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Plankton composition and environmental factors contribute to *Vibrio* seasonality

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Plankton represent a nutrient-rich reservoir capable of enriching *Vibrio* species, which can include human pathogens, at higher densities than the surrounding water column. To better understand the relationship between vibrios and plankton, the partitioning of culturable vibrios, on TCBS, between free living and plankton associated (63–200- and >200-μm-size fractions) was monitored over a 1-year period in coastal waters of Georgia, USA. Seasonal changes in the total *Vibrio* concentration were then compared with changes in environmental parameters as well as changes in the relative composition of the plankton community. Using univariate analyses, *Vibrio* concentrations were strongly associated with temperature, especially when those vibrios were plankton associated ($R^2 = 0.69$ and 0.88 for the water and both plankton fractions; respectively) ($P < 0.01$). Multivariate general linear models revealed that *Vibrio* concentrations in the plankton fractions were also correlated to shifts in the relative abundance of specific plankton taxa. In the 63–200-μm fraction, *Vibrio* concentrations were inversely associated with copepods, cyanobacteria and diatoms. In the >200-μm fraction, *Vibrio* concentrations were positively associated with copepods and negatively associated with decapod larvae. Our results confirm the role of temperature in *Vibrio* seasonality and highlight an important and independent role for plankton composition in explaining seasonal changes in *Vibrio* concentration.

Introduction

Vibrio species are autochthonous members of the bacterial community in warm estuarine and coastal waters worldwide (Thompson *et al.*, 2003a). They are among the most abundant bacteria readily cultured from the marine environment and exhibit distinct seasonal variation in population density and diversity (Thompson *et al.*, 2003a). Ecologically, vibrios play an important role in the degradation of organic matter and act as a link that transfers dissolved organic carbon to higher trophic levels of the marine food web (Grossart *et al.*, 2005). Epidemiologically, at least 12 *Vibrio* species are known to be important pathogens of humans and marine animals (Colwell and Grimes, 1984; Morris Jr, 2003; Panicker *et al.*, 2004).

The association of vibrios with planktonic organisms, especially copepods, has been suggested as an important component of *Vibrio* ecology, especially for *Vibrio cholerae* (Sochard *et al.*, 1979; Huq *et al.*, 1983; Huq *et al.*, 2005). Plankton represent organic-rich microenvironments (Long and Azam, 2001; Grossart *et al.*, 2005), and the high nutrient concentrations of the plankton microhabitat can selectively enrich heterotrophic bacteria, including vibrios (Huq *et al.*, 1983; Tamplin *et al.*, 1990; Lipp *et al.*, 2003; Long *et al.*, 2005). The production of an extracellular chitinase allows vibrios to utilize the chitinous exoskeletons of some plankton taxa as a source of carbon and nitrogen (Thompson *et al.*, 2003a), leading to the hypothesis that some vibrios may show a competitive advantage when plankton associated (Heidelberg *et al.*, 2002a).

Plankton colonized by pathogenic *Vibrio* species can potentially act as a vehicle of disease transmission, as in the case of cholera (Huq and Colwell, 1996; Huq *et al.*, 2005); however, the details of the interactions between other members of the *Vibrio* genus and the plankton community remain largely unknown. Recent studies indicate

that the structure and composition of the heterotrophic bacterial community may be dependent upon the structure and composition of the plankton community (Riemann *et al.*, 2000; Fandino *et al.*, 2001; Pinhassi *et al.*, 2004). It has also been hypothesized that individual plankton species may harbor specific bacterial communities (Grossart *et al.*, 2005).

The objective of this study was to determine whether changes in the composition of the plankton community contribute to the seasonality of the total culturable *Vibrio* population, either synergistically with or independent of temperature. Several studies have described the relationship between vibrios and plankton by characterizing the *Vibrio* population as either free living or plankton associated (Baffone *et al.*, 2006), but few studies have addressed the relationship between the structure and composition of the plankton community and that of the *Vibrio* population (Heidelberg *et al.*, 2002a; Grossart *et al.*, 2005; Huq *et al.*, 2005). We hypothesized that shifts in the composition of the plankton reservoir contribute to the observed seasonality in the *Vibrio* population.

Materials and methods

Sampling sites
This study included 12 sampling sites along the coast of Georgia, USA (Figure 1). The study sites were representative of two distinct estuaries, Sapelo Sound and Wassaw Sound. Typical of the South Atlantic Bight (southeastern USA) and coastal Georgia in particular, mixing in these estuaries is dominated by tidal exchange (Verity *et al.*, 2006). Furthermore, these two estuaries are unique in that they receive little direct river input.

