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Trends in total *Vibrio* spp. and *Vibrio vulnificus* concentrations in the eutrophic Neuse River Estuary, North Carolina, during storm events

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ABSTRACT: *Vibrio* spp. are ubiquitous members of aquatic microbial food webs that can be pathogenic to humans and a range of other organisms. Previously published predictive models for *Vibrio* spp. concentrations in estuarine and coastal waters, based only on salinity and temperature, are 70 to 75% accurate during 'normal' conditions (e.g. not during storms or drought). We have conducted a preliminary comparison of the output from this type of model to the natural concentrations of both total *Vibrio* spp. and the potentially pathogenic *Vibrio vulnificus* when measured during tropical storms. Water samples were collected *in situ* from a deployed platform in the Neuse River Estuary (NRE), North Carolina, USA, during 2 storm events: Hurricane Ophelia and Tropical Storm Ernesto. Total *Vibrio* spp. concentrations were measured using culture-based methods and *V. vulnificus* levels were determined using a newly developed, rapid quantitative polymerase chain reaction (QPCR) assay. Results were analyzed in relation to environmental parameters and to concentrations of the fecal indicator bacteria *Escherichia coli* (EC) and *Enterococcus* spp. (ENT). Total concentrations of *Vibrio* spp. in the NRE were often orders of magnitude higher than those predicted by a previously published model. These large deviations from model predictions may indicate contributions from storm forcing (e.g. resuspension, surges) that are missing from the calm weather observations used to build these models.

KEY WORDS: *Vibrio* spp. · *Vibrio vulnificus* · Storm events · Sediment resuspension · Neuse River Estuary · Water-born pathogens · Quantitative polymerase chain reaction · QPCR

INTRODUCTION

Since the mid-1990s, the United States (US) east coast has experienced an increase in storm activity, which is considered to be part of a multi-decadal change in hurricane activity for the North Atlantic (Goldenberg et al. 2001). Research in the Neuse River Estuary (NRE) has documented significant storm impacts following hurricanes in relation to phytoplankton dynamics, nutrient enrichment and hydrologic perturbations (Peierls et al. 2003, Paerl et al. 2006a,b, Valdes-Weaver et al. 2006). Nutrient over-enrichment of the estuary is a result of proliferating upstream animal (hog and poultry farms) and human populations (Stow et al. 2001, Paerl et al. 2006a). Additionally, post-storm increases in NRE bacterial groups with pathogenic members, particularly the *Vibrio* genus, have been documented (Fries et al. 2008, Hsieh et al. 2008).

Vibrio spp. are ubiquitous, heterotrophic bacteria that are native to coastal, estuarine, and marine environments worldwide. As autochthonous components of aquatic microbial food webs and in symbioses with a range of marine organisms, the more than 50 species in this genus play important ecological roles in aquatic

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environments (e.g. Ruby & Nealson 1976, Colwell et al. 1977). The genus also includes 3 important human pathogens: *Vibrio cholera*, *Vibrio parahaemolyticus* and *Vibrio vulnificus.* All 3 of these pathogens have been demonstrated to have human health impacts on a global scale and are considered to be emerging or re-emerging marine pathogens (Colwell 1996, Tantillo et al. 2004). *Vibrio* spp. are responsible for thousands of infections from both ingestion and contact in the US annually (Altekruse et al. 1997).

Although other *Vibrio* spp. can cause primary septicemia and wound infection, the most severe of these infections are often associated with *Vibrio vulnificus* (Tantillo et al. 2004, Oliver 2005, WHO & FAO 2005). Due to a variety of predisposing conditions (Oliver & Kaper 2007), the Centers for Disease Control and Prevention (CDC) estimate that, every year, between 12 and 30 million people in the US are at risk of serious injury or death from *V. vulnificus* (CDC 2007). From 2003 to 2004, 142 cases of *Vibrio* sp. infections related to water-based recreational activities were reported in the US. Of these, more than half were identified as either *V. vulnificus* (47 cases) or *Vibrio parahaemolyticus* (34 cases). All amputation cases were related to those 2 species (Dziuban et al. 2006). Following hurricane Katrina, 22 cases of *Vibrio* sp. wound infections and 5 resulting deaths were reported (CDC 2005).

Generally, *Vibrio vulnificus* makes up a small portion of the *Vibrio* community in seawater samples. Pfeffer et al. (2003) found that *V. vulnificus* accounted for roughly 8% of the total *Vibrio* spp. cultured from eastern NC estuarine water samples. This proportion agreed closely with 2 studies of culturable *Vibrio* spp. in the Adriatic Sea, which identified less than 10 or 2% as *V. vulnificus*, respectively (Baffone et al. 2006, Masini et al. 2007). *V. vulnificus* has been detected in marine animals, sediments and plankton at levels typically similar to those in surrounding seawater (Oliver et al. 1982, 1983, Aono et al. 1997, Baffone et al. 2006).

