

An Evaluation of the Use of Remotely Sensed Parameters for Prediction of Incidence and Risk Associated with *Vibrio parahaemolyticus* in Gulf Coast Oysters (*Crassostrea virginica*)

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MS 06-472: Received 4 September 2006/Accepted 9 November 2006

ABSTRACT

The U.S. Food and Drug Administration recently published a *Vibrio parahaemolyticus* risk assessment for consumption of raw oysters that predicts *V. parahaemolyticus* densities at harvest based on water temperature. We retrospectively compared archived remotely sensed measurements (sea surface temperature, chlorophyll, and turbidity) with previously published data from an environmental study of *V. parahaemolyticus* in Alabama oysters to assess the utility of the former data for predicting *V. parahaemolyticus* densities in oysters. Remotely sensed sea surface temperature correlated well with previous in situ measurements ($R^2 = 0.86$) of bottom water temperature, supporting the notion that remotely sensed sea surface temperature data are a sufficiently accurate substitute for direct measurement. Turbidity and chlorophyll levels were not determined in the previous study, but in comparison with the *V. parahaemolyticus* data, remotely sensed values for these parameters may explain some of the variation in *V. parahaemolyticus* levels. More accurate determination of these effects and the temporal and spatial variability of these parameters may further improve the accuracy of prediction models. To illustrate the utility of remotely sensed data as a basis for risk management, predictions based on the U.S. Food and Drug Administration *V. parahaemolyticus* risk assessment model were integrated with remotely sensed sea surface temperature data to display graphically variations in *V. parahaemolyticus* density in oysters associated with spatial variations in water temperature. We believe images such as these could be posted in near real time, and that the availability of such information in a user-friendly format could be the basis for timely and informed risk management decisions.

Vibrio parahaemolyticus, a gram-negative, halophilic bacterium indigenous to coastal estuarine and marine environments, is the leading cause of vibrio-associated gastroenteritis in the United States (12, 31). *Vibrio* infections are most common in states bordering the Gulf of Mexico (15) and are usually associated with the consumption of raw shellfish, primarily oysters (5, 8, 13, 15). Outbreaks in 1997 in the Pacific Northwest (3) and in 1998 in Texas (9, 11), Washington, and New York (4, 11) raised the public health concerns throughout coastal states.

The Galveston Bay, Texas, outbreak in 1998, lasting from May until June, was the largest *V. parahaemolyticus* illness outbreak in the United States, with 416 reported cases (9). These outbreaks prompted the Interstate Shellfish Sanitation Conference to develop an interim control plan (ICP) for closing and opening shellfish harvest areas, based on criteria involving measurement of both total and pathogenic *V. parahaemolyticus* in shellfish (29). The current ICP utilizes an enumeration procedure using a colony lift technique and DNA probes that was approved by the American Public Health Association (21) and the U.S. Food and Drug Administration (FDA) (30). These probes target the species-specific thermolabile hemolysin gene (*tlh*) for enumerating total *V. parahaemolyticus*, and the thermostable

direct hemolysin gene (*tdh*) that is associated with pathogenicity (25, 26).

Quantitative data on environmental levels of *V. parahaemolyticus* in oysters and water have shown a positive correlation of *V. parahaemolyticus* densities with water temperature, and thus an apparent seasonality with higher levels during warmer months (5, 10, 12). Salinity is also known to affect *V. parahaemolyticus* densities (5, 10, 20). Possible links between *V. parahaemolyticus* incidence and other environmental parameters, such as turbidity and chlorophyll, are less clear (34). Nevertheless, if one can suitably determine the quantitative effect of environmental parameters favorable for proliferation of this bacterium and then effectively monitor these parameters, the risk of illness could be better predicted and managed. With this in mind, the FDA formulated a risk assessment model in which levels of *V. parahaemolyticus* in oysters at harvest are predicted based on water temperature. The FDA assessment was structured to assess *V. parahaemolyticus* on a regional and seasonal basis. Estimated distributions of water temperature were obtained based on historical (1987 to 1997) measurements from selected fixed site National Oceanographic and Atmospheric Administration (NOAA) monitoring stations and buoys (31). A principal objective of the FDA assessment was to determine the relative importance of the environment and various postharvest practices on