Sampling sites were selected in coordination with an on-going water quality program administered by the Georgia Department of Natural Resources. Sites were selected to represent a range of environments and hydrological conditions including tidal creeks and open-water sounds. Each site was part of a network of oyster beds open for commercial or public harvest. The 12 stations were divided equally between Wassaw Sound (Chatham County) and Sapelo Sound (McIntosh County) and were sampled bi-monthly beginning in January 2006 and ending in February 2007.
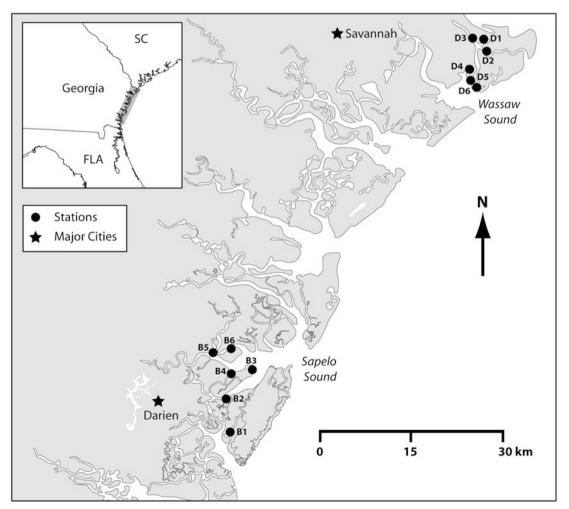

Figure 1 Map of study sites.

Sample collection
Monthly sample collection coincided with the ebb tide of spring tide events. Surface temperature (°C), salinity and dissolved oxygen (DO) (% saturation) were recorded at each sampling site using a YSI Multi-meter (YSI Environmental, model 556, Yellow Springs, OH, USA). Three fractions were collected from each site: surface water and two plankton fractions (63–200 and >200 μm). Briefly, approximately 1 l of surface water was collected in a sterile polypropylene bottle. Plankton fractions were collected by a 5-min horizontal tow of 63- and 200-μm plankton nets (Sea-Gear Corp., model 9000, Melbourne, FL, USA) at <1 m depth. The 63-μm fraction was subsequently filtered through a 200-μm mesh. Plankton samples were then washed and resuspended to a volume of 1 l in a sterile polypropylene bottle using sterile phosphate-buffered saline. Sampling was completed within a 6-h period, beginning early in the morning. Samples were stored in a cooler filled with ambient seawater and transported to the lab, and were processed on the same day. Temperature variability during transit was monitored using a max/min thermometer (SPER Scientific, no. 736690, Scottsdale, AZ, USA).

Plankton identification
A 25-ml aliquot of each plankton sample was fixed (4% v/v, formalin, final concentration) and stored at 5 °C. The wet weights (g ml^{-1}) were determined for the fixed plankton samples. Samples were then preserved for long-term storage in 70% ethanol (v/v) and shipped to EcoAnalysts Inc. (Moscow, ID, USA) for taxonomic identification. For each sample (63–200 and >200 μm), the relative abundance of phytoplankton and zooplankton was determined separately by identifying the first 100 phytoplankton and the first 100 zooplankton encountered to the lowest taxonomical unit. Owing to variability in the lowest taxonomical unit identified across all plankton (for example, some were identified to genus whereas others only to order), identified plankton were subsequently grouped into larger common categories. For phytoplankton, individuals were categorized by class (diatoms (*Bacillariophyceae*)) and phylum (cyanobacteria and green algae (*Chlorophyta*)). For zooplankton, individuals were categorized by subclass (copepods (*Copepoda*)), order (decapods (*Decapoda*) and *Sessilia*) and phylum (*Formanifera* and *Rotifera*).

Enumeration of culturable vibrios
Presumptive *Vibrio* species were enumerated on a selective medium (thio-citrate–bile–sucrose (TCBS) cholera medium, Oxoid CM0333, Basingstoke, Hampshire, UK) (Pfeffer and Oliver, 2003; Thompson *et al.*, 2003a). Briefly, water and plankton samples were serially diluted in phosphate-buffered saline before spread plating 100 μl on duplicate TCBS plates. At least 2–3 dilutions were plated to ensure that the plates were countable. Before dilution, the plankton samples were homogenized for 1 min at 12 000 r.p.m. using a PRO homogenizer (PRO Scientific Inc., model 200, Oxford, CT, USA). Plates were incubated in the dark at 30 °C for 16–20 h and all yellow and green colonies were counted as presumptive vibrios, and reported as colony-forming units (CFUs) per ml of water and CFU per g of plankton.

Data analysis
Bacterial counts were log transformed to fit a normal distribution (Anderson–Darling statistic, $\alpha = 0.10$). In cases in which *Vibrio* levels were below the detection limit a value of zero was assigned for statistical analysis.

Pearson's correlation coefficients were calculated to evaluate relationships between the log-transformed *Vibrio* concentrations (CFU per ml water and CFU per g plankton) and environmental parameters. One-way analysis of variance was used to determine the significance of differences in *Vibrio* concentrations between fractions, sampling sites and collection months; significance was declared when $P < 0.05$. All univariate and bivariate analyses were carried out in MINITAB version 15.0 (MINITAB Inc., State College, PA, USA).

