Title: Differences in total *Vibrio* spp., *V. vulnificus*, and *V. parahaemolyticus* abundance between clams and oysters in North Carolina

Running Title: Vibrio in clams and oysters

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Abstract

Filter feeding shellfish can concentrate pathogenic bacteria, including *Vibrio vulnificus* and *V. parahaemolyticus*, as much as 1000 fold from the overlying water. These shellfish, especially clams and oysters, are often consumed raw, providing a route of entry for a concentrated dose of pathogenic bacteria into the human body. Food-borne infections from these microbes are on the rise, and a better understanding of the conditions that might trigger elevated seafood concentrations of these bacteria are needed. Relatedly, if the bacterial concentrations in water are correlated to those in shellfish, this would simplify sampling regimes, as water samples are more rapidly and easily obtained. After sampling oysters and clams, either simultaneously or separately, for over two years, it was concluded that while *Vibrio* concentrations in oysters and water are related, this is not the case with clams and water. When clams and oysters were collected simultaneously from the same site, the clams were found to have fewer *Vibrio* than oysters. Further, the environmental parameters that correlated with *Vibrio* spp. in oysters and water were found to be quite different than those that correlated with *Vibrio* spp. in clams.

Importance

This study shows that clams are a potential source of infection in North Carolina, especially for *V. parahaemolyticus*. These findings also highlight the need for clam-specific environmental research to develop accurate *Vibrio* abundance models and to broaden ecological understanding of clam/*Vibrio* interactions. This is especially relevant as food-borne *Vibrio* infections from clams are being reported.

Introduction
An estimated 84,000 people contract foodborne Vibrio infections each year in the US, resulting in 500 hospitalizations and 100 deaths (1, 2). Unlike most other major food-borne bacterial pathogens, the number of cases caused by Vibrio spp. are increasing and are currently the highest since national reporting began (2, 3). While there are at least 12 Vibrio spp. that are potentially pathogenic to humans, the two foodborne Vibrio spp. that cause the most infections and the most deaths in the United States are V. parahaemolyticus and V. vulnificus, respectively (2, 4).

V. vulnificus is the single most fatal foodborne pathogen in the United States, and perhaps worldwide (4), accounting for 95% of all US seafood related deaths with a fatality rate approaching 50% (5). Infections resulting from ingestion typically exhibit symptoms including fever, chills, nausea, abdominal pain, hypotension, and the development of secondary lesions on the extremities (5, 6). V. parahaemolyticus infections are far more common, but less severe and generally self-limiting. Infection with this species produces a variety of outcomes, of which gastroenteritis represents ca. 60-80%. Symptoms include diarrhea with cramping, nausea and vomiting, headache, chills, and low-grade fever (7, 8).

Both of these bacterial pathogens occur naturally in estuarine waters worldwide. Molluscan shellfish, such as oysters and clams, concentrate the cells from the surrounding water and efficient filter-feeding ability can lead to levels of $10^5$ CFU/gram of tissue, or more (9, 10). Because these concentrations are up to 100x that of the water column, this makes the consumption of raw and undercooked shellfish the primary route for contracting food-borne Vibrio infections (9, 11).

While there are protective regulations in place in the US to limit the risk of infection from these pathogens, vibriosis is yet increasing (3, 12). Infections follow a seasonal trend, and the
numbers peak in the months of late spring and early summer, corresponding with warm waters and an increased abundance of vibrios (e.g. 10, 13–16). Yet, temperature only partially explains the variation in concentrations seen in shellfish (e.g. 10, 15, 17, 18). To reduce the number of infections, there needs to be a broader understanding of the environmental conditions that contribute to pathogenic \textit{Vibrio} abundance in shellfish. Moreover, while there is a growing oyster/environment/\textit{Vibrio} dataset being produced by the scientific community, there are only a handful of investigations on the occurrence of these pathogens in clams. This is especially relevant as food-borne \textit{Vibrio} infections from clams are being reported (19–21).

