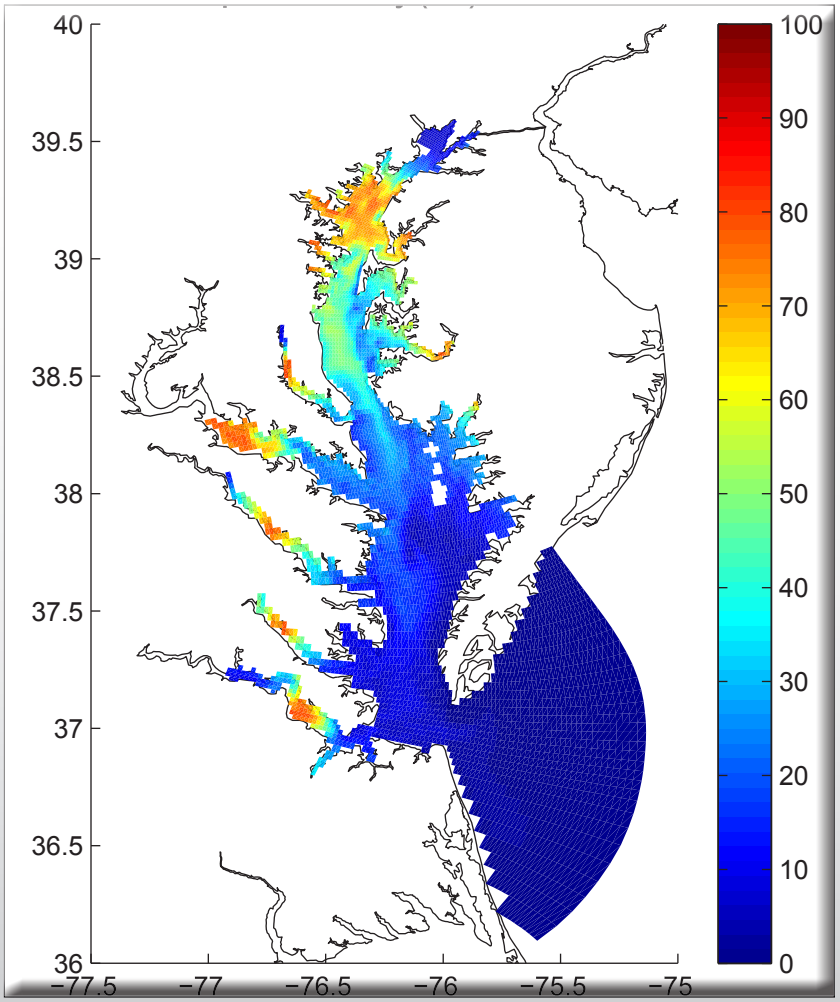
Predicting the Distribution of *Vibrio vulnificus* in Chesapeake Bay



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Abstract

Vibrio vulnificus is a gram-negative pathogenic bacterium endemic to coastal waters worldwide, and a leading cause of seafood related mortality. Because of human health concerns, understanding the ecology of the species and potentially predicting its distribution is of great importance. We evaluated and applied a previously published qPCR assay to water samples (n = 235) collected from the mainstem of the Chesapeake Bay (2007 – 2008) by Maryland and Virginia State water quality monitoring programs. Results confirmed strong relationships between the likelihood of *Vibrio vulnificus* presence and both temperature and salinity that were used to develop a logistic regression model. The habitat model demonstrated a high degree of concordance (93%), and robustness as subsequent bootstrapping (n=1000) did not change model output ($P > 0.05$). We forced this empirical habitat model with temperature and salinity predictions generated by a regional hydrodynamic modeling system to demonstrate its utility in future pathogen forecasting efforts in the Chesapeake Bay.

1. Introduction

Vibrio vulnificus is a Gram-negative, halophilic bacterium endemic to estuarine waters worldwide. *Vibrio* spp. can constitute a large proportion of γ-Proteobacteria in estuarine waters (15-80%), with *V. vulnificus* comprising up to 18% of the total vibrios (Heidelberg et al., 2002b). While functioning as a free-living or particle attached contributor to the heterotrophic microbial community, certain strains are also serious human pathogens. *V. vulnificus* is responsible for 95% of all seafood related deaths in the United States (Oliver and Kaper, 2001). Primary septicemia associated with seafood consumption and wound infections are the most common type of *V. vulnificus* infection in humans, with gastroenteritis occurring relatively infrequently (Strom and Paranjpye, 2000). Cases which become septic have as high as a 50% mortality rate (Rippey, 1994; Oliver and Kaper, 2001). Previous reports by the Centers for Disease Control and Prevention estimated *V. vulnificus* infections annually at 97 total cases in the United States, with 48 associated deaths (Mead et al., 2000). Thus, it is extremely important to understand the ecology, abundance, and distribution of *V. vulnificus* in the interest of human health.

Several efforts have attempted to examine correlations of abundance or presence of *V. vulnificus* with environmental factors (O'Neill et al., 1992; Wright et al., 1996; Lipp et al., 2001; Heidelberg et al., 2002b; Pfeffer et al., 2003; Randa et al., 2004). While various parameters have been reported as being corollated to Vibrio abundance, water temperature, and to a lesser extent salinity, are the only consistently identified variables. In general, growth and abundance is positively correlated with water temperature when greater than 15° C. Salinity may also govern abundance, but the relationship is not as clear. Several researchers have reported estuarine waters of 10-15 ppt to be the preferred salinity (Lipp et al., 2001; Randa et al., 2004). However, they may be cultured from samples of higher salinity waters as well (Tamplin et al., 1982; Oliver et al., 1983), although there have been contradictory results regarding survival and growth of *V. vulnificus* in waters with greater salinity (Randa et al., 2004). The inconsistencies seen in the response of *V. vulnificus* to salinity gradients may result from regional and strain differences between these various studies.