Temperature and salinity are often the most important environmental factors controlling *Vibrio* spp. abundance in estuarine and marine waters. Research in the US as well as worldwide has indicated that storm activity and increasing water temperatures can lead to outbreaks of pathogenic *Vibrio* spp. (Hoi et al. 1998, CDC 2005, Paz et al. 2006). Total *Vibrio* spp. abundance is typically high in summer and low, often undetectable, in cooler months when temperatures are below 10 to 12°C (Kaysner et al. 1987, O'Neill et al. 1992, Lin et al. 2003, Pfeffer et al. 2003, Randa et al. 2004). A temperature range of 15 to 30°C is thought to be ideal for *Vibrio vulnificus* (Oliver 2005).

While mesohaline waters are optimal for the growth of *Vibrio* spp., the influence of salinity on these species appears to be complex and dependent on specific environments (Oliver et al. 1982, 1983, O'Neill et al. 1992, Wright et al. 1996, Lipp et al. 2001, Lin et al. 2003, Pfeffer et al. 2003, Randa et al. 2004, Hsieh et al. 2007). Nonetheless, in most cases, very low (<5) and very high (>25) salinities appear to be detrimental to *Vibrio vulnificus* growth and persistence (Kaspar & Tamplin 1993, Motes et al. 1998). In the NRE, total *Vibrio* spp. concentrations show a positive correlation with salinity seasonally (Hsieh et al. 2007, 2008, Fries et al. 2008), although other authors have found temperature to be a more important factor (Pfeffer et al. 2003).

Previous studies (Pfeffer et al. 2003, Hsieh et al. 2008) have shown distinct seasonal and geographic patterns in *Vibrio* concentrations in the NRE. A relationship between environmental factors and total *Vibrio* spp., based on monitoring data from multiple stations and seasons, has been documented and used to develop predictive models (Hsieh et al. 2008). However, the study of Hsieh et al. (2008) also noted a deviation from the model prediction during Hurricane Ophelia in 2005, with high concentrations of total *Vibrio* spp. documented before and immediately after passage of the storm. Another study documented a coincident increase in total *Vibrio* spp. abundance in NRE sediments following Hurricane Ophelia (Fries et al. 2008). In these cases, storm activity may have introduced sedimentary populations to the water column through resuspension. These observations, in conjunction with increased storm activity for the US east coast, have raised questions about the ecological response of total *Vibrio* spp. and their pathogenic subpopulations during storm events.

In order to gain further understanding of *Vibrio* spp. population response to extreme storm events, we used an *in situ* sampling platform to collect water samples in the NRE when boat access and typical monitoring were not possible (approach outlined in Fries et al. 2008). A range of bacterial, environmental and meteorological data were collected over the duration of each storm, with multiple observations on several different dates. We utilized a newly developed quantitative polymerase chain reaction (QPCR) assay, specific for virulent forms of *Vibrio vulnificus*, to enumerate the pathogen over the course of the 2 storms. Finally, we compared the storm data to the output from the previously published salinity-temperature model for total *Vibrio* spp. in the NRE (Hsieh et al. 2008). This comparison allowed a preliminary examination of the effects of different types of storms on *Vibrio* spp. populations (from *in situ* data collection) in relation to a model developed under 'normal' conditions (not during storms or drought) for the NRE. In addition, this study begins to examine how models for total *Vibrio* spp. perform in relation to pathogenic subsets. As more data is collected, we hope to develop a similar model for *V. vulnificus*.

MATERIALS AND METHODS

Site and sample collection. Two sites for *in situ* instrument deployment (Fig. 1) in the NRE were selected to be co-located with stations sampled regularly by the NRE Modeling and Monitoring (ModMon) program. The NRE is a well-studied, highly eutrophic estuary that has recently experienced extreme storm events (i.e. hurricanes and tropical storms). The selected sites were ModMon Stn NRE30 near New Bern, NC (3.5 m water depth) and ModMon Stn NRE120 (28 km further downstream, 5 m water depth). Samples for this study were collected before, during and after 2 storms: Hurricane Ophelia at Stn NRE30 (August 27 to September 30, 2005) and Tropical Storm Ernesto at Stn NRE120 (August 29 to September 8, 2006).

