperature among the parameters measured (for water samples, r was 0.69 and P was <0.0001; for oyster samples, r was 0.68 and P was <0.0001). Although *V. parahaemolyticus* abundance was tightly coupled with temperature, there were cases in both water and oysters where *V. parahaemolyticus* was undetectable, despite relatively warm water temperatures (Fig. 2C and 3C). There were strong, significant linear relationships between water temperature and *V. parahaemolyticus* abundances in both water and oysters (Table 1). The critical temperature for *V. parahaemolyticus* abundance in water was determined by segmented regression to be ca. 16°C, similar to that for *V. vulnificus* in water. If water temperature is <16.1°C, then log [*V. parahaemolyticus*] = −1.78149 + (0.04163 × water temperature); if water temperature is >16.1°C but <16.8°C, then log [*V. parahaemolyticus*] = −1.1112 + [1.71055 × (water temperature − 16.1)]; and if water temperature is >16.8°C, then log [*V. parahaemolyticus*] = −0.3374 + [0.04782 × (water temperature − 16.8)]; $R^2 = 0.52$. There were no significant differences in the mean *V. parahaemolyticus* concentrations in water or oyster samples among the sites (data not shown).

Genotyping of recovered *V. vulnificus* bacteria. *V. vulnificus* can be subdivided into allelic variants based on the virulence-correlated gene (*vcg*). The two genotypes, termed E-type and C-type, correlate with environmental or clinical isolation, respectively, and can be of use in determining potential virulence via ingestion (38, 39, 42, 43). There were five instances of recovery of PCR-confirmed C-genotype bacteria from water samples, and five instances from oyster samples, during the entire sampling period, from a total of 616 individual presumptive isolates sampled dur-

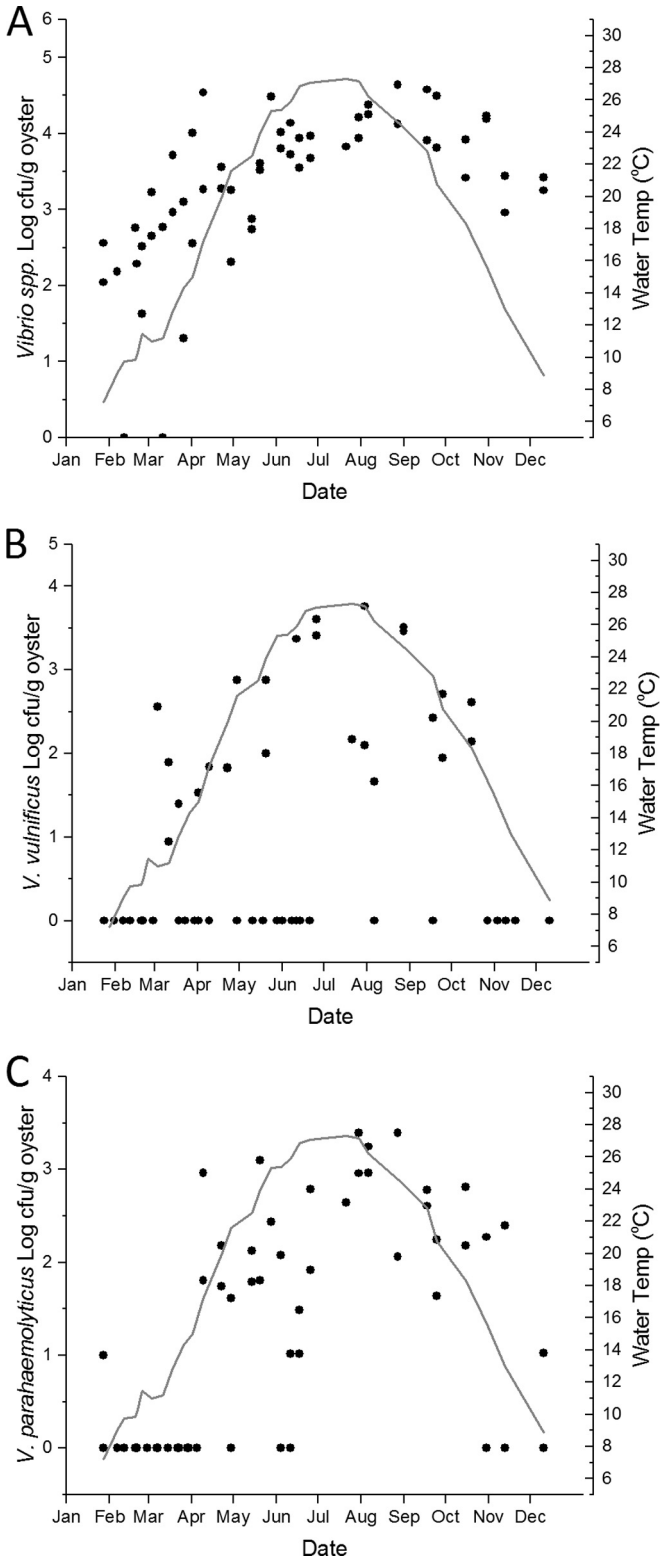

FIG 3 Log_{10} CFU of total *Vibrio* spp. (A), *V. vulnificus* (B), or *V. parahaemolyticus* (C) per gram of oyster sample by collection date (black dots). Results below the limit of detection were assigned the value of 0 log CFU/g. The gray line represents the monthly moving average of the water temperature at the time of sample collection.