General linear regression models (PROC GLM; SAS Institute Inc.; version 9.1, Cary, NC, USA) were developed for each fraction (water, 63–200- and >200-μm plankton) to examine relationships between the relative abundance of each plankton taxon (in the plankton fractions), environmental variables and *Vibrio* concentrations. All variables found to be correlated ($P < 0.10$) in univariate analyses, and their appropriate interaction terms were initially entered into the models. Maximum likelihood estimates of each variable parameter were determined, and variables were eliminated from the model by backward elimination when $P > 0.05$.

Results

Environmental parameters
Among the 70 sample collection events, temperature ranged from 9.3 to 30.8 °C, DO from 48.7 to 105.8% saturation and salinity from 26.3 to 32.2 (Figure 2). Temperature during transport of samples varied by ⩽2 °C, with the exception of samples collected in July 2006, which were inadvertently stored on ice. These 18 samples (six for each fraction) were removed from analysis for culturable *Vibrio* counts.

Temperature and DO exhibited strong seasonal trends, whereas salinity varied less and showed no seasonal pattern. DO and temperature were inversely related ($r = -0.73$), whereas salinity and temperature were directly related ($r = 0.40$) ($P < 0.01$).
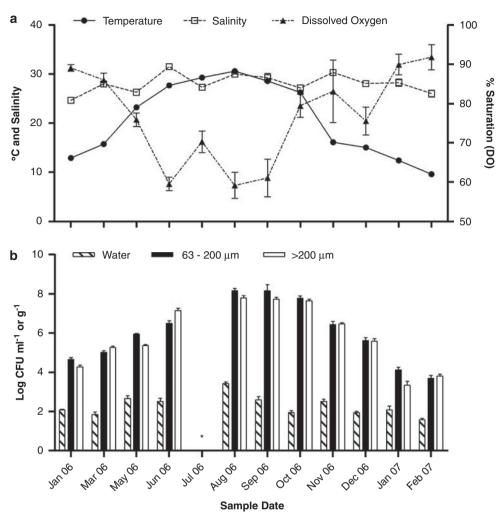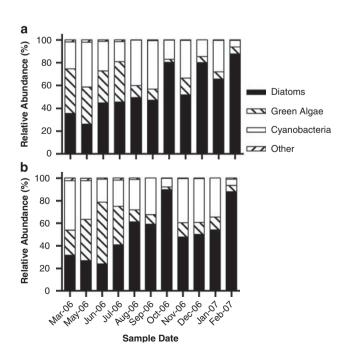
Figure 2 Seasonal fluctuations in mean monthly environmental conditions (temperature, salinity and DO) (**a**) and seasonal fluctuations in mean monthly *Vibrio* concentrations (CFU ml^{-1} in the water column and CFU g^{-1} for the small (63–200 μm) and large (>200 μm) plankton fractions) (**b**).

Analysis of the small plankton fraction (63–200 μm)

The 63–200-μm plankton samples ($N=64$; taxonomic identification was not available for samples collected in January 2006) were composed of phytoplankton and smaller forms of zooplankton. Diatoms (mean relative abundance 55.7%), cyanobacteria (mean relative abundance 26.1%) and green algae (mean relative abundance 17.3%) accounted for 99.1% of all phytoplankton in the 63–200-μm fractions (Figure 3). Across all phytoplankton groups, *Microcystis* (cyanobacteria) was the most commonly identified genus (18.6% mean relative abundance), followed by the diatoms, *Skeletonema* spp. and *Chaetoceros* spp., with mean relative abundances of 10.1 and 8.9%, respectively (Table 1).

The relative abundance of diatoms was inversely related to temperature ($r=-0.40$), whereas the

Figure 3 Seasonal changes in the relative abundance of phytoplankton taxa in the 63–200 μm fraction (**a**) and the >200 μm fraction (**b**).

Table 1 Composition of phytoplankton and zooplankton identified to lowest taxonomic unit over all samples collected ($N = 64$)