A variety of methods have been applied for the detection and identification of Vibrio in seafood or environmental matrices (Tamplin et al., 1982; Oliver et al., 1983; Panicker et al., 2004; Thompson et al., 2004; Panicker and Bej, 2005; Wilkes et al., 2005). Most of these studies have relied on culturing Vibrio from environmental samples on selective media, followed by enumeration. Quantitative PCR (qPCR) has recently gained favor due to its ability to rapidly quantify target organisms in environmental samples. The *V. vulnificus* hemolysin A gene (vvhA) is considered a reliable species-specific target for this purpose and has been evaluated against a large number of isolates and other species (Panicker and Bej, 2005). In addition, qPCR is capable of detecting bacteria in the viable but non culturable state, a particular concern with *V. vulnificus* that has been shown to be able to regain both culturable and pathogenic status under favorable environmental conditions (Oliver, 2005).

Increasingly, efforts are underway to develop and apply empirical habitat models of organisms in support of environmental forecasting (Decker et al., 2007; Jacobs et al., 2009; Constantin de Magny et al., In Press). In the Chesapeake Bay, the Chesapeake Bay Ecological Prediction System (CBEPS) is being developed and implemented by scientists at the National Oceanic and Atmospheric Administration (NOAA), the University of Maryland system, the Chesapeake Research Consortium, and the Maryland Department of Natural Resources (MD DNR). The CBEPS generates Bay-wide nowcasts and three-day forecasts of several environmental variables, including temperature and salinity (Brown et al., 2002). In simulation, these environmental variables can be used to drive empirical habitat mod-

els of target organisms to make first order predictions of their likelihood of occurrence. This general approach has proven to be successful in predicting the likelihood of encountering sea nettles (*Chrysaora quinquecirrha*), a stinging jellyfish (Decker et al., 2007), and *V. cholerae* (Constantin de Magny et al., In Press) in the Bay, and is being implemented for several harmful algal bloom species.

In 2005, researchers at the NOAA Cooperative Oxford Laboratory initiated a Pathogen Monitoring Program in response to NOAA's Ocean's and Human Health Initiative. Samples are collected in partnership with State and Federal water quality monitoring programs to examine the ecology of various human and living resource pathogens throughout the Chesapeake Bay and to develop empirical models for use in ecological forecasting efforts. Here we describe a predictive model, developed for estimating the likelihood of *V. vulnificus* presence in Chesapeake Bay, and demonstrate its application within the CBEPS.

2. Methods

2.1 Sample Collection

Surface water samples (0.5-1m depth) were collected by the Maryland Department of Natural Resources and Virginia Department of Environmental Quality's respective water quality monitoring programs according to Chesapeake Bay Program protocols (USEPA, 1996). Physical parameters are measured at 0.5 meter increments *in-situ* with a YSI dataSonde (YSI Incorporated, Yellow Springs, Ohio, USA). Sterile polypropylene bottles (500ml) were rinsed three times and then filled with surface water from the sample station, and then placed immediately on wet ice. Two replicate bottles were collected at particular stations, as standard protocol of the water quality monitoring programs. All samples were frozen at -20ºC until sampling was completed for the month. Samples used for model development were collected along the mainstem of the Bay during the months of July and October of 2007, and April, July, and October of 2008 (n = 235) (Figure 1).

2.2 DNA Purification

Samples were allowed to thaw, were thoroughly mixed, and 200mL filtered through a 0.22 micron Sterivex filter (Millipore, Billerica, MA). Water was completely removed from each filter by pushing air through it, then the filter housings were wrapped tightly in parafilm, and stored in Whirl-pack (Nasco) bags on dry ice. In heavily turbid areas, water was filtered until no more could pass through the filter and the volume was noted to the nearest milliliter. Sealed filters were then stored in a -80ºC freezer and subsequently extracted using a modified MoBio Soils (MoBio Laboratories, Inc, Carlsbad, CA) protocol previously described and evaluated in detail (Jacobs et al., 2009).

2.3 qPCR and Cycling Parameters *V. vulnificus*

Primers vvh_F (5'-TTCCAACTTCA AACCGAACTATGA-3') and vvh_R (5'-TTCCAGTCGATGC-GAATACGTTG-3') were used in conjunction with the probe vvh874 (5'-/56-FAM/ AACTATCGTGCAC GCTTTGGTACCGT /3BHQ_1/-3') for the detection of *V. vulnificus* (Panicker and Bej, 2005). A unique internal control was incorporated simultaneously into the assay to test for the presence and influence of inhibitors (Nordstrom et al., 2007). qPCR was performed by using 2.50uL of 10X PCR Buffer (Invitrogen, Carlsbad, CA), 1.25uL of 50.00mM MgCl2 (Invitrogen), 0.50uL of 10.00mM dNTP's solution (mixed equal concentration of each) (Roche Diagnostics, Inc, Indianapolis, IN), 1.00 uL of 10.00uM Vvh primer (each), 0.60 uL of 10.00uM Vvh874 probe, 0.19uL of 10.00uM internal control primers (each), 0.38uL of 10uM internal control probe, and 0.40uL of 5U/uL Platinum hot start Taq (Invitrogen) per reaction. DNase/RNase free water was added in quantity sufficient for a 25uL total reaction volume. Two-stage qPCR cycling parameters were optimized to initial denaturation of template at 95ºC