The 2 storms differed in some respects (Fig. 1): Hurricane Ophelia passed closest to the NC coast on September 14, 2005, but the effects of the slow-moving storm began to be felt as strong northeast winds several weeks before passage. These winds perturbed the estuary through mixing and a significant surge of salt water into the upper NRE (Fries et al. 2008). Tropical Storm Ernesto made landfall on the southeastern NC coast on September 1, 2006. Unlike Hurricane Ophelia, this storm moved quickly past the NRE to the west, leading to runoff impacts from inland rainfall after passage. Similarities between the storms include the duration of acute impacts of wind and rain (Fig. 2; data from the National Weather Service, Eastern Region Headquarters, recorded at New Bern Airport: www.erh.noaa.gov). Total rainfall during each storm was similar (12 cm during Hurricane Ophelia and 14 cm during Tropical Storm Ernesto). Peak sustained winds were also comparable (16 and 13 m s^{-1}, respectively) and were sufficient to resuspend sediment into the water column (i.e. >2.2 m s^{-1}, see Fries et al. 2008).

***In situ* sampling and water quality data.** The platform (Time-series Aquatic Contamination Observer or TACO) deployed for this study consists of an ISCO 6712 sampler programmed to collect a total of 24 samples (each 1 l) on a pre-programmed schedule (once every 16 h), plus additional event samples triggered by instruments (see Fries et al. 2008). Sampling depth is approximately 0.5 m above the sediment. A turbidity sensor (Forest Technology Systems Limited, Model DTS-12) was used to trigger the collection of samples with large particle loads (e.g. resuspension, runoff); turbidity (NTU) and temperature (T, in °C) were measured at 10 min intervals. A refractometer (VISTA, model A366ATC) was used in the lab to measure salinity (S). Total suspended solids (TSS) were measured as the dry mass captured onto 0.7 µm glass fiber filters (GF/F, Whatman) after filtering 50 ml of sub-samples (Standard Method 2540D Standard Methods for the Examination of Water and Wastewater, 20th edn, American Public Health Association).

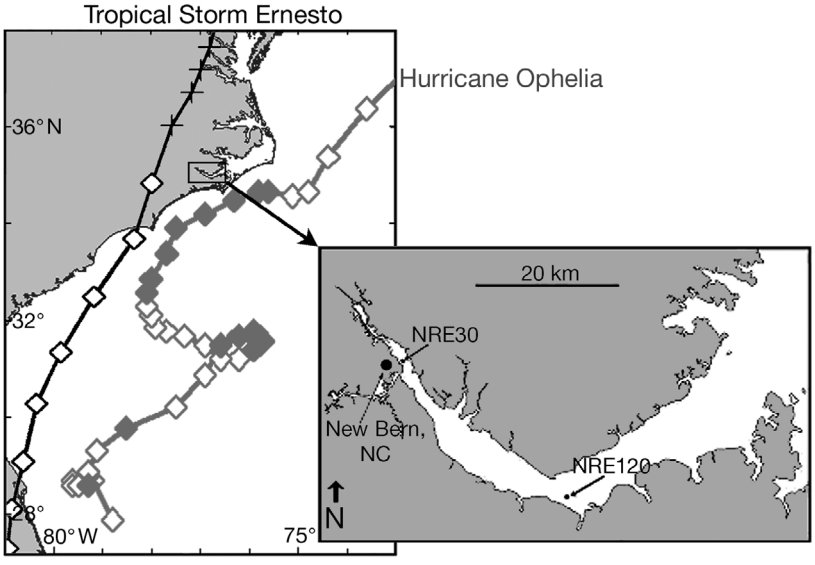

Fig. 1. Map of the study area in North Carolina, USA, and storm tracks for Hurricane Ophelia (September 2005, grey symbols) and Tropical Storm Ernesto (September 2006, black symbols) in relation to the eastern seaboard of the US. On both storm tracks, symbols indicate storm position every 6 h and storm strength (crosses for tropical depression, open diamonds for tropical storm, and closed diamonds for Category 1 hurricane). Samples were taken from Stn NRE30 during Hurricane Ophelia and Stn NRE120 during Tropical Storm Ernesto

Bacterial analyses. The fecal indicator species *Escherichia coli* (EC) and *Enterococcus* (ENT) were isolated and quantified using Colilert-18® and Enterolert™, respectively. High most probable number (MPN) Quanti-tray/2000® (IDEXX Laboratories) quantification trays were used for analyses. After preparing the water samples and pouring into the Quanti-trays, the Colilert-18® and Enterolert™ trays were incubated for 18 h at 35°C and 24 h at 41°C, respectively. MPN per 100 ml and confidence intervals were calculated based on combined numbers of small and large positive wells in the Quanti-trays (Hurley & Roscoe 1983, Fries et al. 2006).

