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Vibrio parahaemolyticus in Rhode Island Coastal Ponds and the Estuarine Environment of Narragansett Bay

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Quantification of the abundance of *Vibrio parahaemolyticus* in water and oysters from Rhode Island showed the presence of environmental strains and low levels of potentially pathogenic strains when water temperatures were ≥18°C, with peak levels in late July to early August. A higher abundance of the *trh* gene than of the *tdh* gene was observed.

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium occurring naturally in estuarine and coastal marine waters that is able to cause illness in humans (12, 14, 18, 19). Filter feeders, such as oysters, are capable of acquiring *V. parahaemolyticus* from the water column and concentrating it in their tissues (10). Because of the potential for human illness through consumption of raw or undercooked shellfish with *V. parahaemolyticus*, these bacteria pose a threat to shellfish aquaculture and fisheries through loss of revenue due to concerns about seafood safety and negative publicity in cases of outbreaks. Illnesses due to *V. parahaemolyticus* from consuming shellfish in Rhode Island and the Northeast United States are rare (3). Because of a strong association between *V. parahaemolyticus* levels and temperature (5, 7, 18, 19), there is an increased awareness of the danger posed by *V. parahaemolyticus* due to a rise in marine and estuarine water temperatures. In Narragansett Bay (Rhode Island), spring-summer sea surface temperatures have warmed by 1.6°C from 1959 to 2005 and are generally over 15.0°C from late May through mid-October (1). Warmer water temperatures could potentially extend the season in which *V. parahaemolyticus* could be a concern.

The total and relative abundances of environmental and potentially pathogenic *V. parahaemolyticus* bacteria in Rhode Island coastal waters have not been determined. A 1985 study showed the presence of *V. parahaemolyticus* in Narragansett Bay, but no pathogenic strains

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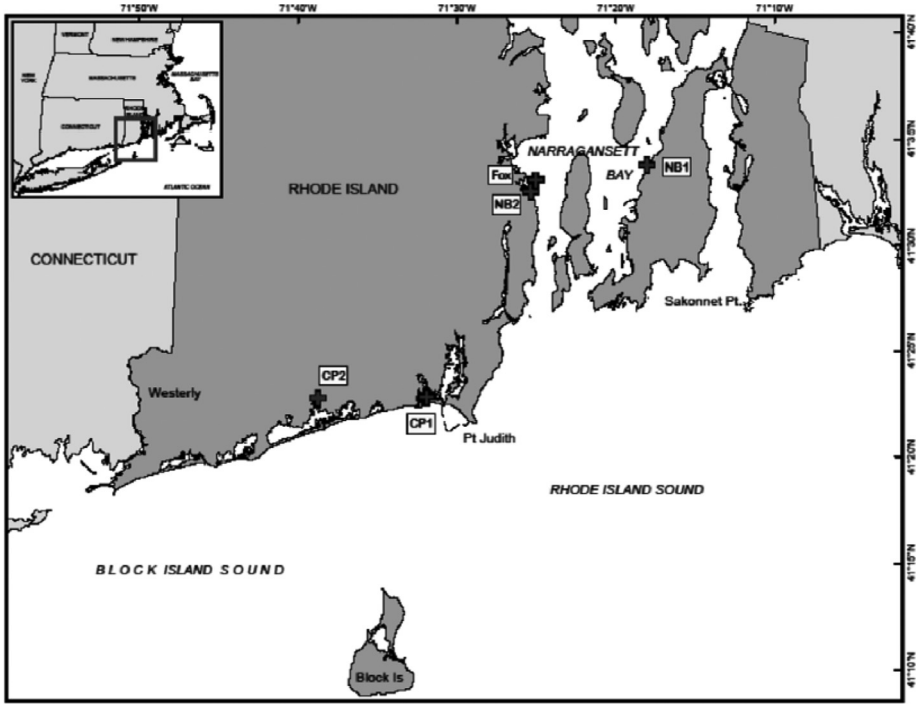

FIG 1 Partial map of Rhode Island showing the locations of the sampling sites in Narragansett Bay (NB1, NB2, and Fox Island) and coastal salt ponds (CP1 and CP2). (Map courtesy of Christopher Damon, adapted from the Rhode Island Geographic Information System [RIGIS; copyright 2011].)