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consumer risk and to make comparisons between different regions and seasons. For this purpose, the use of historical data on water temperature was desirable and, on average, the model-predicted *V. parahaemolyticus* levels based on the fixed site temperature measurements generally agreed with the levels determined by microbiological examination. However, many oyster-harvesting areas are far from fixed-site monitoring stations (i.e., NOAA buoys or similar monitoring stations), and such fixed site data probably do not represent the full range of temperature variations that occur within and across specific oyster-harvesting areas. This is a relevant issue to be addressed if the approach underlying the FDA model is to be utilized for forecasting. Furthermore, the potential for improvements in accuracy of forecasting exist if other determinant factors (e.g., salinity, turbidity, chlorophyll, etc.) substantially influence *V. parahaemolyticus* levels, are spatially variable, and can be monitored via remote sensing technology.

Remote sensing is currently being used to describe and monitor a variety of systems from local to global scales (7, 19). For example, a study described by Lobitz et al. (1, 17, 18, 24) used retrospective remote sensing data to demonstrate a relationship between certain ocean parameters (sea surface temperature [SST] and sea surface height) and cholera (*Vibrio cholerae*) incidence in Bangladesh. In a similar fashion, remotely sensed (RS) data may prove useful to better elucidate and estimate the quantitative effect of factors other than SST in determining *V. parahaemolyticus* densities in U.S. oysters and provide a timely, user-friendly format for risk management. In the present study, we demonstrate the feasibility of this approach by analyzing archived RS data on water temperature, turbidity, and chlorophyll with previously published data of *V. parahaemolyticus* densities in Alabama oysters.

MATERIALS AND METHODS

In situ data. Archived data of biweekly oyster sampling from March 1999 to September 2000 for sampling sites at Dauphin Island Bay Reef (30°16'180"N, 88°05'560"W) and Cedar Point Reef (30°18'262"N, 88°07'519"W) were obtained from the FDA, Gulf Coast Seafood Laboratory, Dauphin Island, Alabama. The following variables were used in this study: total *V. parahaemolyticus* per gram of shellfish meat (geometric mean of two replicate samples), water temperature, and salinity. These data, collected by DePaola et al., were reported in a previous communication (12). The present study compared these *V. parahaemolyticus* density and environmental data with archived RS data. Thus, only in situ observations recorded at times for which RS data were available were used. This occurred for 55 of 78 in situ observations.

RS data. SST (33) and turbidity (beam attenuation) (14) derived from NOAA's Advanced Very High Resolution Radiometer (AVHRR) as well as ocean color (chlorophyll *a*) (28) determined from Sea-Viewing Wide Field-of-View Sensor data (27), are all characterized by a pixel-to-pixel ground sample distance of 1.1 km. SST derived from National Aeronautics and Space Administration's (NASA's) Moderate Resolution Imaging Spectrometer (2) aboard the Aqua (EOS PM) satellite was used to generate Figures 2 and 3. These estimates of RS data were provided by the Naval Research Laboratory, Stennis Space Center, Mississippi, in

processed form via an automated processing system (22). The automated processing system contains programs for sensor calibration, atmospheric correction, geometric registration, and product algorithms (22). Pixel values from the processed files, concurrent spatially and temporally (within 2 h) with in situ data, were extracted using the Environment for Visualizing Images software package (ENVI, version 3.6, Research Systems, Inc., Boulder, Colo.).

Statistical analyses. Effects of environmental parameters on total *V. parahaemolyticus* densities were analyzed by regression analysis. Effects of water temperature and salinity were analyzed by both simple and multiple regressions. The significance of potential effects of chlorophyll and turbidity was ascertained by a two-stage procedure in order to minimize the impact of missing observations. First, residuals (difference in observed versus expected) of a multiple regression of *V. parahaemolyticus* densities against both water temperature and salinity were calculated. These residuals were then considered the dependent variable in simple regressions against chlorophyll and turbidity, respectively. The relationship between in situ water temperature measurements and the corresponding RS data was determined by Pearson correlation. Predictions of spatial variation of mean *V. parahaemolyticus* densities due to variations in water temperature were obtained by combining AVHRR water temperature data with predictions derived from the FDA risk assessment model (31). Specifically, the FDA risk assessment model was run in replicate simulations, with different random number seeds, at fixed water temperature increments. An interpolating regression was then fit to the simulation output to summarize relationship between predicted mean *V. parahaemolyticus* density and water temperature. For the statistical analyses of *V. parahaemolyticus* densities versus environmental parameters, half the limit of detection was substituted when *V. parahaemolyticus* densities were below the limit of detection (10/g) and density estimates were log transformed. All statistical analyses were conducted using the SPSS (SPSS Inc., Chicago, Ill), SAS statistical software (SAS Institute, Cary, N.C.), or a combination thereof.