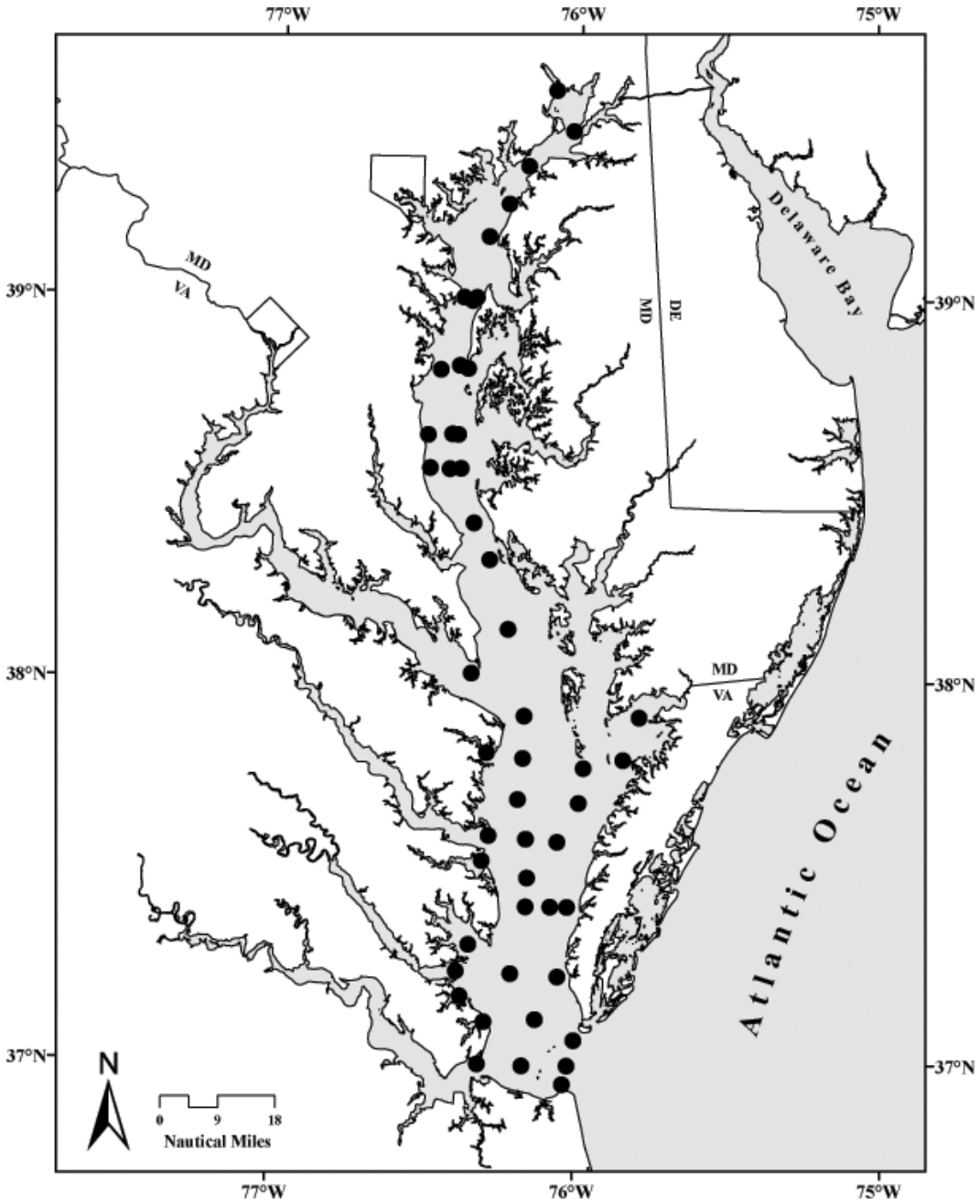

Figure 1. Chesapeake Bay main-stem monitoring stations sampled for *V. vulnificus* abundance in July and October of 2007, and April, July, and October of 2008 (n = 235). Surface water samples were collected by Maryland and Virginia State water quality monitoring programs.

for 60seconds, followed by 50 cycles of denaturation at 95ºC for 5seconds and combined annealing and extension at 59ºC for 45 seconds. On occasion, amplification products were run on a 1.5% agarose gel at 84 volts for 1 hour 45 minutes to ensure proper sized products were being amplified by comparison to a known molecular weight marker.

2.4 Preparation of Standard Curves

Cell suspensions were made from pure cultures taken in active growth phase in alkaline-phosphate water and 200uL from each suspension was plated on tryptone-salt agar (T1N3) to determine cell count. Starting with 2x107 cells/mL, 1:10 dilutions were made down to 2 cells/mL. Two filters were processed for each dilution by spiking 200mL of water with 1mL of the vibrio dilution and all filters were processed as described above. Water used for the standard curve was tested for the presence

of *V. vulnificus* prior to being used to ensure no target bacteria were present using the previously described qPCR assay. Extracted DNA for all dilutions was then run according to the above qPCR parameters and the cycle threshold (Ct) value was plotted against the number of total cells in extraction to determine standard curve.

2.5 Evaluation of Assay Performance

Assay performance testing was carried out as described previously (Jacobs et al., 2009). The assay was evaluated for bottle-to-bottle replication, with-in sample repeatability and assay efficiency (calculated from multiple standard curves using the formula E=-1+10(-1/slope)) (Pfaffl, 2001). In addition, extraction efficiency and the effects of freezing water samples for transportation were evaluated with *Vibrio spp.* Seawater was collected and screened prior to use to ensure no background contamination. Live cells were added to replicate 500mL sterile Nalgene bottles over a 6-log scale. One bottle of each replicate was immediately extracted as described above, while the second replicate was frozen at -20ºC for one week. Bottles were subsequently pulled from the freezer, thawed and extracted in the same manner as all other samples.

2.6 Statistical Analysis

Of the potentially important factors involved in determining the presence of *V. vulnificus,* the CBEPS presently only generates temperature and salinity, so only these two variables were evaluated for use in the development of an empirical habitat model. Logistic regression was employed using temperature, salinity, and interaction terms (SAS Inc, Cary, NC). A better model fit was found for salinity by using optimal salinity rather than the actual value (SALopt). Optimal salinity was determined by binning salinity to the nearest 1 ppt and subsequent examination of the frequency distribution of *V. vulnificus* occurrence. Because prevalence data was not normally distributed (Kolmogorov-smirnov, $D = 0.187$, $p < 0.01$), median salinity was used as SALopt and was determined to be 11.5 ppt (mean = 11.78 ppt). SALopt was calculated as the absolute value of (salinity – 11.5). Final model selection was based on Akaike's Information Criterion (AIC) and concordance. The model was then bootstrapped (n=1000) by randomly sampling with replacement of a subset of data from the entire dataset. Mean parameter estimates and variance were then calculated for the population of bootstrap results. To evaluate the effect of bootstrapping on the model results, probability of occurrence was calculated using the original and bootstrapped model (n=100) and compared using a paired t-test. Probability of occurrence was generated using the equation $p = eLogit/(1 + eLogit)$ where $Logit = \beta 0 + \beta 1\chi 1 + \beta 2\chi 2$.