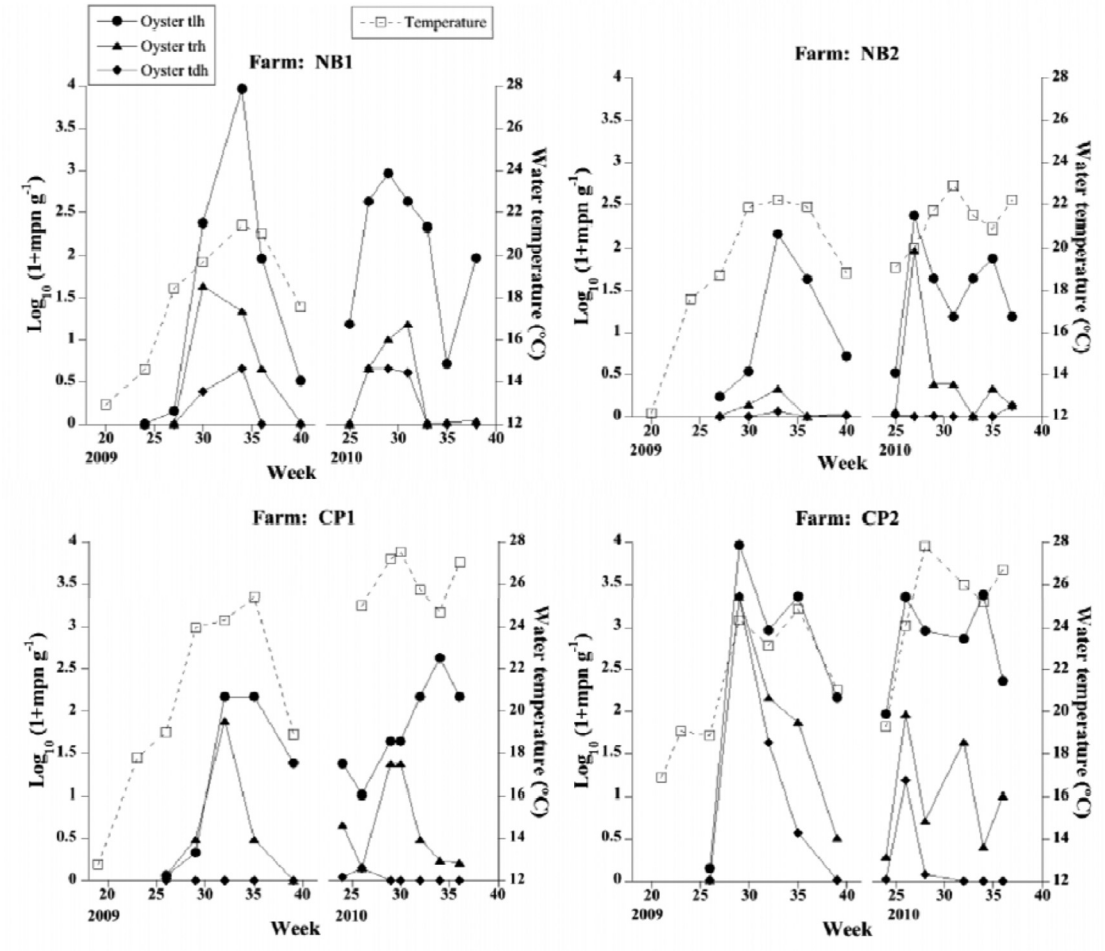

FIG 2 Temporal variation in temperature (right axis, squares, broken line) and levels of *V. parahaemolyticus tlh* (circles, solid line), *trh* (triangles, solid line), and *tdh* (diamonds, solid line) (left axis) in oysters collected from four Rhode Island farms during the summers of 2009 and 2010.

were detected (19). The purpose of this study was to determine the seasonal abundance of environmental and pathogenic *V. parahaemolyticus* in Rhode Island. Water (1 liter) and oyster ($n = 10$ to 15) samples were collected from two farms (NB1, 41°33.678′N, 71°18.431′W; NB2, 41°32.583′N, 71°25.351′W) in Narragansett Bay and two farms (CP1, 41°22.883′N, 71°31.954′W; CP2, 41°22.804′N, 71°33.720′W) in coastal salt ponds (Fig. 1). Water samples were also collected from a site near Fox Island (41°34.2′N, 71°23.4′W) within Narragansett Bay. A 3-tube most-probable-number (MPN) method combined with quantitative PCR detection of the *tlh*, *tdh*, and *trh* genes was used for detection of *V. parahaemolyticus* (16, 20). After incubation of the MPN tubes, a 1-ml portion from each enrichment tube in the serial dilution series was transferred to cryotubes and stored at −80°C. Bacterial DNA was extracted by boiling, and quantitative PCR (qPCR) was used to score the tubes as positive or negative for *tlh*, *trh*, and *tdh*. *Vibrio parahaemolyticus* densities (+1 to account for 0 values) were $\log_{10}$ transformed for statistical analysis using one-way analysis of variance (ANOVA) or Kruskal-Wallis one-way ANOVA on ranks and the appropriate *post hoc* tests (IBM SPSS Statistics 19; IBM, Somers, NY; Sigmastat 3.1; Systat, Chicago, IL).