Total *Vibrio* spp. enumeration was conducted using a 25-fold dilution series based on previously determined relationships between total *Vibrio* spp. concentrations and salinity and temperature (Hsieh et al. 2008). Replicate samples were diluted with sterile phosphate buffered saline (PBS), then

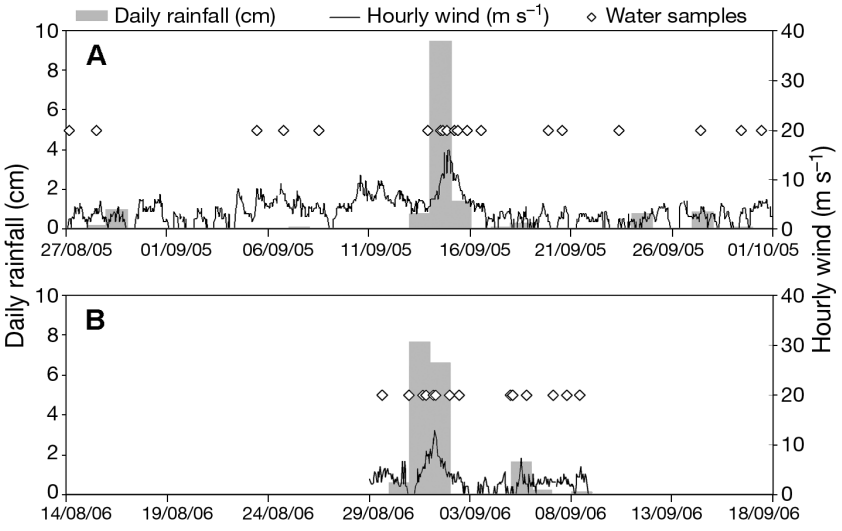

Fig. 2. Meteorological conditions during (A) Hurricane Ophelia and (B) Tropical Storm Ernesto at New Bern, NC. Time axes for both panels are same duration (dates given as dd/mm/yy), but the duration of sampling differed between storms. Data from the National Weather Service, Eastern Region Headquarters, recorded at New Bern Airport (www.erh.noaa.gov)

filtered onto 0.45 µm pore size, sterile gridded filters (GN-6 Metricel Grid, Pall) and plated onto Thiosulfate Citrate Blue Sucrose (TCBS) agar (Difco). Plates were incubated at 37.5°C for 24 h, and yellow or green colonies were counted and reported as colony forming units (CFU) per 100 ml. Resulting CFU are reported in this paper as total *Vibrio* spp.

Molecular analyses. For each water sample, 100 ml of undiluted sample was filtered in duplicate through 47 mm, 0.4 µm polycarbonate (PC) filters (HTTP, Millipore) and stored at –80°C until extraction. DNA extraction was done according to the alternative protocol for maximum yield with the UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories) with a mini bead beater (Biospec Products). A 50 µl volume of eluted DNA was stored at –20°C until further analysis. Standards were prepared by growing *Vibrio vulnificus* (ATCC 27562) in Marine Broth 2216 (Difco) at 37°C for 24 h. The cell suspension was diluted in phosphate buffered saline (PBS) and quantified under epifluorescence microscopy using SYBR Green I (Invitrogen) according to Noble & Fuhrman (1998). Cell suspensions were diluted sufficiently so that aliquots containing 100 000 cells were filtered onto PC filters and stored at –80°C until extraction. Blank filters were also extracted as above and the resulting extracts were used as an additional negative QPCR control.

The QPCR assay was designed to target a 179 bp region of the hemolysin genes, *vvhA* and *vvhB*, of *Vibrio vulnificus* (GenBankM34670). Primers and the TaqMan probe were designed using Primer3 (Rozen & Skaletsky 2000) and were synthesized by MWG Biotech. Primers were 20- and 24-bases, *vvhA* 1973 rev (5′-TCG ACT GTG AGC GTT TTG TC-3′) and *vvhA* 1795 (5′-TGC CT(AG) GAT GTT TAT GGT GAG AAC-3′). The 26-base Taqman® probe was Vvh 1914 (5′-FAM-TAG CCG AGT (AG)GC ATC CGA TCG TTG TT-BHQ-1-3′). Search results from the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST/; National Center for Biotechnology Information) indicated that the primers, probe and amplicon nucleotide sequences were highly unique to the *V. vulnificus* cytolysin/hemolysin gene.

QPCR reactions were carried out in duplicate with a Cepheid Smart Cycler II® using the above oligonucleotide primers and probe and Premix Ex Taq™ (Takara Bio USA). PCR reactions contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 µM of primers, 100 nM of probe, 1.5 U Takara Taq, and 5 µl extracted sample or standard. Positive controls were *Vibrio vulnificus* cell suspensions, grown and extracted as described above and serially diluted for a standard curve with a highest expected concentration of 10 000 cells. Diluted cell suspensions were run in a duplicate 4-log standard curve. Non-template controls (NTC) and negative extraction controls were run in duplicate with each set of samples and standards. All negative controls were negative in resulting reactions. Cycling parameters were: 120 s hot start at 95°C, 40 cycles at 95°C for 15 s, and 60°C for 30 s. Concentrations of *V. vulnificus* cells were calculated using the standard curve generated within the Smart Cycler II® software. In some instances, replicates yielded results consisting of a non-detect and a quantified value. In this case the quantified value was reported.