In this work, oysters and clams were collected from various sites in North Carolina, with several locations being habitats for both oysters and clams. It was hypothesized that because both oysters and clams are filter feeders, the concentrations of \textit{Vibrio} spp. contained within the shellfish would correlate with the concentrations in the water in which the oysters were harvested. Additionally, it was hypothesized that when oysters and clams were harvested from the same site, they would have correlating \textit{Vibrio} abundances. Finally, it was hypothesized that the environmental factors that influence \textit{Vibrio} spp. loads in oysters and clams would be similar.

Thus, our investigations were designed to characterize the presence and levels of \textit{V. vulnificus} and \textit{V. parahaemolyticus} in water, oysters, and clams, and to attempt to correlate these levels with several environmental parameters.

\textbf{Materials and Methods}

\textbf{Sampling Sites}

Oysters (\textit{Crassostrea virginica}), clams (\textit{Mercenaria mercenaria}), and water samples were collected from nine sites along the eastern North Carolina coast (Figure 1). At Calico Creek,
Turnagain Bay, and South River, only oysters were harvested. Only clams were collected at Jumping Run Creek, Bogue Sound, and Spooner Creek. Oysters and clams were collected simultaneously from Hoop Pole Creek, Harlowe Creek, and North River. Water samples were collected from all sites at each sampling event. Sites were chosen to represent a range of salinity minima and maxima, salinity fluctuations, and suitability for growth for either oysters and/or clams.

Shellfish collection and processing

Oyster samples were collected from February 4, 2013 to November 11, 2015. Clams were collected from September 25, 2013 to October 16, 2015. This resulted in 112 sampling events for oysters, 36 for clams, and 125 for water. At each sampling event, five oysters, five clams, or five of both oysters and clams were collected and transported on top of ice to the laboratory and processed within 5 hours. Shellfish were aseptically shucked and the hemolymph drained. Meats were pooled, weighed, and diluted with sterile phosphate buffered saline (PBS) at a 1:1 w:v ratio. Shellfish meats were blended in a paddle blender (Fisher Scientific, Waltham, MA) for 10m at 280 rpm. Homogenates were diluted 1:10 in PBS and 100 µl of both the diluted and undiluted homogenate were spread plated onto media as described below.

Water collection and processing

At each site and sampling event, water was collected simultaneously with shellfish. Water was collected in sterile 1 liter bottles that were rinsed 3x with water immediately above the shellfish. As with shellfish, water samples were placed on top of ice in coolers that were transported to the lab. An HI 96822 digital refractometer (Hanna Instruments, Carrollton, TX) was used to measure salinity. Water samples of 1 to 10 ml were passed through a 0.45 µm pore
size mixed cellulose ester filter (Pall, Port Washington, NY) and placed onto media as described below. To determine total suspended solids (TSS), water samples were also vacuum filtered through a pre-dried, pre-weighed 25mm glass microfiber filter (GE Life Sciences, Pittsburgh, PA), with a minimum of 100ml of water or until the filter was visibly discolored. Filters were placed in a drying oven until water was evaporated, and reweighed.

Measuring environmental parameters

Water temperature and depth of shellfish was measured by hand at the time of each collection. Dissolved oxygen (DO) was measured using an Orion 5 Star handheld probe (Thermo Scientific, Waltham, MA). Measurements of pH were taken with a Denver Instrument (Bohemia, NY) UB-5 pH meter. Data on wind velocity or gusts, precipitation, air temperature, or heating and cooling degree-days were collected from individual weather stations near each sampling site. A list of each weather station is included in Table S1. Heating and cooling degree-days are determined relative to a base temperature of 18°C. Heating degree-days for a particular day were calculated by determining the mean of the maximum and minimum temperature for that day and subtracting that from the base temperature. Cooling days were calculated by subtracting the base temperature from the mean of the daily temperature. Then, the calculated degree-days for individual days were summed for a particular length of time, in this report, 30 days, to determine a cumulative value of degree-days that represent how far and for how long daily temperature was above or below a base temperature.