2.7 Hindcasting

Retrospective predictions or "hindcasts" of the likelihood of *Vibrio vulnificus* presence throughout Chesapeake Bay for the years 1996 and 1999 were generated by applying the empirical habitat model developed here to hindcasts of surface temperature and salinity generated by the Chesapeake Regional Ocean Modeling System (ChesROMS) of the CBEPS to demonstrate the impact of climate variability on *V. vulnificus* distribution. The years of 1996 and 1999 were chosen as representative cool-wet and warm-dry years, respectively, based on previous retrospective analysis of mid-Bay conditions and stream flow (Kimmel et al., 2006).

ChesROMS is an open source Chesapeake Bay implementation of the Regional Ocean Modeling System (ROMS), a community ocean model developed and maintained by Rutgers University (http://www.myroms.org/). ROMS / ChesROMS is a free-surface, terrain following, primitive equations model widely used by the scientific community for a diverse range of applications. ChesROMS uses historical re-analyses, near-real time observations, and forecast data to provide model forcing, such as

atmospheric momentum and heat fluxes, river outflow and ocean sea level, to provide hindcasts, now-casts and short-term (3-day) forecasts of salinity, temperature, and other physical variables in the Bay.

ChesROMS (v1.2) consists of a 150 x 100 cell horizontal grid and 20 layers vertically to yield spatial resolutions in the horizontal that range from 500 meter to five kilometers and in the vertical ranging from 0.2 to 1.5 meters. ChesROMS is the hydrodynamic component of the CBEPS. The CBEPS is comprised of a suite of Unix Shell scripts, Perl scripts, Fortran and C programs, NCL programs, MATLAB scripts and GIS shape files that automatically perform the tasks of compiling the model input files from observations, running the model, processing the model output and displaying the graphical products on a dynamic, interactive web site.

3. Results

3.1 Assay Performance

Assay performance testing was carried out in a manner similar to that as previously described (Jacobs et al., 2009). Although the vvhA gene has been well evaluated for specificity (Panicker and Bej, 2005), we further tested the assay against strains of *Vibrio parahaemolyticus*, *Enterococcus faecium, Hematodinium spp.* and 17 species of the genus Mycobacterium with negative results in all cases. For our extraction protocol, recovery estimates averaged 46.17% ± 7.55 (standard deviation; n = 40) of starting DNA over a 6-log range. High repeatability was found in both replicate water samples from the same station (R2 = 0.92; n = 32 bottles) and replicate samples from the same source (R2 = 0.94; n = 34 samples). Freezing did not significantly affect measured Vibrio concentration with split samples demonstrating a 1:1 relationship (slope = 1.10; R2 = 0.84; n = 20). Standard curves of Ct values versus concentration yielded an assay efficiency of 88.60% ± 5.90 (standard deviation; n = 4), with a sensitivity of <1cell/mL in a 200-mL water sample. No inhibitors were observed in any environmental samples, based on the amplification of the internal control.

3.2 Distribution and Abundance

Overall, *V. vulnificus* was detected in 24% of samples (n=235) from the main-stem Chesapeake during the months examined. Presence followed a strong seasonal trend with 0% positive in April, 32% in July, and 4% in October. Although a single sample was found positive at a water temperature of 15.5°C, 90% of positive observations occurred at water temperatures > 25.2°C (Figure 2). Similarly, the majority of positive observations were found in a relatively narrow salinity range. Eighty percent of positive observations were found between 8.6 and 20.1 ppt (Figure 3). Marine waters were not sampled in this survey. The highest salinity recorded was 27.6 ppt with 90% of samples collected in salinities less than 23.8 ppt. Positive samples ranged from 0.4 to 145 cells/ml.

3.3 Model Development

Binned temperature (2°C) and SALopt (1ppt) data were used to independently fit prevalence data (Figures 4 and 5). Model fit was very good, with R2 values for temperature and salinity 0.70 and 0.94 respectively. The use of both parameters in logistic regression yielded a final model with exceptional concordance (94.2%) and fit (AIC = 164.3, Hosmer and Lemeshow x2 = 6.49, 8 df, p = 0.59, Area under ROC curve (c) = 0.94). An interaction term was evaluated as well, but it was not significant (p > 0.05) and reduced model fit. Bootstrapping (sub-sampling with replacement) was conducted using the same sample size as the original data set (n = 235). Mean intercept and parameter estimates and confidence intervals generated from this exercise are provided in Table 1. The original model and the bootstrapped version were evaluated for consistency in model output. Both models were found to yield similar results (paired t-test, t = -0.954, 1,99df, p = 0.34).