RESULTS

SST derived from AVHRR data and in situ bottom water temperature measurements at the two sampling stations near Mobile Bay, Alabama, correlated well ($R^2 = 0.86$, $P < 0.01$). Generally, SST was slightly higher than bottom water temperature, although this pattern was reversed on rare occasion. Overall, the estimated annual average temperatures based on these data were in close agreement (23.05°C for AVHRR versus 22.94°C for in situ). Table 1 presents the summary statistics for the in situ and RS environmental parameters, as well as actual and predicted mean log total *V. parahaemolyticus* density, based on the FDA risk assessment model (31) and the two water temperature measurements. The following model was used to derive these predictions: mean log *V. parahaemolyticus* per gram = $-0.84 + 0.11 \times (\text{water temperature})$. The predictions based on the in situ data (1.69 log *V. parahaemolyticus* per g) and RS data (1.71 log *V. parahaemolyticus* per g) agreed well with each other (Fig. 1). The observed *V. parahaemolyticus* densities were slightly higher (1.94 log *V. parahaemolyticus* per g) than were either set of predictions, and the observed log *V. parahaemolyticus* densities were more variable. Greater variability of observed densities is expected because these model predictions are of

TABLE 1. Total *Vibrio parahaemolyticus* density in oysters and environmental parameters, collected in situ or via remote sensing^a

Variable	No. of samples	Range	Mean	SD
Actual log total <i>V. parahaemolyticus</i> ^b	55	0.70–3.85	1.94	0.85
Predicted log total <i>V. parahaemolyticus</i> (IS) ^c	55	0.26–2.77	1.69	0.71
Predicted log total <i>V. parahaemolyticus</i> (RS) ^d	49	0.43–2.89	1.71	0.76
IS salinity (CFU/g shellfish)	55	8.70–29.70	21.29	5.24
IS water temp (°C)	55	9.90–32.70	22.94	6.45
RS SST (°C)	49	11.48–33.80	23.05	6.95
RS chlorophyll (mg/m ³)	10	2.45–20.16	9.09	5.65
RS turbidity (m ⁻¹)	34	2.88–49.60	11.55	8.68

^a SD, standard deviation; IS, in situ; RS, remotely sensed; SST, sea surface temperature.

^b Replicate samples averaged together.

^c Based on IS temperature data.

^d Based on RS SST data.

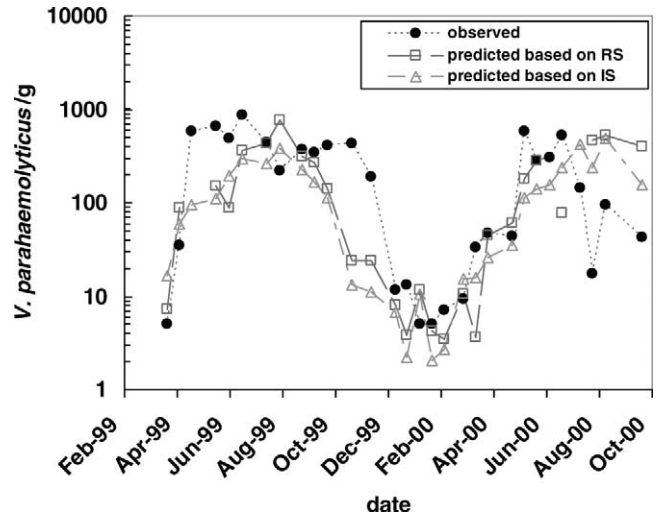


FIGURE 1. Predictions of mean log *V. parahaemolyticus*, based on remotely sensed (RS) and in situ (IS) temperature data versus observed log *V. parahaemolyticus* densities, averaged over both Cedar Point Reef and Dauphin Island Bay sampling sites.