Media and growth conditions

For total presumed total *Vibrio* (VIB) enumeration, thiosulfate-citrate-bile salts-sucrose (TCBS) agar was used, prepared according to the manufacturer’s instructions (Becton,
Dickinson, and company, [BD], Franklin Lakes, NJ). Green and yellow colonies were counted and summed to get total *Vibrio* abundance. CHROMagar *Vibrio* plates (CHROMagar, Paris, France), prepared as instructed, was used to isolate presumptive *V. vulnificus* (dark blue) and *V. parahaemolyticus* (dark purple) colonies. To grow pure cultures of each isolate, heart infusion (HI) agar plates were used (BD, Franklin Lakes, NJ). All media were incubated at 37°C for 24 h. After incubation, colonies on plates were counted, and the data converted to CFU/g of shellfish or CFU/ml of water. This number was then multiplied by the ratio of isolates that were confirmed, using the molecular methods detailed below, to be either *V. parahaemolyticus* or *V. vulnificus*. This resulted in a presumptive value of confirmed bacterial abundance for each sample.

**Molecular confirmation of isolates**

Between 5 and 25 colonies (or all colonies if fewer than 5 were present) from each type of sample (water/shellfish) were isolated from each sampling event. Isolates were grown in pure culture in HI broth overnight and then boiled for 10 min. Centrifugation at 10,000 x g for 10 min separated the aqueous DNA from cellular material. Supernatants, to be used for PCR templates, were stored at -20°C until examined. Using primers described by Tarr et al. (22), *V. parahaemolyticus* was identified using the *flaE* gene. Confirmation of *V. vulnificus* utilized the *vvhA* gene with primers detailed by Warner and Oliver (23).

**Statistics**

All data were combined, disregarding harvest site, and Spearman correlations were determined for all factors. To simplify analysis, the correlation tables were converted into correlation matrices using the igraph package for R (24, 25). Only correlations with an absolute
correlation coefficient of at least .25 were linked with edges in the figures. Vertices were arranged using the Fruchterman-Reingold method to ensure that related vertices were clustered together (26). The correlation coefficients and p-values can be found in Tables S2-S4.

Results

Comparing clams and oysters harvested from the same site

The concentrations of VIB, *V. parahaemolyticus* (VP), and *V. vulnificus* (VV) in clams were significantly lower than those in oysters when the shellfish were harvested simultaneously from the same site (p=0.0002, p=0.0336, and p=0.0093, respectively, paired t-test). The combined results are shown in Figure 2. Each paired sampling event was also examined individually. For VIB, all but three samples were lower in clams than in oysters (Figure S1). For *V. parahaemolyticus* there were generally two patterns observed (Figure S2). Pattern 1 was that oysters contained *V. parahaemolyticus* while clams were non-detectable. The second pattern is that if both types of shellfish contained *V. parahaemolyticus* at the same sampling event, the concentrations in the clams were usually slightly higher than that of the oysters. There was only one paired sampling event in which *V. parahaemolyticus* was present in clams but not in oysters. All but three clam samples collected from the same sites as oysters had non-detectable concentrations of *V. vulnificus* (Figure S3) and only at one of these samples did a clam sample ever have more *V. vulnificus* than oysters.

Harvest season and vibrio risk

Oyster season in North Carolina is from October 15th through March 31st. Figure 3A and Figure 3B respectively show the concentrations of *V. vulnificus* and *V. parahaemolyticus* in oysters harvested both in and out of oyster season (there is no set clam season in NC).
3C and 3D show the concentrations of *V. vulnificus* and *V. parahaemolyticus* in clams. For both *V. vulnificus* and *V. parahaemolyticus* the US Food and Drug Administration (FDA) has determined that *Vibrio* concentrations less than 30/g are sufficient for post-harvest processed shellfish, or for shellfish which are claimed to have “non-detectable levels” (27).

Environmental and biological factors correlating with *Vibrio* spp. abundance

VIB in oysters were correlated with the *Vibrio* abundance in water, however, this was not true for clam VIB (Figure 4). Oyster VIB and water VIB shared all the same environmental correlations with the exception of three day antecedent rainfall, with water temperature and (TSS) having some of the highest coefficients. This similarity in response to environmental conditions is is visualized in figure 4 by a the temperature/TSS/Oyster/Water vertices clustering together. The concentration of VIB in clams and oysters was significantly correlated. Water temperature was less strong for determining clam VIB than it was for oysters or water. Figure S4 shows that while water temperature does appear to influence clam VIB it has more variation than water or oysters. Nevertheless, heating degree days, which are a measure of cumulative cold days, had a tighter negative correlation with clam VIB than the instantaneous water temperature (Figure 4). Clam VIB were apparently affected by salinity, a factor not connected with oysters or water VIB in thus study. Further evidence of this difference is shown in Figure S5, with increases in clam VIB seen above 25‰ salinity.