Inhibition within QPCR samples was tested by spiking the sample extracts with 5000 *Enterococcus casseliflavus* cells per reaction and simultaneously running a second QPCR analysis for *E. casseliflavus* (primers and probe designed by Jorge Santo Domingo, USEPA NERL) on all NRE samples. Replicate NTC samples were also spiked. *E. casseliflavus* has been associated with soils and plant silage (Pinto et al. 1999), and for this study was used as the internal control based upon its previously determined absence in NRE waters (unpubl. data), and its similar behavior to *Vibrio* spp. target DNA. Detection of spiked cells at a predetermined concentration indicated no inhibition of the reaction due to sample matrices and increases our confidence in results with low or null signal in the *Vibrio vulnificus* reaction.

Frozen aliquots of *E. casseliflavus* cells were heat-lysed, and standards were serially diluted from a starting concentration of 50 000 cells per reaction. QPCR reactions were carried out on the Smart Cycler II® with Premix Ex Taq™ as described above. Cycling parameters were: hold at 95°C for 120 s, 45 cycles at 94°C for 30 s, and 60°C for 45 s. Sample inhibition was defined as a cycling threshold (Ct) value that was 1.5 Ct values away from the mean replicate spiked NTC values. None of the samples analyzed over the course of the study appeared to be inhibited.

Statistical analyses. Parameters for correlation analysis included T, S, TSS, NTU, and log-transformed concentrations of EC, ENT, total *Vibrio* spp., and *Vibrio vulnificus.* Parameters were analyzed for individual storm periods, encompassing dates prior to, during and immediately after the storms. Independent 2-tailed *t*-tests assuming equal variances (as a result of Levene's test) were performed on environmental and bacterial data between storm datasets. Multiple regression analyses were done using Pearson's correlations between each *Vibrio* spp. dataset and T, S, or S and T combined. Correlations were considered significant at $p \leq 0.05$. All analyses were conducted using the software SPSS v.11. Non-detects for EC, ENT and *V. vulnificus* were not included in statistical analyses.

RESULTS AND DISCUSSION

Environmental data during storms

Comparison of data collected during the periods surrounding Hurricane Ophelia and Tropical Storm Ernesto revealed both similarities and differences between the 2 storms (Table 1). The T range was similar between events, with a small amount of fluctuation. However, T was higher during Tropical Storm Ernesto (*t*-test, $p < 0.05$). Range and mean of S during Hurricane Ophelia were lower (*t*-test, $p < 0.01$) than for Tropical Storm Ernesto, but this is typical of Stn NRE30, which is further upstream in the estuary (Fig. 1). Note that almost all of the data fall in the S (5 to 25) and T (15 to 30°C) ranges considered ideal for the growth of *Vibrio vulnificus* in the NRE and elsewhere (Kaspar & Tamplin 1993, Motes et al. 1998, Oliver 2005). TSS and NTU during both storms were more variable than the same parameters measured during monitoring (authors' unpubl. data), highlighting the ability to sample episodic suspensions using the platform. Suspensions appeared to be sediment resuspension events, based on the high average ratio (>1) of TSS to NTU (Fries et al. 2007). Concentrations of fecal indicator bacteria varied during both storms, with higher concentrations of EC in the time period surrounding Hurricane Ophelia (*t*-test, $p < 0.05$). The US Environmental Protection Agency recommended single sample water quality standards (104 MPN 100 ml^{-1} ENT and 320 MPN 100 ml^{-1} for EC) were exceeded in 2 samples collected following the storm events, indicating a likelihood of contamination from stormwater runoff. Total *Vibrio* spp. and *V. vulnificus* concentrations were an order of magnitude higher during Tropical Storm Ernesto than during Hurricane Ophelia (*t*-tests, $p < 0.01$). *V. vulnificus* was also detected much more frequently in the time period surrounding Tropical Storm Ernesto (80% of samples), compared to Hurricane Ophelia (31% of samples).

Table 1. Environmental parameters and bacterial concentrations (*Vibrio* spp., *Vibrio vulnificus*, and fecal indicator bacteria) immediately prior to, during, and after Hurricane Ophelia (max. n = 19) and Tropical Storm Ernesto (n = 15). Range, mean, standard deviation (SD) of values. Results of *t*-tests (*t* and p values) for storm comparisons are also presented. Mean ± SD for bacterial concentrations calculated using log-transformed data resulting in ± SD range (in parentheses). TSS: total suspended solids; EC: *Escherichia coli*; ENT: *Enterococcus* spp.; MPN: most probable number; CFU: colony forming unit; ns: not significant