***Vibrio parahaemolyticus* densities in oysters and water.** *V. parahaemolyticus* was detected in Rhode Island oyster and water samples for approximately 15 weeks during the summers of 2009 and 2010 (Fig. 2 and 3). For most locations, densities of *V. parahaemolyticus* increased rapidly in a period of 2 to 3 weeks after water temperatures reached approximately 18°C, starting in early July for 2009 or mid-June for 2010. This study confirmed July to August as the period of highest risk for *V. parahaemolyticus*. This is similar to the case with the Chesapeake Bay, where *V. parahaemolyticus* first peaked in June and July in the water column after water temperature rose to 19°C (13, 18). The maximum level of total *V. parahaemolyticus* seen in oysters in this study (9×10^3 MPN g^{-1} oyster tissue) corresponds to a very low risk of gastroenteritis from oysters (10). Levels of total *V. parahaemolyticus* bacteria detected in Rhode Island oysters are comparable to levels observed in shellfish from other U.S. and European coastal areas ($<10^4$ MPN g^{-1}) (2, 5, 6, 11, 15, 16, 17, 18, 20).

Densities of *V. parahaemolyticus* in Narragansett Bay water samples in 2009 and 2010 ($\leq$15 MPN ml^{-1}) are comparable to the maximum densities found in a 1985 study (4.95 CFU ml^{-1} [19]) (Fig. 3). Levels of *V. parahaemolyticus* in water collected from oyster farms were in general slightly higher than levels at the non-farm reference site at Fox Island. Maximum densities of *tlh* in water were significantly higher in 2009 than in 2010 (Kruskal-Wallis one-way ANOVA; $P = 0.049$). The summer of 2009 was a colder summer with more precipitation, possibly explaining some of the differences between these 2 years.