For *V. vulnificus*, once again oysters and water shared nearly all correlating factors, with water depth having a small but significant correlation with *V. vulnificus* in oysters (Figure 5). These factors included temperature and TSS, similar to the VIB cluster. Salinity was negatively correlated with *V. vulnificus* concentrations in water and oysters, but not in clams. In both water and oysters, *V. vulnificus* increased up to ca. 17‰ salinity, and then decreased above that, while
clam concentration altered very little along the salinity gradient (Figure S6). DO and pH were correlated with clam *V. vulnificus* abundance but not with water or oysters (Figure 5).

Once again, the clam *V. parahaemolyticus* vertex did not cluster with oysters and water (Figure 6). As with *V. vulnificus*, temperature and TSS were the most critical correlating environmental factors for oysters and water. Water temperature and wind weakly correlated with *V. parahaemolyticus* in clams.

**Discussion**

There are several approaches to reducing human infections caused by the seafood-borne bacteria *V. vulnificus* and *V. parahaemolyticus*. One approach is the development of easy-to-use predictive models that can provide shellfish consumers or producers an early warning of when shellfish harvested from a particular site might contain dangerous concentrations of either bacterium. To develop these models, an understanding of the biological and ecological parameters that influence bacterial abundance in shellfish is required. To that end, there have been numerous recent studies that compare environmental conditions to *Vibrio* concentrations in oysters. A recent review on the topic presents the data from these reports and also highlights the site to site differences observed in these types of studies (12). Infections from these bacteria are most commonly acquired by eating raw oysters, but a significant number of infections are also caused by the consumption of raw or undercooked clams (19–21). There are strikingly few reports on environmental levels of these pathogens in the hard clam (*Mercenaria mercenaria*), and such data are needed to begin developing predictive models. Initially, one might assume, as they are both filter-feeding shellfish, that clams and oysters would have correlated, if not similar, concentrations of *Vibrio* spp., especially when these shellfish are harvested from the same site and growing area. After collecting samples from three sites in eastern NC, where clams and
oysters could be simultaneously harvested, we found that clams had significantly fewer *V.
parahaemolyticus*, *V. vulnificus*, and VIB. than oysters. A study by Jones et al. (28), conducted
in Long Island Sound, also found that these human pathogens, as well as *V. cholerae*, were lower
in clams than in oysters.