Parameter	Hurricane Ophelia		Tropical Storm Ernesto		Storm comparison	
	Range	Mean ± SD	Range	Mean ± SD	*t*	p
Temperature (°C)	24.5 to 28.3	26.0 ± 1.1	25.0 to 29.4	27.1 ± 1.6	–2.41	0.022
Salinity	3 to 11	6 ± 2	4 to 15	12 ± 4	–5.84	<0.001
TSS (mg l^{-1})	4.0 to 35.6	11.9 ± 9.8	4.7 to 84.5	16.8 ± 20.6	ns	>0.05
Turbidity (NTU)	3.8 to 28.6	10.0 ± 7.4	3.4 to 55.5	12.6 ± 13.4	ns	>0.05
EC (MPN 100 ml^{-1})[a]	10 to 407	35 (13 to 94)	5 to 12	9 (5 to 14)	2.35	0.033
ENT (MPN 100 ml^{-1})[b]	8 to 123	32 (16 to 66)	5 to 892	17 (4 to 80)	ns	>0.05
Total *Vibrio* spp. (CFU 100 ml^{-1})	93 to 30 900	2790 (500 to 15 500)	205 to 271 000	28 200 (2380 to 335 000)	–3.18	0.003
Vibrio vulnificus (cells 100 ml^{-1})[c]	2 to 91	10 (2 to 53)	13 to 2044	199 (43 to 920)	–3.57	0.003

Due to no detection, the number of samples included in the analyses differed from total number of samples taken as follows: [a]n = 14 for Hurricane Ophelia / n = 3 for Tropical Storm Ernesto, [b]n = 17 / n = 9, [c]n = 4 / n = 12

Although the storms were similar in some respects (TSS, NTU, rainfall totals, peak wind speeds), sampling location may play an important role in the bacterial response and may explain the differences seen in total *Vibrio* spp. numbers and the detection of *Vibrio vulnificus*. During summer and fall, Stn NRE30 is often upstream of the mesohaline region of the NRE. Wetz & Paerl (2008) found that this region, which encompasses Stn NRE120, is often significantly affected by storms. Phytoplankton blooms occur frequently in this region, generating low nutrient levels in the upper water column. Vertical fluxes of nutrient-replete waters through wind-driven mixing and resuspension during storms can quickly replenish the upper water column, inducing post-storm phytoplankton blooms. Areas of high concentrations of both total *Vibrio* spp. and *V. vulnificus* have been found to coincide with the peak phytoplankton region of the NRE (Hsieh et al. 2007). Similarly, storm-driven mixing could introduce sediment-bound bacteria (Lipp et al. 2001, Randa et al. 2004, Fries et al. 2008). However, if pre-storm conditions favor a well-mixed, high-nutrient water column, storm effects may have less of an impact on water column ecology (Wetz & Paerl 2008). Several days of along-channel northeast winds prior to the landfall of Hurricane Ophelia generated a significant storm surge and multiple mixing events prior to our sampling. In contrast, Tropical Storm Ernesto was preceded by mostly southerly winds, which limited resuspension and did not prevent water column stratification. Mixing due to storm-induced northeasterly winds could then have introduced both sediment-bound bacteria and nutrients, leading to a more pronounced change in *Vibrio* spp. concentrations during Tropical Storm Ernesto.

Correlations of *Vibrio* concentrations with salinity and temperature, and comparison to model

Correlations between *Vibrio* concentrations (total *Vibrio* spp. and *Vibrio vulnificus*) and environmental and bacterial data during Hurricane Ophelia were not significant ($p \geq 0.05$, Table 2). The lack of a relationship may be due to data limitations or site-specific characteristics unrelated to S and T that may influence *Vibrio* populations at Stn NRE30. During Tropical Storm Ernesto, in contrast, significant and positive correlations were found between total *Vibrio* spp. and S and T, similar to the predictions from other salinity-temperature models, and between *V. vulnificus* and temperature (Table 2). This result is consistent with numerous studies that have found S and T to be important factors in predicting *Vibrio* concentrations (e.g. Pfeffer et al. 2003, Randa et al. 2004, Hsieh et al. 2008). In the NRE, previously published models accurately predict total *Vibrio* spp. concentrations under normal environmental conditions. However, total *Vibrio* spp. concentrations (in CFU 100 ml^{-1}) during both storms were often greater than those predicted for the same S and T using the model developed by Hsieh et al. (2008) for the NRE ($n = 417$, $R = 0.77$, $p < 0.01$; Fig. 3):

$$\log(\textit{Vibrio}) = -0.304 + (0.116 \times S) + (0.0739 \times T)$$

The data for Tropical Storm Ernesto from this study was regressed against both S and T and generated a comparable model ($n = 15$, $R = 0.92$, $p < 0.001$; Fig. 4):

$$\log(\textit{Vibrio}) = -3.889 + (0.196 \times S) + (0.221 \times T)$$

However, the predictions from our model are significantly higher for the range of conditions observed (Fig. 4); i.e. during storms, the Hsieh et al. (2008) model underestimates total *Vibrio* spp. concentrations. Total *Vibrio* spp. concentrations prior to Hurricane Ophelia (August 28) and the post-storm data for Tropical Storm Ernesto (September 6, 7 and 8) agree much more closely with the Hsieh et al. (2008) model (Fig. 3). During the storms, the Hsieh et al. (2008) model does appear to track measured total *Vibrio* spp. concentration; however, the