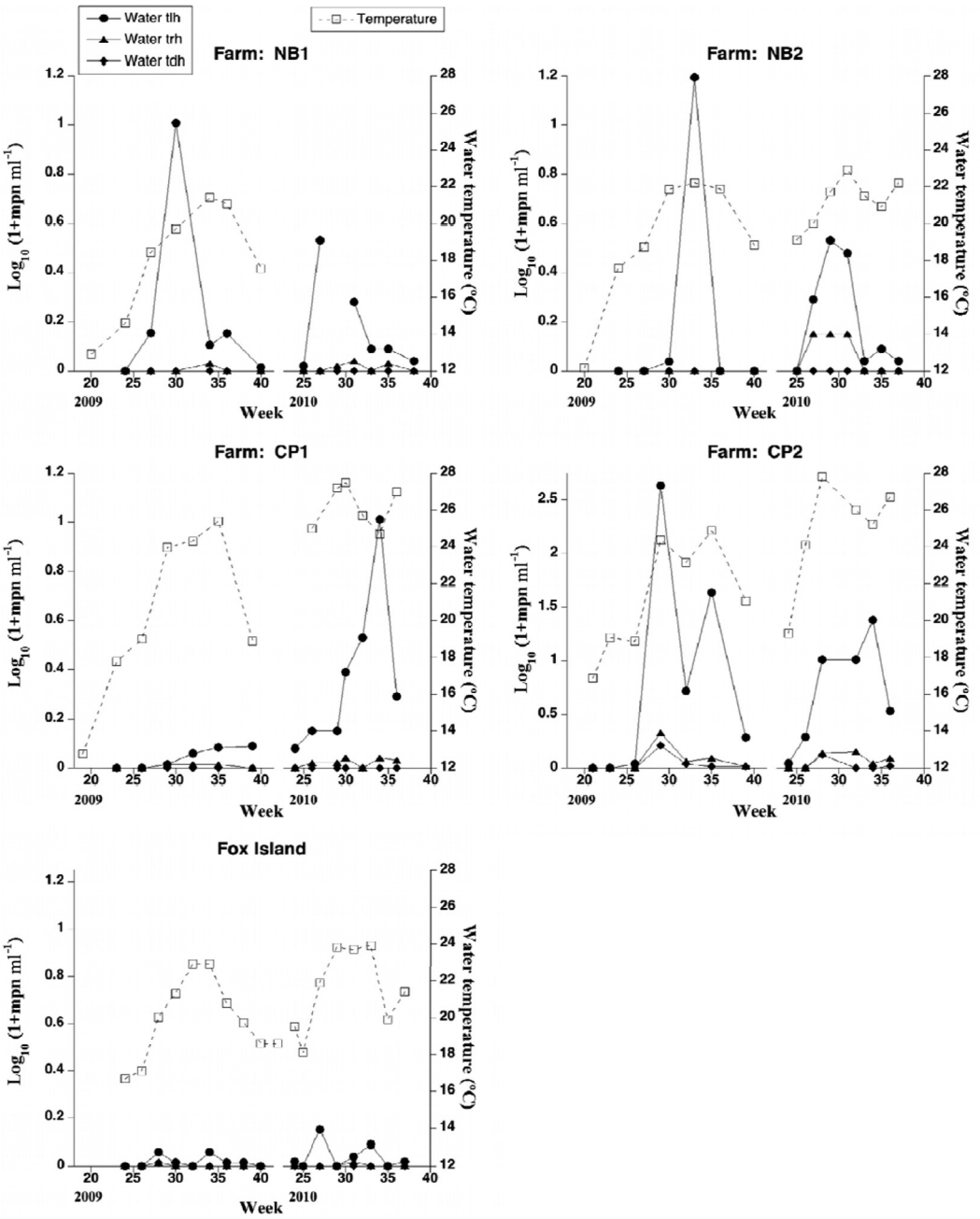

FIG 3 Temporal variation in temperature (right axis, squares, broken line) and levels of *V. parahaemolyticus tlh* (circles, solid line), *trh* (triangles, solid line), and *tdh* (diamonds, solid line) (left axis) in water collected from four Rhode Island farms and Fox Island station during the summers of 2009 and 2010.

Abundance of pathogenic *V. parahaemolyticus* relative to total *V. parahaemolyticus*. For 21 of 48 (43.8%) oyster samples, only the *trh* gene was detected (*tdh* was nondetectable [Table 1]), showing a predominance of trh^+ strains in Rhode Island. The percentage of pathogenic *V. parahaemolyticus* relative to total *V. parahaemolyticus*, calculated by dividing *trh* and *tdh* values by that for *tlh*, varied greatly both spatially and temporally for both years, and changes occurred in a matter of weeks (Table 1 and Fig. 2 and 3). Studies of other U.S. coastal waters (Gulf Coast and Pacific) typically show that most samples contain either a majority of tdh^+ strains lacking *trh* or of tdh^+ trh^+ strains (6, 16, 20). Only one other study of U.S. waters (Chesapeake Bay) has shown a higher prevalence of trh^+ over tdh^+ (18). However, studies from other countries, such as Norway, India, and France, have also shown a higher relative prevalence of trh^+ (4, 8, 9). The relative percentage of pathogenic *V. parahaemolyticus* in oysters in Rhode Island (2.5 to 31.9% on average) (Table 1) is higher than what has been reported for other coastal locations in the United States (0.3 to 3.2%) (10). However, recent studies have shown that the percentage of pathogenic *V. parahaemolyticus* can be high (16).

Conclusions. This study provides a range of *V. parahaemolyticus* densities found in Rhode Island oysters and water samples. We detected a higher abundance of the *trh* gene than of the *tdh* gene. It is unknown if the levels of pathogenic *V. parahaemolyticus* mea-

TABLE 1 Percentages of pathogenic *V. parahaemolyticus* relative to total *V. parahaemolyticus* in water and oyster samples from Fox Island, Narragansett Bay, and two farms in the coastal ponds for 2009 and 2010 combined

Farm[a]	% *trh* or *tdh* prevalence [avg ± SD (range)] in:			
	Oysters		Water	
	trh	*tdh*	*trh*	*tdh*
NB1	2.5 ± 5.0 (0–17.8)	0.2 ± 0.3 (0–0.8)	6.2 ± 11.8 (0–31.8)	0 (0)
NB2	7.2 ± 11.2 (0–39.8)	0.4 ± 0.8 (0–2.4)	10.5 ± 16.7 (0–45.8)	0 (0)
CP1	31.9 ± 39.2 (0–100)	0.4 ± 1.3 (0–4.6)	15.7 ± 29.0 (0–100)	0 (0)
CP2	6.3 ± 4.7 (0.1–24.8)	3.5 ± 7.6 (0–24.8)	2.4 ± 3.0 (0–4.6)	1 ± 1.5 (0–3.8)
Fox Island	NT[b]	NT	7 ± 14.4 (0–38.9)	2.7 ± 8.1 (0–24.3)
Total	12.1 ± 23.4 (0–100)	1.1 ± 3.9 (0–24.8)	8.2 ± 17.1 (0–100)	0.7 ± 3.4 (0–24.3)

[a] NB1 and NB2 are farms in Narragansett Bay; CP1 and CP2 are farms in coastal ponds.
[b] NT, not tested.

sured in this study by qPCR were due to the presence of one or a few trh^+ strains lacking *tdh* in relatively higher abundance or to the accumulation of low levels of multiple tdh^+ trh^+ strains and of trh^+ strains lacking *tdh*. This study provides a baseline for further studies of the ecology of *V. parahaemolyticus* in coastal waters in a temperate estuary in the Northwest Atlantic.

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