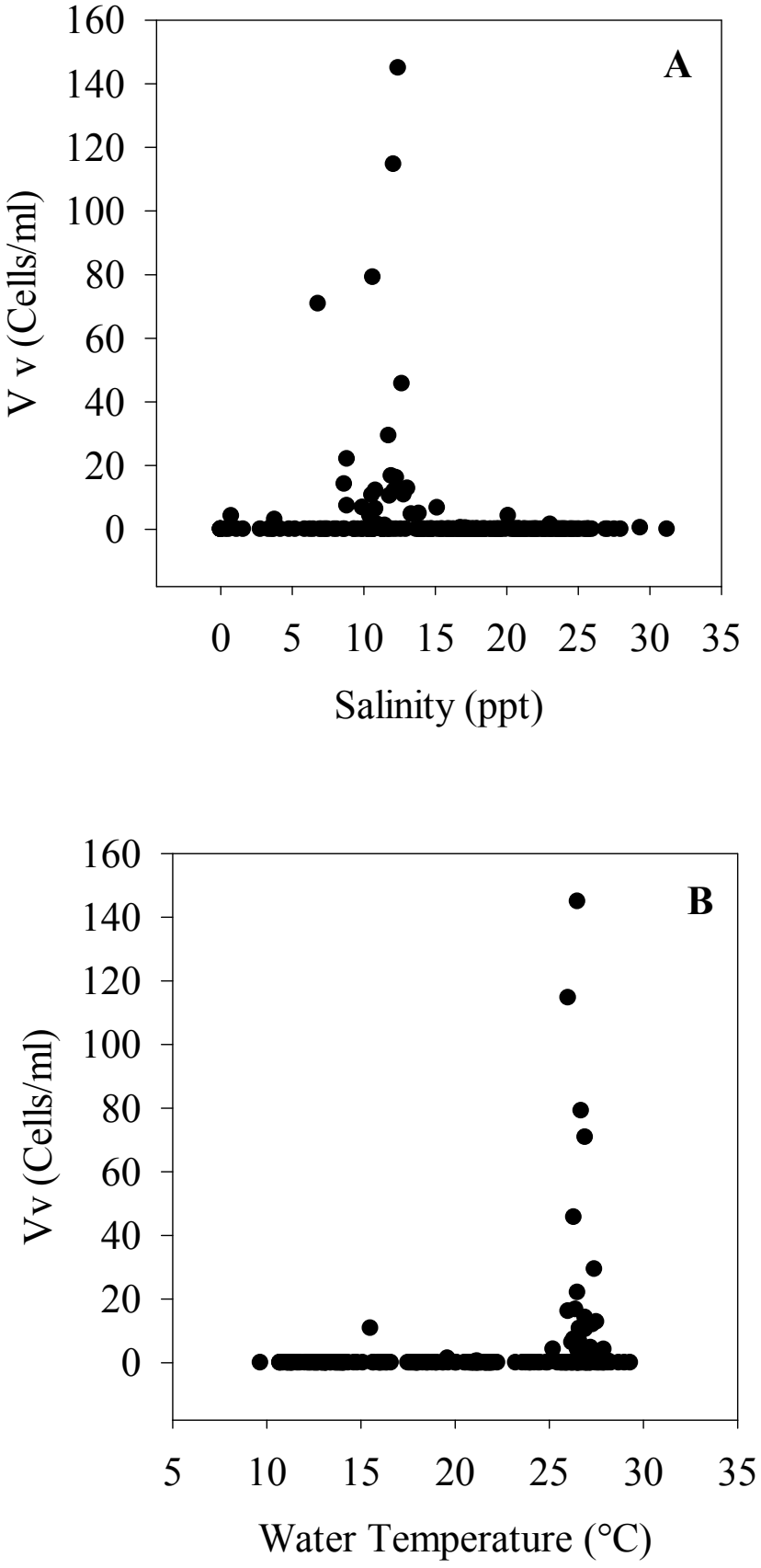

Figure 2. V. vulnificus abundance plotted against surface salinity (A) and surface water temperature (B) over the course of the study period in Chesapeake Bay.

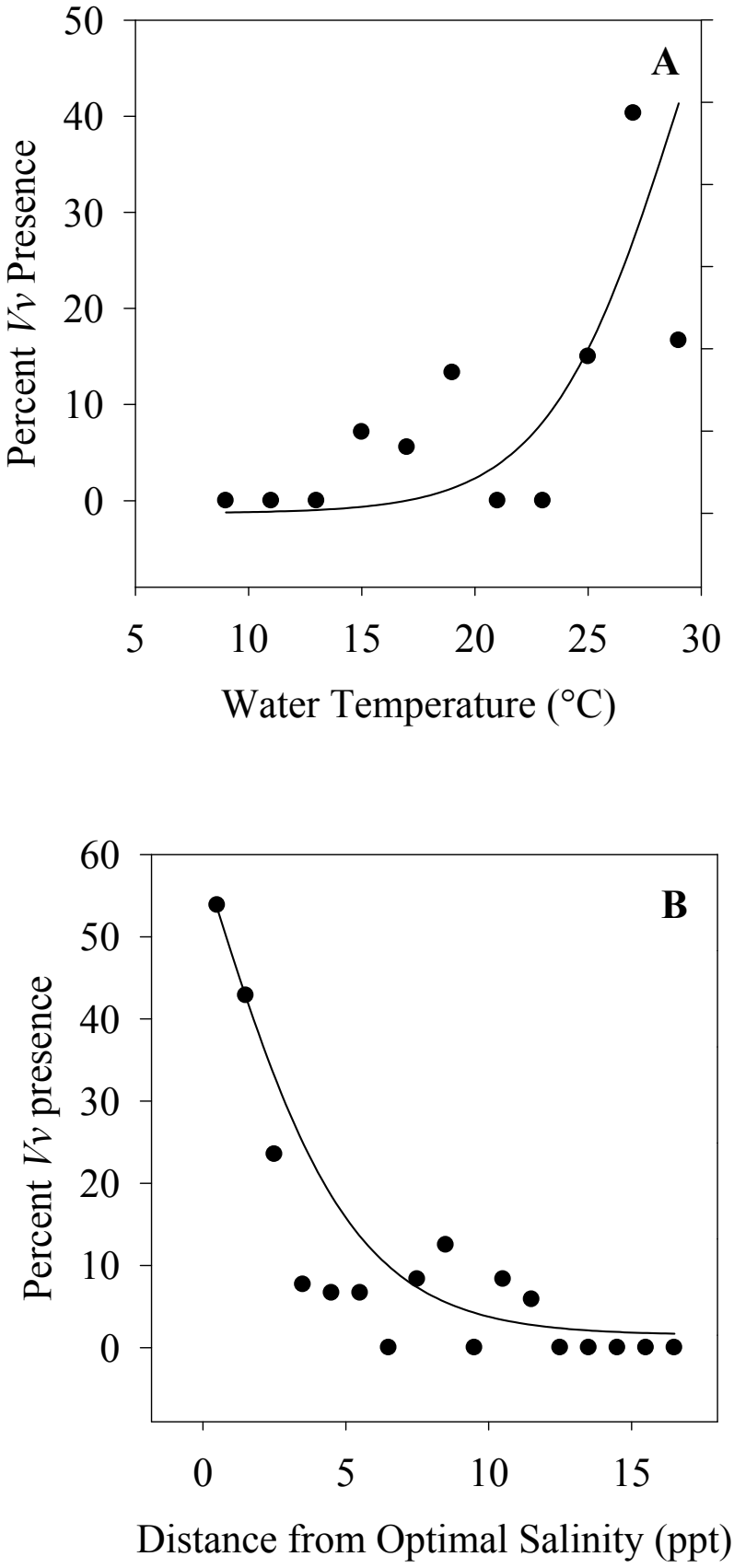

Figure 3. The relationship between the percentage of samples where *V. vulnificus* was detected and (A) binned surface temperature and (B) binned surface salinity. Data were fit using logistic regression analysis.