Fraction	63–200 μm		>200 μm	
	Genus	*Mean relative abundance (%)*	*Genus*	*Mean relative abundance (%)*
Phytoplankton	*Microcystis*[a]	18.55	*Microcystis*[a]	16.62
	Skeletonema[b]	10.12	*Thalassiosira*[a]	12.70
	Chaetoceros[b]	8.86	*Oocystis*[c]	9.65
	Oscillatoria[a]	7.37	*Oscillatoria*[a]	9.14
	Coscinidiscus[b]	7.15	*Coscinidiscus*[b]	5.73
	Oocystis[c]	7.10	*Navicula*[b]	4.72
	Rhizosolenia[b]	3.96	*Nitzschia*[b]	4.00
	Synedra[b]	3.92	*Melosira*[b]	3.81
	Biddulphia[b]	3.69	*Sphanerocystis*[c]	3.46
	Nitzschia[b]	3.35	*Biddulphia*[b]	3.30
	Sphanerocystis[c]	3.13	*Achnanthes*[b]	3.24
	Rhizoclonium[c]	2.87	*Chaetoceros*[b]	2.82
	Navicula[b]	2.68	*Rhizosolenia*[b]	2.75
	Achnanthes[b]	2.32	*Synedra*[b]	2.69
	Chlorella[c]	1.85	*Chlorella*[c]	2.58
	Melosira[b]	1.71	*Lyngbya*[a]	1.81
	Pseudonitzschia[b]	1.48	*Pseudonitzschia*[b]	1.65
	Thalassiosira[a]	1.15	*Chalmydononas*[c]	1.34
	Chalmydononas[c]	1.11		
	Fragilaria[b]	1.03		
	Lowest identified taxonomic unit	*Mean relative abundance (%)*	*Lowest identified taxonomic unit*	*Mean relative abundance (%)*
Zooplankton	*Copepoda*[d]	58.78	*Calanoida*[d]	74.64
	Foraminifera[e]	14.41	*Brachyura*[f]	10.35
	Calanoida[d]	10.88	*Decapoda*[f]	5.32
	Rotifera[g]	6.34	*Sessilia*[h]	3.11
	Harpacticoida[d]	3.16	*Harpacticoida*[d]	2.14
	Cyclopoida[d]	2.35	*Balanus*[h]	1.63
	Gastropoda[i]	1.24		
	Bivalvia[j]	1.24		

Phytoplankton were identified to genus level; zooplankton were identified to genus, order, class or phylum. Only those groups with a mean relative abundance of ⩾1% are shown.
[a]Cyanobacteria.
[b]Diatom.
[c]Green algae.
[d]Class Copepoda.
[e]Phylum Formanifera.
[f]Order Decapoda.
[g]Phylum Rotifera.
[h]Order Sessilia.
[i]Class Gastropoda; phylum Mollusca.
[j]Class Bivalvia; phylum Mollusca.

relative abundance of cyanobacteria varied directly with temperature ($r = 0.41$) ($P < 0.05$ for each). No other significant associations were found.

Copepods (mean relative abundance 75.2%), *Foraminifera* (mean relative abundance 14.4%), *Rotifera* (mean relative abundance 6.4%), *Sessilia* (mean relative abundance 0.8%) and decapods (mean relative abundance 0.02%) accounted for 99.8% of all zooplankton in this fraction (Figure 4). Among this size fraction, most zooplankton could only be identified to the level of subclass or higher; however, among the copepods for which a finer level identification was determined, calanoid and harpacticoid were the most commonly noted at 10.9 and 3.16% relative abundance, respectively (Table 1).

The relative abundance of copepods was directly related to temperature ($r = 0.42$), whereas the relative abundance of foraminiferida was inversely related to temperature ($r = -0.42$) ($P < 0.05$ for each). No other significant associations were found.

Analysis of the large plankton fraction (>200 μm)
The >200-μm plankton samples ($N = 64$) were composed primarily of zooplankton; however, some phytoplankton were also captured in this size fraction. Diatoms (mean relative abundance 52.2%), cyanobacteria (mean relative abundance 27.8%) and green algae (mean relative abundance 19.1%) accounted for 99.0% of all phytoplankton in this size fraction (Figure 3). Among all phytoplank-
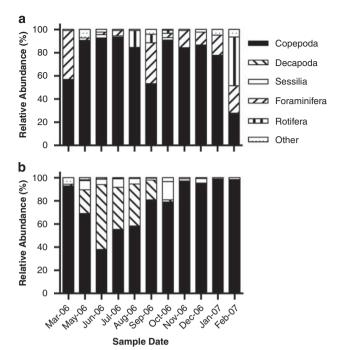
Figure 4 Seasonal changes in the relative abundance of zooplankton taxa in the 63–200 μm fraction (**a**) and the >200 μm fraction (**b**).

Table 2 General linear model describing changes in the log *Vibrio* concentrations in the water fraction ($R^2 = 0.97$; $P < 0.001$)

Parameter	Estimate ± s.e.[a]	P-value	Type III SS[b]	% Variation described[c]
Temperature	0.043 ± 0.0078	<0.001	5.82	33.36
Salinity	0.049 ± 0.0062	<0.001	11.62	66.64

[a]Standard error.
[b]Sums of squares.
[c]The additional variability (expressed as percent) explained by each parameter in the full model based on the type III sums of squares (SS).

ton in this size fraction, *Microcystis* (cyanobacteria) was the most commonly noted at 16.6% mean relative abundance, followed by *Thalassiosira* and *Oocystis* at 12.7 and 9.7% mean relative abundance, respectively (Table 1). No significant correlations between the phytoplankton groups and environmental parameters were found.