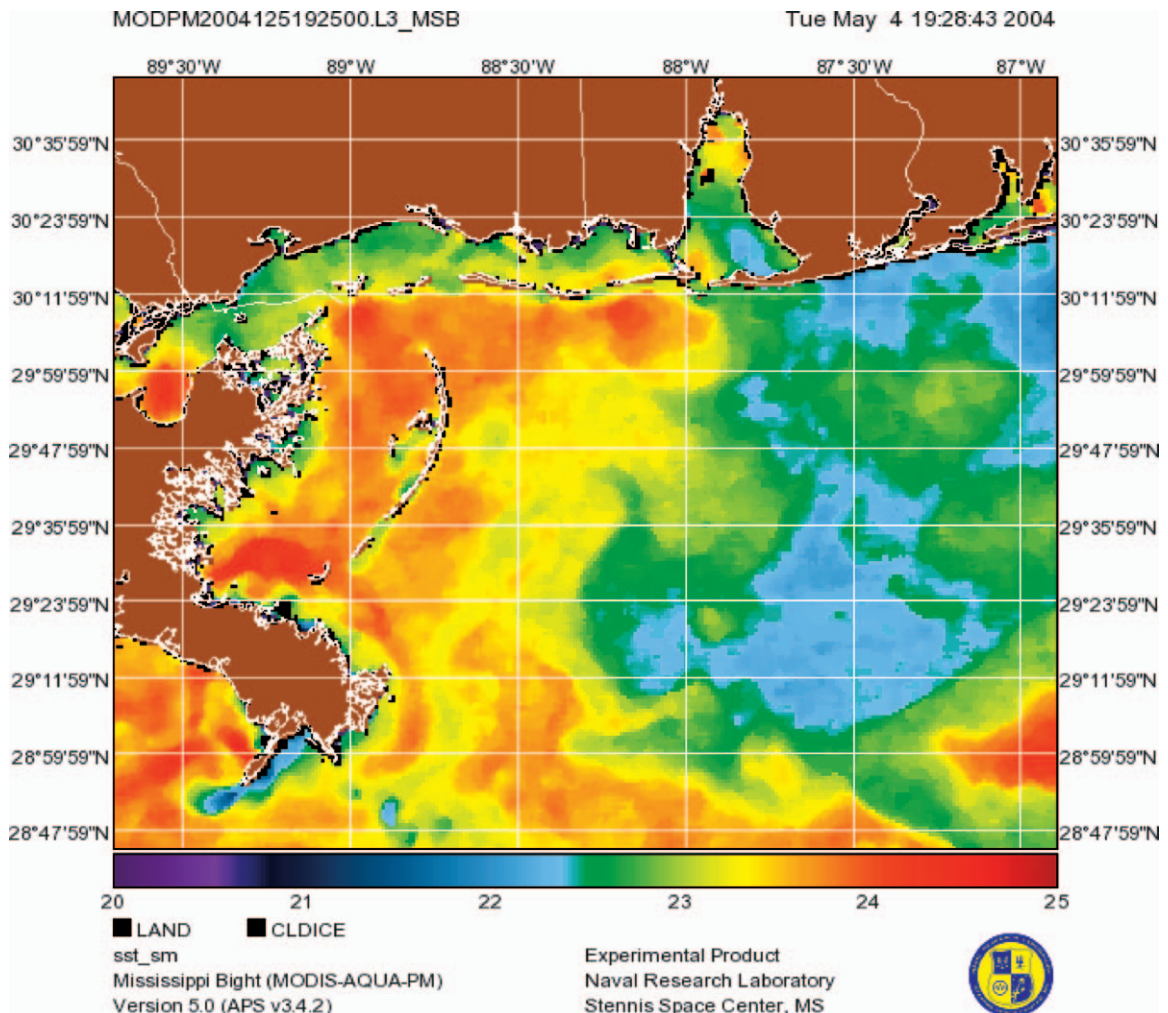


FIGURE 2. NASA's Moderate Resolution Imaging Spectrometer (MODIS) SST image of SST along the Louisiana, Mississippi, and Alabama coasts on 4 May 2004.