Table 1. Logistic model parameter estimates and 95% upper (UCL) and lower (LCL) confidence intervals derived from bootstrapping (n=1000).

	Mean	LCL	UCL
Intercept	-7.867	-7.638	-8.096
Temp	0.316	0.308	0.325
SALopt	-0.342	-0.349	-0.334

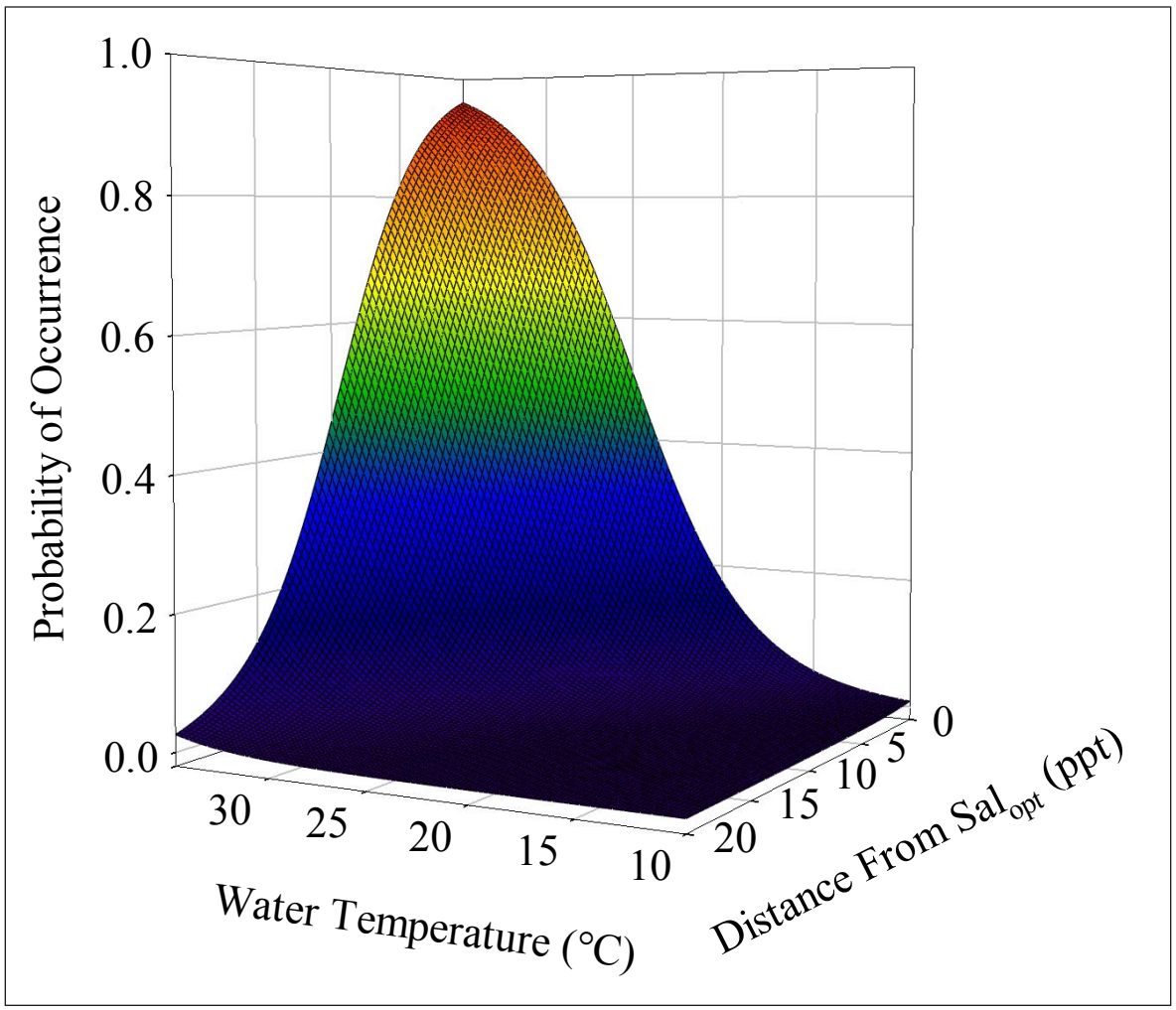

Figure 4. Probability plot generated using the logit function and parameter estimates derived from logistic regression analysis and bootstrapping. Color scale represents probability of occurrence with red high (100%) and blue low (0%).