Lower concentrations of these pathogens in clams is welcome news, as this suggests clams
are less likely to result in human infections. North Carolina’s oyster season runs from October
15th to March 31st. The timing of the oyster season was put in place at a time when, historically,
oysters were eaten mostly in the winter months to avoid the spawning period during the summer
months. The reason the timing of the oyster season exists today, is due to the scarcity of the
oysters (Shannon Jenkins, NCDMF, pers comm). Still, this timing likely has a strong effect on
keeping the number of oyster-based *Vibrio* infections low. For *V. vulnificus*, the out-of-season
period corresponds to the period when the majority of NC oysters that are above 30 cfu per gram
are found. For *V. vulnificus* and *V. parahaemolyticus*, 30 cfu per gram of oyster is a value that
the FDA considers to be below the limit of detection, and therefore safe (27). Note that
concentrations above 30/g are not labeled “unsafe”, but do carry increased risk of infection. In
this study, only seven “in season” oyster samples were above this limit for *V. vulnificus*, found in
February and October/November. *V. parahaemolyticus* concentrations above 30/g occurred “in
season” only in October and November, again showing the effectiveness of the oyster season
regulations for reducing potential infections. It is important to note that private culture oyster
harvests are permitted in the summer “off season”. These shellfish pose a potential infection
risk, and research is currently underway to understand the risk differences in farmed vs. wild
shellfish.
While the *Vibrio* spp. concentrations were found to be lower in clams, there is no clam season in NC, as clams are more abundant than oysters. While there is no FDA limit for *Vibrio* in clams, if the same 30/g value that is used as “non-detectable” in oysters is applied, the potential for infection appears greater. Interestingly, there were only five clam samples in this study that contained detectable *V. vulnificus*, and one of these was below the 30/g threshold. Those above the threshold were found from mid-June through mid-October. The reason for the low number of *V. vulnificus* isolates recovered in clams could partially be due to the relatively higher (>20‰) salinity of clam growing sites, considering that *V. vulnificus* is sensitive to high salinity. Yet, when examining the clam and oyster samples that were collected simultaneously from the same sites, it is evident that oysters more often contain these pathogens. Thus, the higher salinity is certainly not the sole cause, and clam biology must play a role. Nevertheless, these data suggest that *V. vulnificus* infection arising from clam consumption will be rare in NC. The *V. parahaemolyticus* data tell a different story. Many of the clam samples from mid-March to mid-October had concentrations well above 30/g, and these are a potential health risk. Unsurprisingly, water temperature, and temperature related factors such as heating and cooling days, had strong significant influence on VIB in oysters and water, but less so for clams. For both oysters and water, VIB increased as temperature increased, though this increase slowed at ca. 22°C. In clams, VIB did increase with temperature but the data were highly varied, making the relationship less obvious. Interestingly, salinity was positively correlated with VIB in clams, but not in water or oysters. This is striking as a previous study, in a different NC body of water, did show a correlation between salinity and VIB in water as well (29). Furthermore, salinity is one of the variables that is most often correlated with VIB in water in other studies (30–35) The concentrations of VIB in oysters along the salinity gradient remained mostly flat,
with salt-tolerant *Vibrio* spp. and other bacteria likely occupying oyster matrices vacated by less
salt tolerant species like *V. vulnificus* (36, 37). In clams, VIB began increasing at ca. 25‰
salinity, but currently we have no explanation for this observation. The temperature factor that
had a tighter relationship with clam VIB was heating days, rather than water temperature at time
of collection. Heating degree-days are a measure of how long (in days) the air temperature was
below a base temperature; cooling degree days are the opposite. These are a quickly calculated,
and simple, measurement of cumulative hot or cold days. The data in this study provide
evidence that lengthy strings of cold days are more important (negative correlation) in
determining clam VIB than instantaneous water temperature.

Like VIB, clam *V. vulnificus* clustered more distantly than oysters and water in the
Fruchterman-Reingold layout of the correlation network map, indicating it is less related than the
others. Water temperature and TSS were positively correlated with oyster/water *V. vulnificus*,
while salinity was negatively correlated. For both oysters and water, the number of *V. vulnificus*
appeared to increase until ca. 17‰ salinity, and then began to decline, as is commonly reported
(10, 36–39). Clam concentrations remained mostly flat even as salinity increased. Once again,
water temperature did not appear to have a significant effect on clam *V. vulnificus* concentrations
in this study. Also surprisingly, salinity was not related to clam bacterial abundances. These
conclusions must be considered carefully, however, as there were few clam samples with
detectable *V. vulnificus*.

There were fewer *V. parahaemolyticus* in clams than in oysters, but the number of clam
samples with confirmed *V. parahaemolyticus* isolates vastly outnumbered clam samples with
confirmed *V. vulnificus*. Once again, temperature was not as related to bacterial concentrations
in clams as it was for water and oysters. Again, TSS was related to oysters/water but not clam *V.
289 *parahaemolyticus* abundance. Finally, wind related factors were unique in relating to clam
290 abundances, suggesting perhaps that wind-driven mixing or resuspension plays a larger role in
291 clam uptake of *Vibrio* than in oysters.

292 This study confirms and expands on an earlier finding by Jones et al. (28) that clams and
293 oysters harvested simultaneously from the same location have differing, and often uncorrelated,
294 concentrations of *Vibrio* spp., including human pathogens. VIB, *V. vulnificus*, and *V.
295 parahaemolyticus* concentrations in oysters and water are significantly correlated and share most
296 of the same environmental determinates as well. Clam *Vibrio* concentrations, on the other hand,
297 are either unrelated or only weakly related to water column *Vibrio* numbers. Furthermore, clam
298 *Vibrio* always had influential environmental parameters that were unique, not shared by oysters
299 or water. Similarly, oysters and water have common correlates that clams did not have. This
300 appears to indicate that oysters contain a high number of transient *Vibrio*, which is why they are
301 correlated with the *Vibrio* in the surrounding water. Clams, however, with weaker correlations
302 with water *Vibrio*, might have a more stable population of *Vibrio*. This stable population is
303 further evidenced by the resistance to change from daily water temperature. With so many
304 unique and unshared environmental variables contributing to either oyster or clam bacterial
305 concentrations, we hypothesize that clam biology plays a larger factor in internal *Vibrio
306 abundance than originally thought. More research is clearly needed, specifically regarding
307 clam/*Vibrio* uptake and depuration, to better understand this interesting relationship.