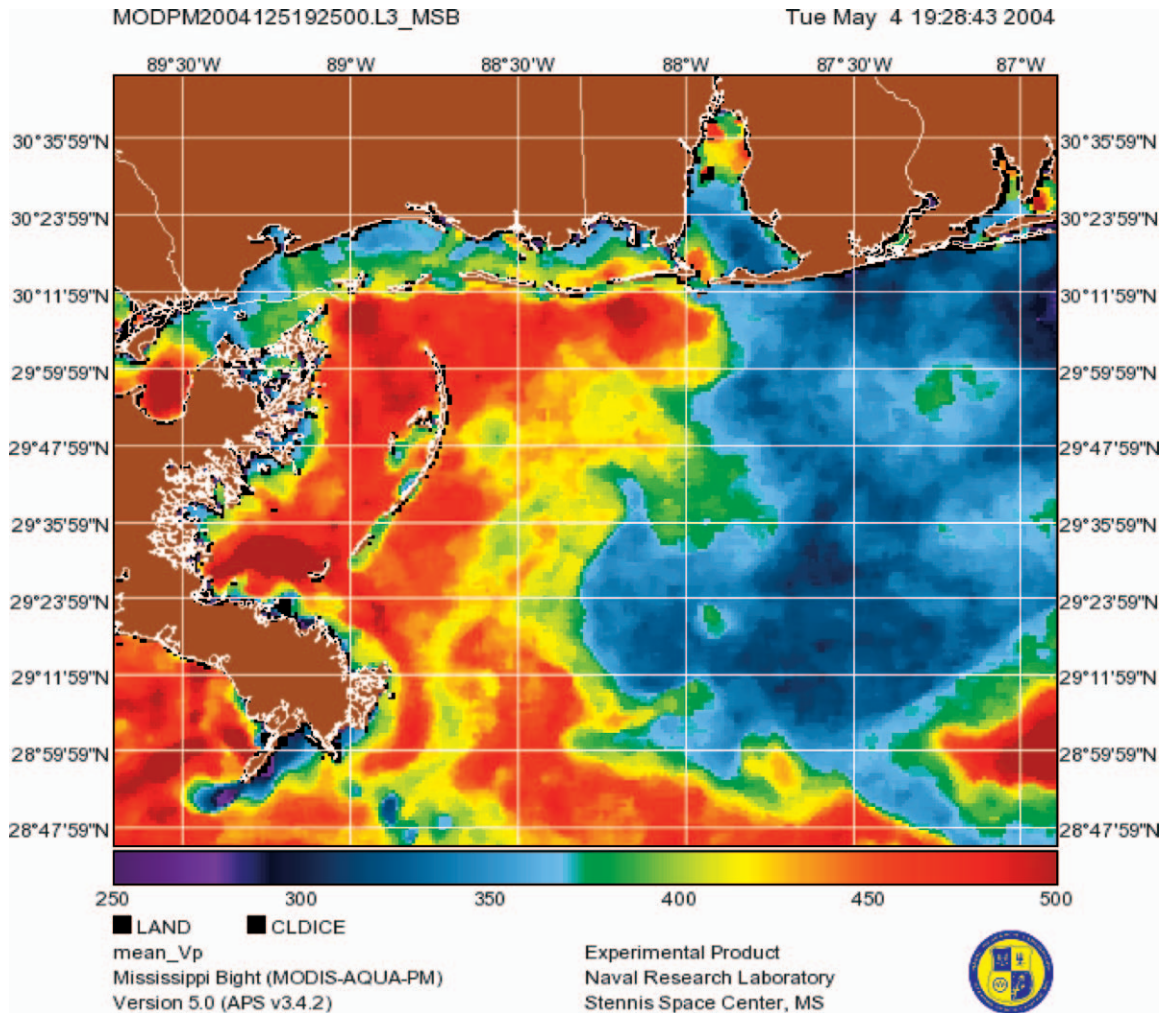


FIGURE 3. Mean *V. parahaemolyticus* per gram prediction image, based on RS MODIS SST values for 4 May 2004.

mean log *V. parahaemolyticus* per gram, based on water temperature alone. The predictions do not include the effect of additional environmental factors (e.g., salinity) or account for the natural sample-to-sample variability exhibited in samples collected under identical conditions. Correlation between the observed and predicted values was similar irrespective of whether predictions were based on the in situ ($r = 0.692$) or the RS ($r = 0.673$) water temperature data.