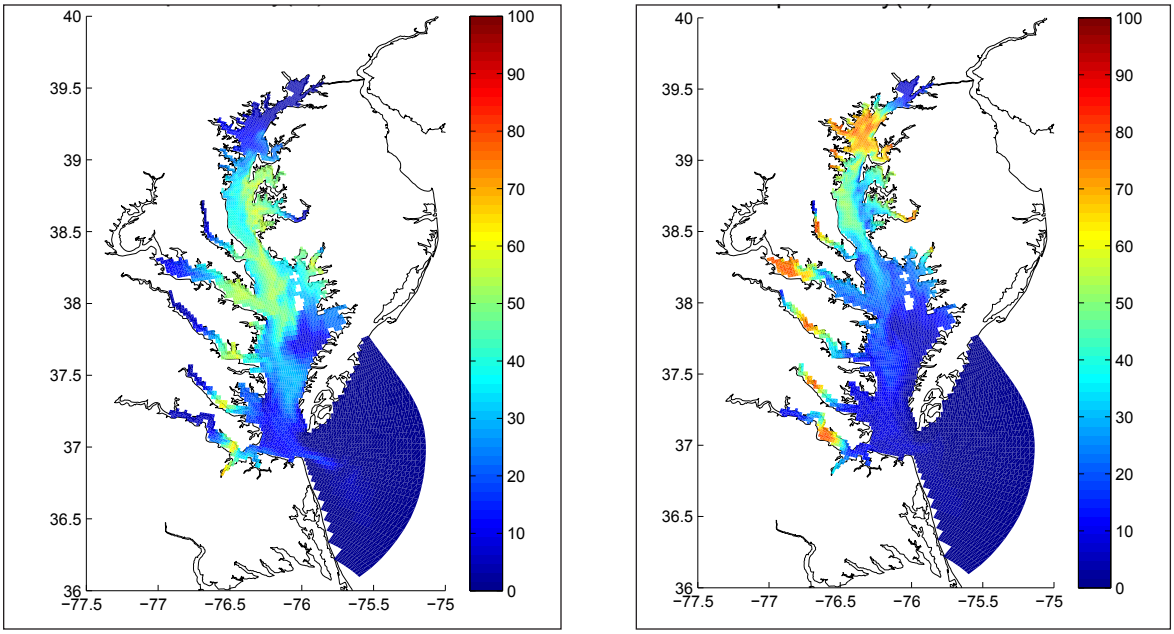

Figure 5. Hindcast depicting probability of occurrence of *V. vulnificus* in wet (1996) and dry (1999) years. Both figures represent conditions present on August 1st of each year. Color scale represents probability of occurrence with red high (100%) and blue low (0%).

3.4 Hindcasts

Hindcasts of the likelihood of *V. vulnificus* presence in Chesapeake Bay in 1996, representative of cool-wet years, suggests a lower overall probability of occurrence and a general southward shift of their presence in the bay. Conversely, hot-dry years, as represented by 1999, indicates an enhanced probability of occurrence and a northward shift of areas of greater risk (Figure 5).

4. Discussion

To our knowledge, this effort represents the first attempt to empirically model and predict the distribution of *Vibrio vulnificus* in a large estuarine system. By using only temperature and distance from optimal salinity, our model correctly classified *V. vulnificus* presence 93% of the time. Incorporation of the habitat model into the Chesapeake Bay Ecological Prediction System allows hindcasting, nowcasting and short-term (3 day) forecasting of possible locations of likely *V. vulnificus* presence. These hindcasts, nowcasts and forecasts can be used to better understand the ecology and the influence of environmental variability on this pathogen, and they can guide pathogen monitoring programs and enhance first responder capabilities.

This effort also represents one of the first approaches to rely strictly on molecular techniques for enumeration of *V. vulnificus* from water. The assay developed by Paniker et al. (Panicker and Bej, 2005) proved to provide strong specificity. When coupled with our modified extraction technique, highly repeatable standard curves were obtained from spiked matrix controls with very good efficiency. Replicate sample and replicate extractions from the same source were also highly repeatable. Thus, this effort offers a complete method for enumeration of *V. vulnificus* from aquatic sources with estimates of both recovery and sampling error.

While molecular techniques are currently coming to the forefront in the investigation of the diversity and abundance of bacterioplankton (Smythe et al., 2002; Crump et al., 2004; He and Jiang, 2005), there are still some limitations. One key issue is the subject of live versus dead cells in the environment. It has been shown that DNA may remain intact within bacteria for days or even weeks (Nocker et al., 2006; Nocker et al., 2007). In general, PCR does not have the ability to separate living members of the bacterioplankton community from recently deceased members, possibly with inflated abundance estimates as a result. Conversely, PCR does have advantages over standard culture methods when applied to environmental samples. Due to the ability of Vibrio spp. to enter in a viable but non-culturable (VBNC) state, current culture methods may be underestimating the abundance of these organisms, especially when water temperature begins to decrease (Colwell et al., 1985; Oliver, 1995; 2005). In contrast, PCR does not rely on the state of the organism, but rather that the target gene is present.

One limitation of the current model is that it does not separate clinically significant from environmental strains of *V. vulnificus*. Three biotypes have been recognized; biotype I associated with human clinical cases, biotype 2 affecting fish, with one serovar (E) affecting humans, and recently biotype 3, zoonotic pathogens causing wound infections in Israel (Tison et al., 1982; Amaro and Biosca, 1996; Bisharat et al., 1999; Sanjuan et al., 2009). Molecular methods have been evaluated for the purpose of segregating clinical from environmental strains (Nilsson et al., 2003; Panicker et al., 2004; Vickery et al., 2007; Sanjuan et al., 2009). However, there is no consensus on a particular approach, and efforts to date have not been consistent. For example, Sanjuan et al. (2009) compared several pheonotypic and genotypic methods for typing and found that none were capable of reliably distinguishing biotypes or clinical from environmental strains. This is an important consideration for assessing risk and understanding virulence determinants in the protection of human health, and future efforts in typing are warranted.