Copepods (mean relative abundance 77.8%), decapods (mean relative abundance 15.7%), *Sessilia* (mean relative abundance 4.7%) and *Foraminifera* (mean relative abundance 0.3%) accounted for 99.9% of all zooplankton in this size fraction (Figure 4). Among all zooplankton in this size fraction, calanoid copepods were the most frequently identified order (74.64% relative abundance), followed by *Brachyura* (decapod) larvae at 10.4% mean relative abundance (Table 1).

The relative abundance of decapods and *Sessilia* was directly related to temperature ($r = 0.78$ and 0.38, respectively), whereas the relative abundance

of copepods was inversely related to temperature ($r = -0.72$) ($P < 0.05$ for each).

Analysis for total culturable Vibrio
Of the 210 samples collected and processed, 192 were used in statistical analyses (samples collected in July 2006 were not included because of improper storage conditions). Fluctuations in mean monthly total culturable *Vibrio* concentrations followed a strong seasonal trend (Figure 2). Univariate analyses (Pearson's correlation coefficient) revealed that *Vibrio* concentrations in all fractions were directly related to temperature ($r = 0.69$ for water and $r = 0.88$ for both plankton fractions ($P < 0.01$ for each)). Conversely, *Vibrio* concentrations were inversely related to DO in all fractions ($r = -0.49$, -0.67 and -0.68 for the water, 63–200- and >200-μm fractions, respectively ($P < 0.01$ for each)). Salinity showed a positive, although weak, correlation with *Vibrio* concentrations ($r = 0.36$, 0.35 and 0.39 for the water, 63–200- and >200-μm fractions, respectively ($P < 0.01$ for each)).

The mean *Vibrio* concentration in the water fraction (500 CFU ml^{-1} ($N = 64$)) was up to 6 orders of magnitude less than the mean concentration of vibrios in the plankton fractions (4.91×10^7 and 1.58×10^8 CFU g^{-1} for the 63–200- and >200-μm fractions, respectively ($N = 64$ for both)) (Figure 2). *Vibrio* concentrations in the water fraction ranged from 20 CFU ml^{-1} in February 2007 to 6150 CFU ml^{-1} in August 2006. Concentrations in the 63–200-μm fractions ranged from 1.83×10^3 CFU g^{-1} in February 2007 to 5.98×10^8 CFU g^{-1} in September 2006. Concentrations in the >200-μm fraction ranged from 7.36×10^2 CFU g^{-1} in January 2007 to 1.58×10^8 CFU g^{-1} in August 2006 (Figure 2). *Vibrio* concentrations were not significantly different between the two plankton fractions or between the different sampling sites (analysis of variance, $P < 0.001$).

General linear models to describe changes in total culturable Vibrio *levels over time*
Multivariate general linear models (GLMs) were constructed for the water fraction (Table 2) and each plankton fraction (Tables 3 and 4 for the 63–200- and >200-μm fractions, respectively). In the 63–200-μm fraction, only models based on copepods, diatoms and cyanobacteria were significantly predictive (Table 3), and in the >200-μm fraction, only models incorporating copepods and decapods were significantly predictive (Table 4).

Water fraction model. Temperature and salinity were the only significant correlates of *Vibrio* concentration in the water fraction (Table 2). DO and the concentration of vibrios in both plankton fractions (63–200 and >200 μm), as well as their interaction terms, were included in the model as potential drivers; however, these variables were not

Table 3 Taxon-specific plankton models describing changes in the log *Vibrio* concentration in the 63–200-μm plankton fraction

Model	Parameter	Estimate ± s.e.[a]	P-value	Type III SS[b]	% Variation described[c]
Copepod	Copepod abundance	−6.41 ± 2.42	0.0108	3.47	8.11
$R^2 = 0.99$	Water *Vibrio*[d]	3.80 ± 0.99	<0.001	7.25	16.97
	Temperature	0.28 ± 0.043	<0.001	20.90	48.90
	DO	0.0031 ± 0.011	0.787	0.037	0.09
	Copepod × DO	0.083 ± 0.031	0.009	3.64	8.52
	Water *Vibrio* × Temperature	−0.056 ± 0.020	0.008	4.06	8.74
	Water *Vibrio* × DO	−0.032 ± 0.011	0.0008	3.70	8.66
Diatom	Diatom abundance	−16.19 ± 5.42	0.004	3.63	18.36
$R^2 = 0.85$	Water *Vibrio*	0.043 ± 0.21	0.044	1.73	8.76
	Temperature	−0.12 ± 3.57	0.098	1.15	5.84
	DO	−0.093 ± 0.031	0.004	3.59	18.19
	Diatom × Temperature	0.042 ± 0.098	<0.001	7.35	37.18
	Diatom × DO	0.012 ± 0.049	0.021	2.31	11.68
Cyanobacteria	Cyanobacteria abundance	−28.11 ± 3.90	0.008	3.90	7.36
$R^2 = 0.81$	Water *Vibrio*	−1.97 ± 0.93	0.040	2.31	4.36
	Temperature	0.18 ± 0.022	<0.001	34.55	65.19
	Salinity	−0.29 ± 0.11	0.012	3.49	6.58
	DO	−0.067 ± 0.032	0.042	2.25	4.24
	Cyanobacteria × Salinity	1.02 ± 0.36	0.007	4.04	7.62
	Water *Vibrio* × DO	0.028 ± 0.013	0.034	2.46	4.65