308 **Acknowledgements**

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**Table and Figure Captions**

Figure 1: Locations of shellfish harvest sites. Color of site name indicates type of shellfish harvested. Yellow: clams, blue: oysters, green: both clams and oysters. (The main map is reprinted from Google Earth; the inset map is reprinted from reference 38.)

Figure 2: Box plots of total *Vibrio* spp. (VIB), *V. parahaemolyticus* (VP), or *V. vulnificus* (VV), in Oysters (Oys) or Clams. Black line is mean, box represents 25th to 75th percentile, and whiskers are minimum and maximum. Asterisks indicate significant difference in means.

Figure 3: Concentration of *V. vulnificus* in oysters (A) or clams (C) and concentration of *V. parahaemolyticus* in oysters (B) or clams (D) by time of year collected. Horizontal lines in A and B mark the dates of opening (Oct. 15th) and closing (March 31st) of oyster season in North Carolina. Vertical line indicates concentration of bacteria that the FDA considers “non-detectable” in oysters. Gray boxes represent times of the year when the shellfish are in season and there are *Vibrio* concentrations greater than the FDA “non-detectable level”. Actual limit of detection was 1 log CFU/g. Values below limit of detection were assigned a value of 0 log CFU/g.

Figure 4: Total *Vibrio* correlation network map. Network is based on Spearman’s rank correlation coefficients. Vertices are connected by edges only if the correlation coefficient is greater than 0.25. Edges are colored based on positive (green) or negative (magenta) correlation. Thickness of edges represents correlation strength. Vertices are arranged using the Fruchterman-Reingold method, which clusters similar vertices together. Three day antecedent precipitation
(X3.precip) is summed while three date antecedent wind speed (X3.wind) and wind gusts
(X3.gust) are averaged. The value of heating degree-days (Hot 30) or cooling degree-days (Cool 30) was obtained by summing the degree-days 30 days before (and including) the collection date. Vertices representing total *Vibrio* concentrations in clams (Clam.VIB), oysters (Oys.VIB), and water (Wat.VIB) are colored only for ease of viewing.

Figure 5: *V. vulnificus* correlation network map. Network is based on Spearman’s rank correlation coefficients. Vertices are connected by edges only if the correlation coefficient is greater than 0.25. Edges are colored based on positive (green) or negative (magenta) correlation. Thickness of edges represents correlation strength. Vertices are arranged using the Fruchterman-Reingold method, which clusters similar vertices together. Three day antecedent precipitation
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Figure 6: *V. parahaemolyticus* correlation network map. Network is based on Spearman’s rank correlation coefficients. Vertices are connected by edges only if the correlation coefficient is greater than 0.25. Edges are colored based on positive (green) or negative (magenta) correlation. Thickness of edges represents correlation strength. Vertices are arranged using the Fruchterman-Reingold method, which clusters similar vertices together. Three day antecedent precipitation
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Figure S1: Paired concentrations of total *Vibrio* spp. in oysters and clams from samples harvested simultaneously at the same site.

Figure S2: Paired concentrations of *V. parahaemolyticus* in oysters and clams from samples harvested simultaneously at the same site.

Figure S3: Paired concentrations of *V. vulnificus* in oysters and clams from samples harvested simultaneously at the same site.

Figure S4: Log total *Vibrio* in oysters (blue), clams (magenta), and water (green) compared to water temperature in Celsius at time of sample collection. Data is displayed in CFU/g for shellfish samples and CFU/ml for water. Circles are individual data while lines are smoothed averages.