ing 56 sampling events. The dates, sites, and sampling conditions for these occurrences are listed in Table 3. The average salinity of sites containing PCR-confirmed C-type isolates was 28‰, while the mean water temperature was 19°C. Only on one occasion (7 August 2014), at one site (Hoop Pole Creek), were confirmed C-type *V. vulnificus* bacteria isolated from both the oyster meat and water column portions of the samples. In total, only 5% of confirmed *V. vulnificus* isolates belonged to the C-genotype. When the isolates were separated by source, 9% of confirmed water column isolates and 5% of confirmed oyster isolates belonged to the C-genotype.

DISCUSSION

Four North Carolina oyster-harvesting sites were chosen that provided high and low, broad and narrow salinity ranges, in order to maximize the range of collection conditions encountered during the study. There were no observable relationships between log total-*Vibrio*, *V. parahaemolyticus*, or *V. vulnificus* concentrations and precipitation or wind speed at the collection sites. All bacterial species tested exhibited significant seasonality, as has been observed previously across many members of this genus. We detected *Vibrio* spp. in all water samples and all but two of the oyster meat samples over the course of the study. Total-*Vibrio* abundance in water was related to water temperature, but in contrast to previous studies on total-*Vibrio* abundance in North Carolina waters, no relationship with salinity was observed (44–46). Furthermore, there was no difference in the mean abundance of total-*Vibrio* spp. observed in either water or oysters at any of the sampling sites, despite the differences in tidal influence on environmental conditions. This lack of difference is likely due to the ability of bacteria in the *Vibrio* genus to flourish at various temperatures and salinities ranging from freshwater to full-strength marine water, and while the numbers of individual species might differ at each location, the total number of vibrios remains relatively unchanged.

V. vulnificus in both oyster and water samples exhibited significant positive correlations with water temperature and negative correlations with salinity. We found that in North Carolina coastal water, 16°C was the minimum temperature at which culturable *V. vulnificus* bacteria were commonly isolated, a finding similar to a report by Pfeffer et al. (47), although our study did not find a maximum temperature at which *V. vulnificus* could be isolated. Despite the correlation with water temperature, there were numerous oyster and water samples collected in very warm waters in which no detectable *V. vulnificus* were observed. A reason for such lack of detection of this species at these times is apparent when the effect of salinity is also incorporated into the analysis. The sampling site with the highest average salinity, Hoop Pole Creek, was also the site with the lowest average number of recoverable *V. vulnificus* bacteria from the water. Interestingly, there was no statistical difference in the average abundance of *V. vulnificus* in oysters among the sites, indicating that colonization of oyster matrices could have a protective effect against external environmental conditions, such as the high levels of salinity reported in this study. However, this appears to be true only up to a point, since extreme or prolonged periods of elevated salinity have been shown to alter the oyster microflora, including *V. vulnificus* (31, 48–50). It was found that the combination of salinity and water temperature provided the best-fitting linear regression models for *V. vulnificus* in both water and oysters, and these models were used to generate

TABLE 2 Statistics from significant[a] multiple linear regression analysis of abundance of *V. vulnificus* bacteria from either oyster or water samples with water temperature and salinity

V vulnificus abundance	Intercept (SE)	Slope from multiple linear regression analysis with:		Adjusted R^2
		Salinity (SE)	Water temp (SE)	
In water samples (log CFU/ml)	−0.38346 (0.5486)	−0.08876 (0.018)	0.12896 (0.01969)	0.51726
In oyster samples (log CFU/g)	0.20309 (0.71867)	−0.02648 (0.02188)	0.08758 (0.02414)	0.17928

[a] At a P value of <0.05.

matrix tools that provide easy-to-interpret visual references about the potential concentrations of *V. vulnificus* in North Carolina oysters. The use of such tools could allow oyster harvesters and water quality managers to make rapid decisions as to whether the oysters collected from these sites should be served raw or as a shucked product meant to be cooked.