Abbreviation: DO, dissolved oxygen.
[a]Standard error.
[b]Sums of squares.
[c]The additional variability (expressed as percent) explained by each parameter in the full model based on the type III sums of squares (SS).
[d]Log concentration of vibrios in the water fraction.

Table 4 Taxon-specific plankton models describing changes in the log *Vibrio* concentration in the >200-μm plankton fraction

Model	Parameter	Estimate ± s.e.[a]	P-value	Type III SS[b]	% Variation described[c]
Copepod	Copepod abundance	16.69 ± 4.61	<0.001	5.20	12.61
$R^2 = 0.99$	Water *Vibrio*[d]	−6.40 ± 1.77	<0.001	5.19	12.59
	Temperature	0.79 ± 0.17	<0.001	8.25	20.01
	Salinity	−0.62 ± 0.18	0.001	4.65	11.29
	DO	0.065 ± 0.035	0.074	1.33	3.22
	Copepod × Temperature	−0.38 ± 0.13	0.005	3.34	8.12
	Copepod × DO	−0.08 ± 0.040	0.045	1.67	4.06
	Water *Vibrio* × Temperature	−0.11 ± 0.032	<0.001	4.91	11.92
	Water *Vibrio* × Salinity	0.31 ± 0.075	<0.001	6.67	16.18
Decapod	Decapod abundance	−1.32 ± 0.53	0.017	2.41	7.65
$R^2 = 0.84$	Water *Vibrio*	−7.27 ± 2.30	0.0026	3.99	12.68
	Temperature	0.38 ± 0.063	<0.001	14.54	46.27
	Salinity	−0.60 ± 0.23	0.011	2.78	8.85
	Water *Vibrio* × Temperature	−0.08 ± 0.029	0.0064	3.22	10.23
	Water *Vibrio* × Salinity	0.31 ± 0.093	0.0015	4.50	14.31

Abbreviation: DO, dissolved oxygen.
[a]Standard error.
[b]Sums of squares.
[c]The additional variability (expressed as percent) explained by each parameter in the full model based on the type III sums of squares (SS).
[d]Log concentration of vibrios in the water fraction.

significant and were removed from the final model. The model estimated that each 1-°C rise in temperature corresponded to a 0.043-fold increase in the log concentration of vibrios. Meanwhile, each unit increase in salinity corresponded to a 0.049-fold increase in the log concentration of vibrios. Although the magnitude of these parameter estimates was small, temperature accounted for 33.36% of the model variance, whereas salinity accounted for 66.64% of the model variance (Table 1).

63–200-μm fraction copepod model. This model estimated that each 1% increase in the relative abundance of copepods corresponded to a 6.41-fold decrease in the log concentration of vibrios (Table 3). Copepods accounted for 16.63% of the model

variation (8.11% of this variation was independent and an additional 8.52% was through interactions with DO). Each one-log increase in *Vibrio* concentration in the water fraction corresponded to a 3.80-fold increase in the 63–200-μm plankton fraction. The log concentration of vibrios in the water fraction accounted for 34.37% of the model variation (16.97% independently and an additional 8.74 and 8.66% from interactions with temperature and DO, respectively). Each 1-°C increase in temperature was responsible for a modest 0.28-fold increase in the log *Vibrio* concentration. Independently, temperature accounted for 48.90% of the variation. Although DO was retained in the final model because it was an important interaction variable, there was no significant independent relationship between plankton-associated *Vibrio* concentrations and DO (Table 3).

63–200-μm fraction diatom model. This model estimated that each 1% increase in the relative abundance of diatoms corresponded to a 16.19-fold decrease in log *Vibrio* concentration (Table 3). Diatoms accounted for 67.22% of the model variance (18.36% independently and an additional 37.18 and 11.68% because of interactions with temperature and DO, respectively). Each one-log increase in the concentration of vibrios in the water fraction corresponded to a 0.43-fold increase in log *Vibrio* concentration in the 63–200-μm fraction, and accounted for 8.76% of the model variance. Each 1-°C change in temperature and each 1.0% change in DO corresponded to 0.12- and 0.093-fold decreases in log *Vibrio* concentrations, respectively. Independently, temperature and DO accounted for 5.84 and 18.19% of the model variance, respectively (Table 3).