A series of regressions were performed to evaluate whether or not some of the differences between the observations and temperature-based predictions were significantly related to the other environmental parameters (salinity, turbidity, chlorophyll). First, consistent with what is already known about the effects of temperature (31) and salinity (5, 20), a multiple regression of log *V. parahaemolyticus* per gram against both RS SST and salinity parameters showed that both were significant ($P < 0.05$). The effect of salinity was determined to be quadratic. The linear regression model that best fit the data was mean log *V. parahaemolyticus* per gram = $-1.904 + 0.084 \times (\text{RS SST}) + 0.242 \times (\text{salinity}) - 0.006 \times (\text{salinity}^2)$. Residuals from the fit of this equation were then used to assess the significance of turbidity and chlorophyll. The residuals of the regression fit were defined as the observed minus the predicted values.

A positive residual corresponds to an observed level greater than that predicted based on water temperature and salinity. These residuals did not show a significant relationship with RS turbidity, but there was a significant correlation ($R^2 = 0.55$, $P < 0.05$) with RS chlorophyll. The association of the residuals with RS chlorophyll was positive. Occasions when observed log *V. parahaemolyticus* per gram were greater than predicted corresponded to higher levels of chlorophyll, and this relationship appeared consistent across both sampling sites. However, these observations are based on a very limited number of samples.

Despite some differences between remotely sensed and in situ bottom temperature measurements, the spatial variations in water temperature across a given region or estuary on a given day appears to be of equal or greater magnitude. For example, RS SST data for 4 May 2004 are shown in Figure 2. Here, one can see a variation of between 22 and 25°C for oyster-harvesting areas across Alabama, Mississippi, and Louisiana. By linking image data such as this with model-based predictions, the effect of this spatial variation on predicted *V. parahaemolyticus* densities can be effectively depicted. Based on the FDA risk assessment model, the relationship between mean *V. parahaemolyticus* per gram and water temperature is summarized by the equa-

tion: mean *V. parahaemolyticus* per gram = $0.871 \times \exp[0.2648 \times (\text{water temperature})]$. Predictions for oyster harvesting areas across Alabama, Mississippi, and Louisiana on 4 May 2004 are shown in Figure 3, based on the SST data and the prediction equation.

DISCUSSION

This study investigated the utility of remote sensing data for prediction of *V. parahaemolyticus* levels in raw oysters at harvest. RS SST data were found to relate to in situ temperature data with relatively high correlation, and observed systematic differences were small and consistent with expectations (23). Thus, predictions based on RS water temperature appear feasible now and provide much greater coverage than available with sporadically located weather buoys. Additionally, the imagery provides a user-friendly format for the interpretation and use of risk assessment outputs for risk management. Cloud cover and ground clutter are major limitations of this approach and interfere with turbidity and chlorophyll measurements more often than with SST data. However, the limited archived data available for chlorophyll did show significant correlation with *V. parahaemolyticus* levels and merits further examination. Approaches that may be used to mitigate the effect of remote sensing data gaps include compositing data collected over multiple previous days and/or statistically interpolating, both spatially and temporally (6, 16). Use of additional sources of data, including buoys and vessels, may also help overcome the remote sensing data gaps.

Factors such as salinity and air temperature that affect *V. parahaemolyticus* levels at harvest and postharvest, respectively, were not included. The RS data-based predictions shown in Figure 3 do not include effects of salinity, and predictions have not been extended to include postharvest levels. Currently, there are no data sources (AVHRR or other) that can provide information on these two parameters with the same spatial resolution as for water temperature. Whereas daytime (e.g., mid-day) air temperatures may be relatively constant over large areas (e.g., 100 km² or more) on a given day and could be obtained from fixed sites (e.g., airports), salinity is more spatially variable. Absent a data source for salinity with spatial resolution equal to or better than that of water temperature, RS water temperature-based predictions are either contingent on specified salinity levels, or such predictions can be averaged over the likely distribution of salinity in oyster harvesting areas, considered independent of location. The latter "averaging" approach was adopted in the FDA risk assessment model, and hence the basis on which Figure 3 was constructed. This approach could be improved if a source of information for spatial variation of salinity were available. Alternatively, a range of salinity-specific predictions may be of value in the absence of such information.