The influence of temperature and salinity on Vibrio spp. abundance has been well documented (Tamplin et al., 1982; Oliver et al., 1983; O'Neill et al., 1992; Lipp et al., 2001; Pfeffer et al., 2003; Thompson et al., 2004). Our model demonstrates that these two variables are highly constraining for *V. vulnificus* within the range we sampled in Chesapeake Bay. While positive correlation with water temperature is a consistent finding in the literature (generally elevated at > 20°C), the relationship of *V. vulnificus* with salinity has been more variable. Our results suggest an optimal salinity of 11.5 ppt, with abundance declining with distance from optimal. In previous work in Cheasapeake Bay, Wright et al. (1996) found an inverse correlation with salinity. However, a limited, meso-haline (~8-20 ppt) range was sampled, all in potentially good *V. vulnificus* habitat, according to our model. Our results are in fact identical throughout this range, with *V. vulnificus* abundance tapering off along a down-Bay transect. Similar results were also reported by Lipp et al. (2001) in Charlotte Harbor, Florida where a positive correlation with salinity below 15 ppt and negative above 15 ppt was found, essentially defining an optimal salinity for *V. vulnificus* within this system. Randa et al. (2004) reported elevated levels of *V. vulnificus* at 5-10 ppt, along with a positive correlation at higher salinities (20-25 ppt). While most studies support a mesohaline salinity distribution, *V. vulnificus* has been isolated from higher salinity environments (Tamplin et al., 1982; Oliver et al., 1983). In our efforts, we did not encounter salinities > 28 ppt, and rarely > 25 ppt. Thus model interpretation should be limited to these areas within Chesapeake Bay.

Development of the habitat model was attempted solely with temperature and salinity, due to their known influence on the distribution of *V. vulnificus* as well as current limitations in forecast capabilities using the ChesROMS model. However, other work has investigated the link between *V. vulnificus* and

several other environmental parameters with mixed results. For example, Pfeffer et al. (2003) showed an inverse correlation with dissolved oxygen (DO) and no correlation between *V. vulnificus* and fecal coliforms, turbidity or pH. In contrast, Barbieri et al. (1999) found no correlation between *V. vulnificus* and DO, while Tamplin et al. (1982) suggests a negative correlation with fecal coliforms. Oliver et al. (1983) showed a positive correlation with pH and a weak positive correlation with turbidity. Randa et al. (2004) found strong correlations between chlorophyll a, total bacteria counts, and *V. vulnificus* densities, but reasoned that they were likely just correlated with temperature. Measurements of DO, turbidity and pH were obtained from our sample stations along with a full suite of nutrient and chemical parameters. Future work will focus on tuning this model with other variables in order to further refine *V. vulnificus* prediction.

Using the ChesROMS hydrodynamic model, we briefly demonstrated the potential utility of our approach in evaluating *V. vulnificus* response to climate variability. The year 1996 had higher than average freshwater input in January and July causing surface temperatures and salinity to remain low, particularly in the upper Bay region (Kimmel et al., 2006). According to our model, this scenario should result in a down-Bay shift in the distribution of *V. vulnificus*, encompassing a large area of the mid-Bay main-stem, but with low probability of occurrence (Figure 5). In contrast, from the summer of 1998 through early 2000 the Bay experienced lower than average freshwater input resulting in elevated salinity and surface temperature (Kimmel et al., 2006). In this scenario, *V. vulnificus* are predicted to be more prevalent, with concentrated areas in the upper Bay and tributaries (Figure 5). With global concerns of climate change, pathogens that respond to increasing temperature are of great concern in that increased temperatures could affect their density, distribution, and perhaps virulence. Indeed, climate has been suggested to play a role in both *V. vulnificus* and *V. cholera* disease outbreaks (Colwell, 1996; Paz et al., 2007). We anticipate using this model to evaluate climate change scenarios in Chesapeake Bay with respect to *V. vulnificus* abundance and distribution. The University of Maryland, in collaboration with other institutions and agencies, is implementing the Chesapeake Bay Forecast System (CBFS), a regional Earth System model of the Chesapeake Bay (Murtugudde, 2009). The CBFS is a fully integrated, biogeochemical model of the bay watershed and estuary and consists of a coupled atmospheric/land/ocean model complete with biological and geochemical components that would allow longer-term (> 14 days) predictions of *V. vulnificus* and other pathogens (Decker et al., 2007; Constantin de Magny et al., In Press) to explore the potential ramifications of management policies on pathogens in the Bay and anticipate their potential response to climate scenarios.

The similarity of the *V. vulnificus* habitat model and those previously published for jellyfish (*Chrysaora quinquecirrha*) in Chesapeake Bay is remarkable (Decker et al., 2007). Salinity and temperature constraints are nearly identical for the two species, resulting in similar parameter estimates. A potential explanation for their co-occurrence may lie in a common connection through zooplankton. Vibrio spp. are known to colonize zooplankton, particularly those with chitonous exoskeletons such as copepods (Venkateswaran et al., 1989; Tamplin et al., 1990; Heidelberg et al., 2002a). Copepod abundance in Chesapeake Bay is regulated to some extent by grazing by the ctenophore *Mnemiopsis leidyi* (Feigenbaum and Kelly, 1984; Purcell and Decker, 2005; Kimmel et al., 2006). *C. quinquecirrha* in turn is a major predator of *M. leidyi*. Because *M. leidyi* does not appear to be constrained by physical factors within the estuarine portion of Chesapeake Bay, in areas where the two species overlap, *M. leidyi* abundance is reduced (Feigenbaum and Kelly, 1984; Purcell and Decker, 2005; Kimmel et al., 2006). This reduction in ctenophore abundance, and subsequent grazing pressure, has been shown to enhance copepod abundance (Feigenbaum and Kelly, 1984; Purcell and Decker, 2005; Kimmel et al., 2006). Thus the potential exists for Vibrio abundance to be regulated to some extent by food web dynamics in Chesapeake Bay.

To our knowledge, this effort marks the first attempt to forecast the probability of occurrence of a potential human pathogen in Chesapeake Bay. While the potential of this approach is great for protecting human health, the responsibility of forecasting human pathogens is enormous. Risk assessment and communication represents one of the largest challenges in marine-associated illness. The consequences to user groups and local Bay economies could be dramatic if the *V. vulnificus* predictions are misinterpreted as was seen previously in the region with information regarding Pfiesteria sp. (Magnien, 2001). Thus, use of this algorithm or interpretation of the data by those unfamiliar with public health issues in Chesapeake Bay is strongly discouraged. Forecasts and hind-casts generated using our model are being evaluated by an Ad-Hoc committee of Federal, State, and County health officials for potential application in communicating Vibrio spp. risk and guiding monitoring efforts.

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