63–200-μm fraction cyanobacteria model. This model estimated that each 1% increase in the relative abundance of cyanobacteria corresponded to a 28.11-fold decrease in the log concentration of vibrios (Table 3). The relative abundance of cyanobacteria accounted for 14.98% of the model variance in log *Vibrio* concentrations in the 63–200-μm fraction (7.36% independently and an additional 7.62% through interaction with salinity). Each one-log increase in the concentration of vibrios in the water fraction corresponded to a 1.97-fold decrease in the 63–200-μm fraction and explained 9.01% of the model variation (4.36% independently and an additional 4.65% through interaction with DO). Each 1-°C change in temperature corresponded to a 0.18-fold increase in the log concentration of vibrios and explained 65.19% of the model variance. Salinity and DO also showed significant negative associations with log *Vibrio* concentrations in this fraction (Table 3).

>200-μm fraction copepod model. This model estimated that each 1% increase in the relative abundance of copepods corresponded to a 16.68-fold increase in the log concentration of vibrios; however, this relationship was modified by interactions between copepods and temperature and DO that were inversely related to log *Vibrio* concentrations (parameter estimates = −0.38 and −0.08, respectively). The relative abundance of copepods accounted for 24.79% of the model variance (12.61% independently and an additional 8.12 and 4.06% through interactions with temperature and DO, respectively). Each one-log increase in the concentration of vibrios in the water fraction corresponded to a 6.40-fold decrease in the log concentration of vibrios in the >200-μm fraction, and accounted for 40.69% of the model variance (12.59% independently and an additional 11.92 and 16.18% through interactions with temperature and salinity, respectively). Each 1-°C increase in temperature corresponded to a 0.79-fold increase in log *Vibrio* concentration, and accounted for 20.01% of the model variance. Each unit increase in salinity corresponded to a 0.62-fold decrease in log *Vibrio* concentration, and accounted for 11.29% of the model variance (Table 4).

>200-μm fraction decapod model. This model estimated that each 1% increase in the relative abundance of decapods corresponded to a 1.32-fold decrease in the log concentration of vibrios (Table 4). The relative abundance of decapods accounted for 7.65% of the model variance. Each one-log increase in the concentration of vibrios in the water fraction corresponded to a 7.27-fold decrease in the log concentration of vibrios in the >200-μm fraction, and accounted for 37.22% of the model variance (12.68% independently and an additional 10.23 and 14.31% through interactions with temperature and salinity, respectively). Each 1-°C increase in temperature corresponded to a 0.38-fold increase in log *Vibrio* concentration, and accounted for 46.27% of the model variance. Each unit increase in salinity corresponded to a 0.60-fold decrease in the log *Vibrio* concentration, and accounted for 8.85% of the model variance (Table 4).

Discussion

Most of the earlier research investigating *Vibrio*–plankton interactions have been focused on the relationship between *V. cholerae* and copepods (for example, Huq *et al.*, 1983, 1984, 2005; Tamplin *et al.*, 1990); however, less is known about the more general response of *Vibrio* populations to natural plankton communities and specifically whether plankton community composition can explain some of the seasonal variabilities in total culturable *Vibrio*. Results of this study show that seasonal variations in *Vibrio* concentrations can be modeled using changes in both environmental variables and the composition of the plankton reservoir.

Although this study relied on a culture-based approach to quantify total *Vibrio* abundance (which likely underestimated the total population due to non-culturable cells), the selective medium (TCBS) has been used extensively in the past to recover a large range of *Vibrio* spp. and provides standard approach for comparison with other studies (Massad and Oliver, 1987; Pfeffer and Oliver, 2003; Pfeffer *et al.*, 2003). Despite the limitation in relying only on abundance of culturable *Vibrio*, the results presented here support earlier findings that temperature, salinity and DO are significant drivers of *Vibrio* seasonality (Heidelberg *et al.*, 2002b; Lipp *et al.*, 2003; Thompson *et al.*, 2003b; Baffone *et al.*, 2006), but suggest an independent role for plankton in describing the seasonal patterns. Multivariate GLMs showed that seasonal changes in the composition of the plankton community were significantly associated with changes in co-measured total culturable *Vibrio* concentrations.