In the re-analysis of previously published data on *V. parahaemolyticus* in Alabama oysters, differences in *V. parahaemolyticus* levels not attributable to differences in temperature and salinity were found to be significantly associated with RS chlorophyll. Although this finding is based on a very limited number of observations, the exist-

ence of a positive association of *V. parahaemolyticus* densities with chlorophyll suggest a relationship between *V. parahaemolyticus* and phytoplankton, the primary source of chlorophyll in aquatic environments. Phytoplankton are a major component of the oyster diet, and attachment of *V. parahaemolyticus* to some species of phytoplankton may occur but has not been reported. Zooplankton also feed on phytoplankton and vibrios including *V. parahaemolyticus*; these have been shown to attach and multiply on copepods (1, 17, 18, 24, 34). This could increase *V. parahaemolyticus* densities in the waters overlying oyster reefs.

On the other hand, RS turbidity showed a nonsignificant relationship with *V. parahaemolyticus* after correcting for the effects of temperature and salinity. This observation is not consistent with the findings of Watkins and Cabelli (34), who found that *V. parahaemolyticus* was highly correlated with turbidity in Narragansett Bay, Rhode Island. It may be that RS turbidity is not a sufficiently accurate measure of actual (i.e., in situ) turbidity or that the cause or composition of turbidity of the Alabama waters in the present study differed from that in the Narragansett Bay study. For example, freshwater runoff, resuspension of bottom sediments, and blooms of phytoplankton or zooplankton can all contribute to turbidity and yet have different impacts on *V. parahaemolyticus* levels. It is also possible that, given the range of turbidity variations at the Alabama sampling sites, the effect of turbidity was too small to be effectively estimated based on the limited number of observations available from the archived data.

Availability of RS data to investigate potential correlations of chlorophyll and turbidity to *V. parahaemolyticus* densities was found to be problematic. RS chlorophyll and turbidity measurements are reliant on sunlight to produce accurate reflectance data, and even slight cloud coverage over a particular area of interest at the time of satellite pass over can yield invalid data or none at all. For example, at least one RS data product concurrent spatially and temporally was available for 55 (71%) of the 78 in situ observations. Lack of data was usually due to cloud cover, but on three occasions, data were missing due to sensor outages. Of those 55 occasions for which RS data were available, SST, turbidity, and chlorophyll were available on 50, 34, and only 10 occasions, respectively. SST was the most reliable data product; it is based on emitted (not reflected) thermal infrared energy, and therefore not as vulnerable to effects by clouds as are chlorophyll and turbidity measurements. These limitations of the data made statistical evaluation of the importance of turbidity and chlorophyll on *V. parahaemolyticus* density difficult because of the reduction in the sample size.

Spatial predictions such as that shown in Figure 3 can potentially be obtained in near real time. One can and should expect that temporal variations in conditions (i.e., from one day or week to the next) are equally important, and that the spatial pattern of risk across different oyster-harvesting areas is not constant. The use of RS data may also be of value for retrospective investigation of past outbreaks such as that due to oysters harvested from Galveston Bay in 1998 or the more recent outbreak in Alaska in 2004.

With accurately specified and estimated prediction models, RS data can be used for risk assessment, both to investigate past outbreaks and illness patterns and to provide a basis for forecasting and rapid, near real-time dissemination of pertinent information for risk management.

ACKNOWLEDGMENTS

This study was funded by a NOAA Oceans and Human Health Initiative grant (NA-04-OAR4600214), and partial support to A.M.B.P. was provided by NASA, Applied Sciences Directorate. We thank the following agencies and individuals for providing laboratory equipment, space, and/or aid in sample collection and analyses: Mr. John Tennyson and Ms. Angela Ruple, NOAA, NMFS, Seafood Inspection Laboratory; Mr. Scott Gordon, Mississippi Department of Marine Resources; Mr. George Blackstone and Ms. Jessica Nordstrom, FDA, Dauphin Island Seafood Laboratory; Dr. Greg Carter, USM, Gulf Coast Geospatial Center; and Dr. Crystal Johnson and Ms. Dawn Rebarchik, USM, Gulf Coast Research Laboratory.

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