Each *V. vulnificus* isolate that was molecularly confirmed was subjected to *vcg* genotyping in order to determine the proportions, in each oyster or water sample, of the more-virulent C-genotype strains and the less-virulent E-genotype strains. Only five of the samples collected from water throughout the year, and five separate samples collected from oysters, contained PCR-confirmed C-genotype *V. vulnificus* cells. Furthermore, on only one sample date did both the oyster and water samples contain C-genotype cells. No correlation was found between the environmental parameters measured and the occurrence or abundance of C-genotype strains, but this is likely due to the low recovery of C-type strains.

The only environmental parameter that correlated with the concentration of *V. parahaemolyticus* bacteria, in both water and oyster samples, was water temperature. Previous studies have differed in showing salinity to be correlated or not correlated with salinity, and this study supports the latter finding (32, 34). The minimum temperature for *V. parahaemolyticus* to be detectable in North Carolina oyster-harvesting waters was 16°C, with no detectable difference in mean *V. parahaemolyticus* abundance in water or oysters at any of the sampling sites. Thus, in this study, salinity does not appear to play a significant role in driving the occurrence or concentration of *V. parahaemolyticus*. Yet, remarkably, although there was a tight coupling of *V. parahaemolyticus* abundance with water temperature, there still remained instances

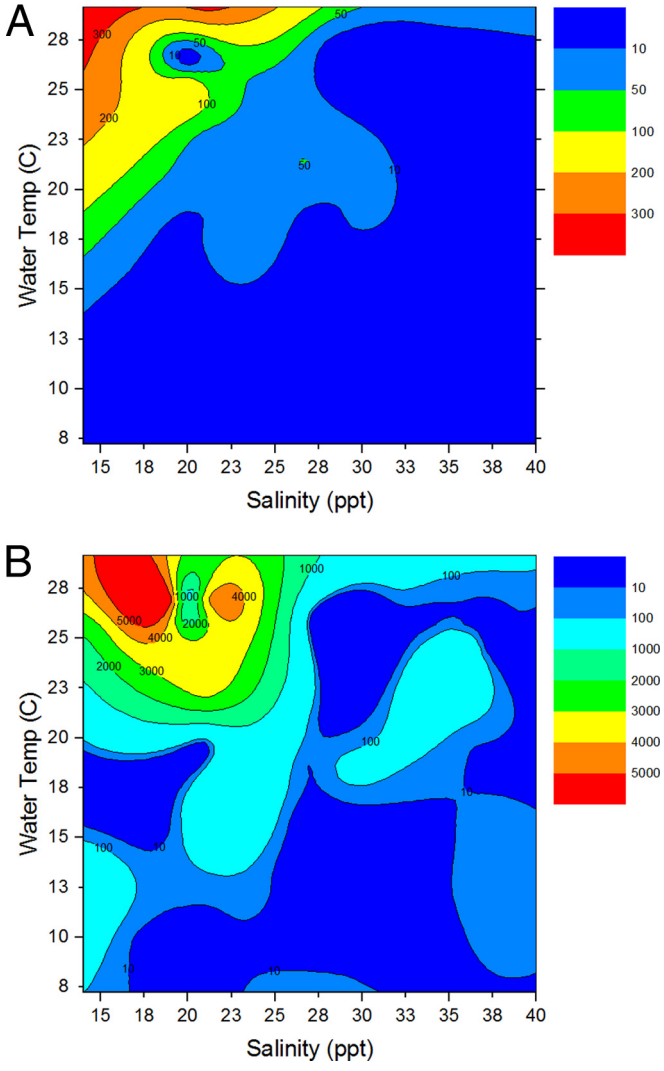

FIG 4 Heat maps of *V. vulnificus* abundances in water (A) or oyster (B) samples by salinity and water temperature during the collection period. Numbers on heat maps and in keys represent bacterial counts in CFU/ml for water and CFU/g for oysters.