Factors affecting Vibrio *in water.* Salinity is known to be correlated with some *Vibrio* spp., but its significance can vary across the *Vibrio* genus (Thompson *et al.*, 2003a) and change depending on the range of temperature in the system (Lipp *et al.*, 2001; Randa *et al.*, 2004). In this study, we noted a moderate range in temperature over the year but a very small range in salinity, which may have been due to the selection of tidally dominated study sites and collections restricted to outgoing tides. Therefore, based on earlier studies (for example, Lipp *et al.*, 2001; Louis *et al.*, 2003), we expected that temperature would be the dominant correlate for *Vibrio* abundance, which was supported by the results from Pearson Product Moment Correlation (that is, univariate analysis). However, when the more robust GLMs were applied to develop predictive models for *Vibrio* abundance, temperature and salinity, the only significant predictors of free-living *Vibrio* concentrations, both showed weak parameter estimates and salinity accounted for a greater proportion of the model variance in *Vibrio* concentration compared with temperature. This suggests that the relationship between temperature and salinity may be more complex than that reported earlier based on studies using univariate analysis alone. It also suggests that the free-living *Vibrio* community may be a distinct group of bacteria, as the GLM showed no significant relationship or interaction between *Vibrio* levels detected in the plankton fractions.

Factors affecting Vibrio *in plankton.* Although temperature and salinity remained important correlates with *Vibrio* abundance in both plankton fractions, the independent role of the specific composition of plankton within those fractions also significantly affected the levels of *Vibrio,* which may

indicate a specific relationship between plankton dynamics and the *Vibrio* population. In both fractions (63–200 and >200 μm), the relative abundance of copepods was significantly associated with the concentration of *Vibrio*. In particular, parameter estimates indicated a strong direct relationship between *Vibrio* concentration and the relative abundance of copepods in the >200-μm fraction. Copepods have been implicated as a potential vector of *V. cholerae* (Huq *et al.*, 1996; Colwell *et al.*, 2003), and an ecological relationship between *V. cholerae* and copepods has been suggested earlier (Huq *et al.*, 1983, 1984; Tamplin *et al.*, 1990; Thomas *et al.*, 2006). Conversely, a negative association was noted between copepods and *Vibrio* in the 63–200-μm fraction, which may reflect differences in copepod life stages between the fractions. The majority of copepods in the 63–200-μm fraction represented earlier life stages (naupliar and copepodite), which displayed a bimodal distribution, peaking in May and October 2006, when water temperatures were not optimal for the growth of most *Vibrio* spp. (Thompson *et al.*, 2003a). These earlier copepod life stages also differ physiologically in that earlier stages are continuously molting and shedding attached vibrios in the process (Tamplin *et al.*, 1990).

In addition to copepods, within the 63–200-μm plankton fraction, phytoplankton (diatoms and cyanobacteria) were shown to be significant correlates of *Vibrio* abundance. In both cases, an increase in the relative abundance of diatoms or cyanobacteria corresponded with a significant decrease in co-measured *Vibrio* concentration. *V. cholerae* have been shown to attach to certain cyanobacteria and other algal species (for example, Tamplin *et al.*, 1990); however, the total *Vibrio* community may respond differently to the presence of phytoplankton and the inverse relationship may reflect a release of nutrients that corresponds with the decay of a diatom or cyanobacterial bloom (Middelboe *et al.*, 1995; Riemann *et al.*, 2000). The decline of the bloom could then stimulate an increase in secondary production by heterotrophic bacteria, including *Vibrio* (Lancelot and Billen, 1984; Pomeroy and Wiebe, 2001). In particular, cyanobacterial-derived organic matter has been reported as an important growth factor for *V. cholerae* and *V. vulnificus* (Eiler *et al.*, 2007*).* Alternately, the increase in the abundance of certain cyanobacteria, which are known to produce a range of antibacterial metabolites, could have contributed directly to a decline in the growth of total culturable *Vibrio* in that plankton fraction (Lam *et al.*, 2008; Singh *et al.*, 2008).

Within the >200-μm-size fraction, the only other plankton type, besides copepods, significantly associated with *Vibrio* levels were decapods, primarily crab larvae. Among the plankton models described for this study, the decapod model was unique in that changes in parameters other than relative plankton abundance, namely temperature, salinity and the

concentration of *Vibrio* in the water fraction, were the primary drivers of *Vibrio* abundance (that is, parameter estimates were higher for these parameters than for the relative abundance of decapods). The association with decapods may have been weak for two reasons: (1) decapods comprised a relatively small proportion of this fraction and (2) the occurrence of decapods coincided with warm water temperatures, which were also optimal for the growth of *Vibrio*. In other words, the magnitude of change in the abundance of decapods was relatively small compared with the magnitude of change occurring in the total culturable *Vibrio* counts; however, this result does not preclude the idea that a rare member of the plankton community could not have a profound affect on the *Vibrio* population.

Our results confirm the importance of temperature, salinity and DO as independent drivers of both free-living and plankton-associated *Vibrio* concentrations. In addition, we have shown that plankton composition plays an important and independent role as a driver of the total culturable *Vibrio* community in natural estuarine systems. These findings highlight the potentially complex relationship between seasonal shifts in plankton composition and the net effect on total *Vibrio* levels and suggest that *Vibrio* concentrations should be considered within the context of bloom formation and decline as well as with single-point determinations of plankton composition.

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