Table 2. *Vibrio* spp. and *Vibrio vulnificus*. Correlations with environmental parameters and log-transformed fecal indicator bacteria concentrations during Hurricane Ophelia (n = 19) and Tropical Storm Ernesto (n = 15). R: Pearson product-moment correlation coefficients for log-transformed total *Vibrio* spp. (in CFU 100 ml^{-1}) and *Vibrio vulnificus* (in cells 100 ml^{-1}); n: number of samples; p: significance, with $p \leq 0.05$ considered significant (*); TSS: total suspended solids; EC: *Escherichia coli*; ENT: *Enterococcus* spp.; MPN: most probable number. Variance in sample size due to non-detects

	Total *Vibrio* spp.			*Vibrio vulnificus*		
	R	n	p	R	n	p
Hurricane Ophelia						
Temperature (°C)	–0.45	19	0.053	–0.03	4	0.96
Salinity	0.28	19	0.24	0.19	4	0.76
TSS (mg l^{-1})	0.22	19	0.28	0.25	4	0.64
Turbidity (NTU)	0.20	19	0.41	0.1	4	0.96
EC (MPN 100 ml^{-1})	0.19	14	0.52	0.2	4	0.80
ENT (MPN 100 ml^{-1})	0.16	17	0.57	0.41	3	0.50
Tropical Storm Ernesto						
Temperature (°C)	0.75*	15	0.001	0.62*	12	0.031
Salinity	0.89*	15	<0.001	0.44	12	0.16
TSS (mg l^{-1})	0.49	14	0.069	0.24	12	0.37
Turbidity (NTU)	0.24	14	0.42	0.18	12	0.57
EC (MPN 100 ml^{-1})	–0.13	3	0.94	0.88	3	0.29
ENT (MPN 100 ml^{-1})	–0.49	9	0.22	0.67	7	0.079

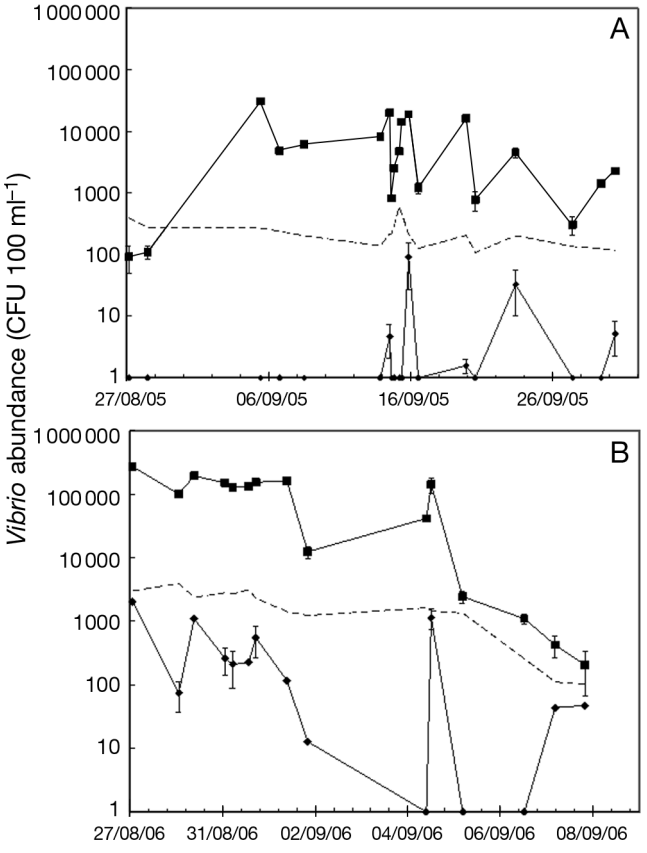

Fig. 3. *Vibrio* spp. (■) and *Vibrio vulnificus* (◆). Total concentrations during (A) Hurricane Ophelia and (B) Tropical Storm Ernesto. Error bars = SD. Dashed lines show predicted values for total *Vibrio* spp. using a model, based on salinity and temperature (Hsieh et al. 2008). Dates given as dd/mm/yy