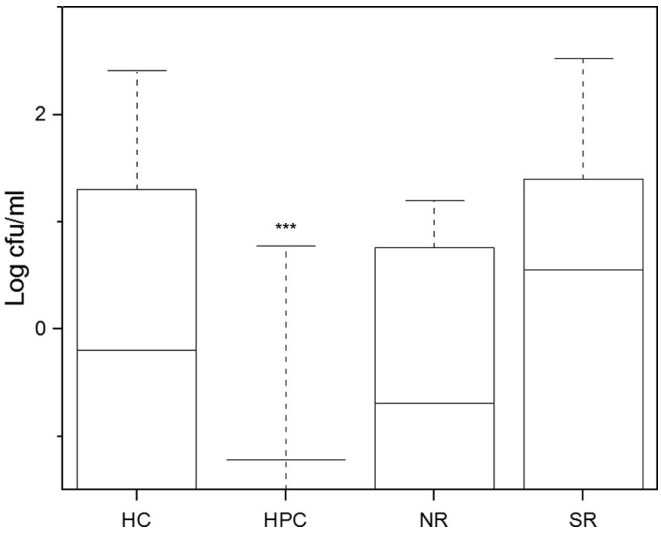

FIG 5 Average yearly concentrations of *V. vulnificus* recovered in water samples at different collection sites. HC, Harlowe Creek; HPC, Hoop Pole Creek; NR, North River; SR, South River. Boxes represent 25th to 75th percentiles; whiskers represent the maximum; and solid horizontal lines within boxes represent the means. HPC had numerous points below the limit of detection; therefore, the box containing the 25th to 75th percentiles is a single line at −1.5 log. Asterisks represent means significantly different from the others (***, $P < 0.001$).

TABLE 3 Dates, salinities, and water temperatures of samples from which C-type *Vibrio vulnificus* strains were isolated

Source of C-type *V. vulnificus*	Date collected (mo/day/yr)	Site[a]	Salinity (‰)	Water temp (°C)	% C-type strains[b]
Oysters	7/29/13	SR	21	29	20
	8/7/14	HPC	28	27	50
	8/7/14	HC	20	27	25
	9/4/14	HC	15	25	10
	10/2/14	HC	27	21	100
Water	4/17/14	HC	21	20	25
	6/5/14	NR	36	24	20
	8/7/14	HPC	28	27	100
	8/14/14	SR	19	27	33
	9/4/14	HC	15	26	25

[a] SR, South River; HPC, Hoop Pole Creek; HC, Harlowe Creek; NR, North River.
[b] Percentage of all confirmed *V. vulnificus* strains in each sample that were identified as C-type.

in both water and oyster samples in which there were no detectable *V. parahaemolyticus* bacteria when water temperatures were relatively warm. This suggests that there are yet unrevealed or possibly stochastic factors that contribute to the frequency and distribution of *V. parahaemolyticus* bacteria.

The maxima of the bacterial concentrations encountered in this study were compared to those in another, similarly performed study that observed *Vibrio* counts for a year in shellfish and oysters, with sites that included the Gulf Coast (51). The maximum *V. vulnificus* concentrations recovered from water and oysters in that study were, respectively, 332 CFU/ml and 25,000 CFU/g (51). In this study, the maximum *V. vulnificus* concentrations were found to be 191 CFU/ml in water and 5,740 CFU/g in oysters, or nearly one-half of the concentrations in water from the Gulf Coast study, and one-fifth of the concentrations in oyster meats. For *V. parahaemolyticus*, the same study found concentrations of 204 CFU/ml in water and 22,000 CFU/g in oyster tissues, while this study of North Carolina water samples had a maximum of 88 CFU/ml and oysters contained a maximum of 2,479 CFU/g (51). These lower concentrations of vibrios in water and oysters in North Carolina could partially explain the relatively low number of vibrio-related infections in North Carolina.

It should be noted that the U.S. Food and Drug Administration administers the *Bacteriological Analytical Manual* (BAM), wherein the agency's preferred media for isolating *V. vulnificus* are given as modified cellobiose-polymyxin-colistin (mCPC) and cellobiose-colistin (CC) (52–54). While the newer medium CPC+ is not preferred, the FDA found no difference in efficacy (55). In this study, the CHROMagar *Vibrio* medium was used. The use of the CHROMagar *Vibrio* medium for the isolation of *V. vulnificus* has been compared with that of CPC+ previously, and the former has been found to yield fewer false-positive results, making environmental analysis easier (56). For *V. parahaemolyticus*, the CHROMagar *Vibrio* medium has been shown to produce the best results of culture-based isolation methods (57).

The matrix tools presented here for *V. vulnificus* in water and oysters are potentially useful for North Carolina commercial or recreational shellfishermen, water quality managers, and consumers (especially those at risk for *V. vulnificus* infections), informing decisions about the consumption of raw or undercooked oysters harvested from particular sites on particular days. The aim has been to use environmental measurements that are easy to achieve, using simple, cost-effective tools such as thermometers and refractometers. These results indicate interesting patterns, particularly in the strength (or lack thereof) of the relationships between important potential *Vibrio* pathogens and salinity. Continued data collection over the next few years will result in the capture of a wider range of environmental conditions over which to examine relationships and will improve our understanding of the combined effects of salinity and temperature on vibrios. Further data collection will also permit the examination and continued comparison of the density maxima of the two pathogenic *Vibrio* spp. examined here and their Gulf Coast counterparts. The findings of this study highlight apparent regional differences and indicate the need for *Vibrio*-related shellfish-harvesting regulations to be tailored to the state or region in which the oysters are actually harvested, rather than using a "one-size-fits-all" approach.

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