Figure S5: Log total *Vibrio* in oysters (blue), clams (magenta), and water (green) compared to salinity at time of sample collection. Data is displayed in CFU/g for shellfish samples and CFU/ml for water. Circles are individual data while lines are smoothed averages. Salinity is in parts per thousand.

Figure S6: Log *V. vulnificus* in oysters (blue), clams (magenta), and water (green) compared to salinity at time of sample collection. Data is displayed in CFU/g for shellfish samples and CFU/ml for water. Circles are individual data while lines are smoothed averages. Salinity is in parts per thousand. Vertical black line indicates 17 ppt salinity.
Table S1: Weather stations used for meteorological data for each site and sampling date. HPC = Hoop Pole Creek, HC = Harlow Creek, TB = Turnagain Bay, SR = South River, CC = Calico Creek, NR = North River, JNR = Jumping Run Creek, SC = Spooners Creek, BS = Bogue Sound

Table S2: Spearman’s rank correlation matrix for total *Vibrio* spp. with significance and correlation coefficients.

Table S3: Spearman’s rank correlation matrix for total *V. vulnificus* with significance and correlation coefficients.

Table S4: Spearman’s rank correlation matrix for total *V. parahaemolyticus* with significance and correlation coefficients.

Literature Cited


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Figure 3: Concentration of *V. vulnificus* in oysters (A) or clams (C) and concentration of *V. parahaemolyticus* in oysters (B) or clams (D) by time of year collected. Horizontal lines in A and B mark the dates of opening (Oct. 15th) and closing (March 31st) of oyster season in North Carolina. Vertical line indicates concentration of bacteria that the FDA considers “non-detectable” in oysters. Gray boxes represent times of the year when the shellfish are in season and there are *Vibrio* concentrations greater than the FDA “non-detectable level”. Actual limit of detection was 1 log CFU/g. Values below limit of detection were assigned a value of 0 log CFU/g.
Figure 4: Total Vibrio correlation network map. Network is based on Spearman’s rank correlation coefficients. Vertices are connected by edges only if the correlation coefficient is greater than 0.25. Edges are colored based on positive (green) or negative (magenta) correlation. Thickness of edges represents correlation strength. Vertices are arranged using the Fruchterman-Reingold method, which clusters similar vertices together. Three day antecedent precipitation (X3.precip) is summed while three date antecedent wind speed (X3.wind) and wind gusts (X3.gust) are averaged. The value of heating degree-days (Hot 30) or cooling degree-days (Cool 30) was obtained by summing the degree-days 30 days before (and including) the collection date. Vertices representing total Vibrio concentrations in clams (Clam.VIB), oysters (Oys.VIB), and water (Wat.VIB) are colored only for ease of viewing.
Figure 5: V. vulnificus correlation network. The network is based on Spearman's rank correlation coefficients. Vertices are connected by edges if the correlation coefficient is greater than 0.25. Edges are colored based on positive (green) or negative (magenta) correlation strength. Vertices are arranged using the Fruchterman-Reingold method, which clusters similar vertices together. Three day antecedent precipitation (X3.precip) is summed while three day antecedent wind speed (X3.wind) and wind gusts (X3.gust) are averaged. The value of heating degree-days (HDD) or cooling degree-days (CDD) was obtained by summing the degree-days 30 days before and including the collection date. Vertices representing total Vibrio concentrations in clams (Clam.VIB), oysters (Oys.VIB), and water (Wat.VIB) are colored only for ease of viewing.
Figure 6: *V. parahaemolyticus* correlation network map. Network is based on Spearman’s rank correlation coefficients. Vertices are connected by edges only if the correlation coefficient is greater than 0.25. Edges are colored based on positive (green) or negative (magenta) correlation. Thickness of edges represents correlation strength. Vertices are arranged using the Fruchterman-Reingold method, which clusters similar vertices together. Three day antecedent precipitation (X3.precip) is summed while three date antecedent wind speed (X3.wind) and wind gusts (X3.gust) are averaged. The value of heating degree-days (Hot 30) or cooling degree-days (Cool 30) was obtained by summing the degree-days 30 days before (and including) the collection date. Vertices representing total *Vibrio* concentrations in clams (Clam.VIB), oysters (Oys.VIB), and water (Wat.VIB) are colored only for ease of viewing.