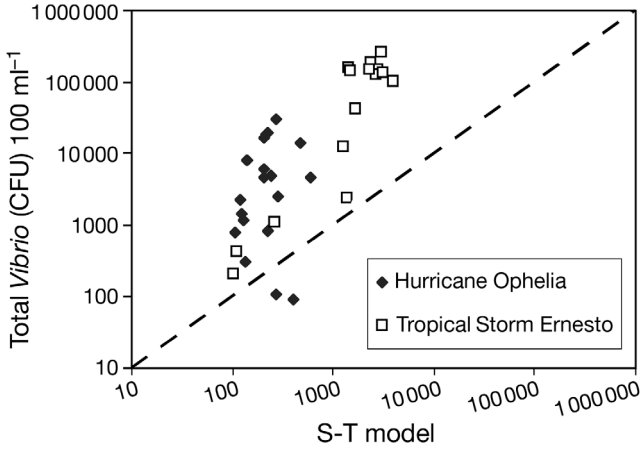

Fig. 4. *Vibrio* spp. Comparison of total concentrations with previously published model for *Vibrio*, based on salinity and temperature (S–T) (Hsieh et al. 2008). Bacterial abundance reported in CFU or cells 100 ml^{-1}. Concentrations from Hurricane Ophelia and Tropical Storm Ernesto plotted with 1:1 line (dashed) indicative of agreement between measurements and predictions

magnitude of the changes is not accurately predicted. Concentration peaks for the bacterial data were similar, and a relationship between total *Vibrio* spp. and *Vibrio vulnificus* was apparent during Tropical Storm Ernesto, for which total *Vibrio* spp. and *V. vulnificus* concentrations were correlated ($R = 0.66$; $p = 0.02$; $n = 12$). Lower values for total *Vibrio* spp. (≤1000 CFU 100 ml^{-1}) seem to co-occur with the non-detection of *V. vulnificus*, indicating the likelihood that *V. vulnificus* represents a small, but significant, portion of the total *Vibrio* spp. concentration.

CONCLUSIONS

Extreme climate events, namely storms and droughts, are important drivers of the function of estuarine systems, especially those prone to shallow, wind-driven mixing such as the NRE (e.g. Paerl et al. 2001, Wetz & Paerl 2008). The current pattern of elevated hurricane and storm activity is expected to last for several decades (Goldenberg 2001), and as a byproduct of global climate change, storms are predicted to increase in incidence and intensity (Emanuel 2005, Webster et al. 2005). Some attention has been invested in understanding the effect of rainfall on human health, largely focused on the input of microbial contaminants via stormwater to coastal and estuarine environments (Curriero et al. 2001). In contrast, little attention and research has been devoted to understanding the impact of storms on bacterial pathogens that are naturally found in estuarine environments, including the *Vibrio* genus. Given the strong predictive relationships previously found between salinity/temperature and total *Vibrio* spp. and the impact of storms in the form of estuarine mixing and 'freshening' of the water column, one might naturally expect that storms would have an important effect on *Vibrio* spp. populations. We present a novel scheme for assessing the ecology of *Vibrio* spp. and specific pathogenic species subsets, such as *Vibrio vulnificus*, during extreme storm events, where in the past we have observed deviation from previously published predictive models (Hsieh et al. 2008). The approach combines (1) *in situ* sample collection as detailed in Fries et al. (2008) during extreme storm events, when boat-based sampling is not possible; (2) use of new QPCR methods for the quantification of *V. vulnificus* in estuarine water samples; and (3) comparison of data collected during storm events to assess the differential response of *Vibrio* spp. to salinity and temperature regimes during storms. This initial comparison demonstrated deviations from models and indicated that storms can have substantial effects on *Vibrio* spp. populations within an estuarine environment. The dataset is small, but further research will

provide the sample size necessary for creating new predictive models for individual *Vibrio* species, in relation to the entire genus, during specific times of extreme climatic influences. Furthermore, comparison of the total *Vibrio* spp. and *V. vulnificus* models will provide insight into how salinity and temperature affect individual species differently than the entire population. In the future, as the dataset expands and as more storms are characterized, it will be possible to conduct further analyses that will increase the usefulness of the observed relationships. For example, it will be possible to use the ratio of *V. vulnificus* to the total *Vibrio* spp. from the respective models to demonstrate the relative importance of environmental factors on the pathogenic *Vibrio* species and to improve methods for the detection of sedimentary *Vibrio* spp. concentrations. This additional information will empower modelers to assess the consequences of the increased estuarine water temperature and storm frequency likely to occur with global climate change. Future research will be necessary to understand the role of a range of climatic events, including extreme droughts, storms and floods — the frequency and intensity of which appear to be increasing — on the growth/success and transport of autochthonous pathogens within highly productive estuarine environments. Research of this nature could provide data to model the fate, transport and presence of pathogens in relation to the risk to human health. More extensive data collection will improve the diagnosis of *Vibrio* spp. population dynamics, but the scheme presented here should prove to be a useful tool to employ in future studies.

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