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Recreational swimmers' exposure to *Vibrio vulnificus* and *Vibrio parahaemolyticus* in the Chesapeake Bay, Maryland, USA



Kristi S. Shaw^{a,b,*}, Amy R. Sapkota^b, John M. Jacobs^c, Xin He^d, Byron C. Crump^{a,e}

^a University of Maryland, Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, MD 21601, USA

^b University of Maryland, School of Public Health, Maryland Institute for Applied Environmental Health, 2234P SPH Building, College Park, MD 20742, USA

^c National Ocean Service (NOS), National Centers for Coastal Ocean Science (NCCOS), Cooperative Oxford Laboratory (COL), 904 S. Morris Street, Oxford, MD 21654, USA

^d University of Maryland, School of Public Health, Department of Epidemiology and Biostatistics, 2234H SPH Building, College Park, MD 20742, USA

^e Oregon State University, College of Earth, Ocean, and Atmospheric Science, 104 CEOAS Administration Building, Corvallis, OR, USA

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ABSTRACT

Vibrio vulnificus and *Vibrio parahaemolyticus* are ubiquitous in the marine–estuarine environment, but the magnitude of human non-ingestion exposure to these waterborne pathogens is largely unknown. We evaluated the magnitude of dermal exposure to *V. vulnificus* and *V. parahaemolyticus* among swimmers recreating in *Vibrio*-populated waters by conducting swim studies at four swimming locations in the Chesapeake Bay in 2009 and 2011. Volunteers ($n = 31$) swam for set time periods, and surface water ($n = 25$) and handwash ($n = 250$) samples were collected. Samples were analyzed for *Vibrio* concentrations using quantitative PCR. Linear and logistic regressions were used to evaluate factors associated with recreational exposures. Mean surface water *V. vulnificus* and *V. parahaemolyticus* concentrations were 1128 CFU mL⁻¹ (95% confidence interval (CI): 665.6, 1591.4) and 18 CFU mL⁻¹ (95% CI: 9.8, 26.1), respectively, across all sampling locations. Mean *Vibrio* concentrations in handwash samples (*V. vulnificus*, 180 CFU cm⁻² (95% CI: 136.6, 222.5); *V. parahaemolyticus*, 3 CFU cm⁻² (95% CI: 2.4, 3.7)) were significantly associated with *Vibrio* concentrations in surface water (*V. vulnificus*, $p < 0.01$; *V. parahaemolyticus*, $p < 0.01$), but not with salinity or temperature (*V. vulnificus*, $p = 0.52$, $p = 0.17$; *V. parahaemolyticus*, $p = 0.82$, $p = 0.06$). Handwashing reduced *V. vulnificus* and *V. parahaemolyticus* on subjects' hands by approximately one log (93.9%, 89.4%, respectively). It can be concluded that when Chesapeake Bay surface waters are characterized by elevated concentrations of *Vibrio*, swimmers and individuals working in those waters could experience significant dermal exposures to *V. vulnificus* and *V. parahaemolyticus*, increasing their risk of infection.

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1. Introduction

Vibrio vulnificus and *Vibrio parahaemolyticus* are normal functioning members of natural bacterioplankton communities in estuarine and

marine waters that are routinely used for swimming and other recreational activities. These microorganisms can also cause mild to severe infections, including wound infections, gastroenteritis, and septicemias, among individuals who are exposed to contaminated waters (Dziuban et al., 2006; Hlavsa et al., 2011; Yoder et al., 2008). In the Chesapeake Bay region, the Centers for Disease Control and Prevention (CDC) reported 65 illnesses associated with *Vibrio* spp. infections in 2011 (CDC, 2013a). Foodborne Diseases Active Surveillance Network (FoodNet) data showed a 43% increase (CI: 16%–76%) in the incidence of *Vibrio* infections at ten U.S. sites in 2012 compared with 2006–2008 (CDC, 2013b).

Specifically, there are approximately 93 serious (requiring hospitalization) cases of *V. vulnificus* reported in the United States annually (Scallan et al., 2011). A study of non-foodborne *Vibrio* infections (NFVIs) from 1997 to 2006, before Vibriosis became a nationally notifiable disease, reported that *V. vulnificus* was responsible for 35% of all NFVIs and 78% of NFVI deaths in the United States (Dechet et al., 2008). For immunocompromised individuals infected with *V. vulnificus*, there is an estimated 50% mortality rate (Oliver, 2005). In contrast, *V. parahaemolyticus* infections

Abbreviations: ANOVA, analysis of variance; ATCC, American Tissue Culture Collection; CDC, Centers for Disease Control and Prevention; CFU, colony forming unit; CI, confidence interval; Ct, cycle threshold; DNA, deoxyribonucleic acid; FAO, Food and Agricultural Organization; FDA, Food and Drug Administration; HIV, human immunodeficiency virus; ID₅₀, median infective dose; NFVI, non-foodborne *Vibrio* infection; PBS, phosphate buffered saline; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; TBSA, total body surface area; *tsh*, thermostable direct hemolysin; *trh*, thermostable related hemolysin; *vcgC*, virulence correlated gene, clinical; WHO, World Health Organization; YSI, Yellow Springs Instruments.

* Corresponding author at: Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, 2234P SPH Building #255, College Park, MD 20742, USA. Tel.: +1 443 521 4814.

E-mail address: krististevensshaw@gmail.com (K.S. Shaw).

¹ Present address: University of Maryland, School of Public Health, Maryland Institute for Applied Environmental Health, 2234P SPH Building, College Park, MD 20742, United States.

are not as severe as those caused by *V. vulnificus*, rarely progressing to septicemias (5%). However, the percentage of *V. parahaemolyticus* manifesting as wound infections (34%) is comparable to that of *V. vulnificus* (45%), and the percentage of *V. parahaemolyticus* infections manifesting as gastroenteritis (59%) is significantly higher than that of *V. vulnificus* (5%) (Dechet et al., 2008).

Routes of exposure to *V. vulnificus* and *V. parahaemolyticus* include ingestion of contaminated seafood, dermal contact with contaminated estuarine/marine water and, in the case of *V. vulnificus*, dermal contact with contaminated fish (CDC, 2013c; CDC, 2013d). While the non-ingestion infectious dose is largely unknown for both *V. vulnificus* and *V. parahaemolyticus* (FDA, 2012), risk assessments from the U.S. Food and Drug Administration suggest that the ingestion infectious dose, producing a 50% probability of illness for *V. parahaemolyticus*, is approximately 10^6 to 10^8 CFU g^{-1} (FDA, 2005). Risk of illness modeled by the Food and Agricultural Organization of the World Health Organization (FAO/WHO) approximated an ingestion infectious dose of 10^3 to 10^7 CFU g^{-1} oyster tissue for *V. vulnificus* (WHO, 2005). Meanwhile, the use of sub-cutaneous *V. vulnificus* inoculums in murine models has demonstrated a non-ingestion infectious dose of 1000 CFU, with an ID₅₀ of approximately 10 CFU for iron-dextran treated mice (Thiaville et al., 2011). Therefore, it is conceivable that the non-ingestion human infectious dose, encountered from direct contact between an open wound and *Vibrio*-populated media (e.g., water, surfaces, seafood products), may equate to a fraction of the estimated ingestion infectious dose.

While non-ingestion, dermal exposures to *Vibrio* are likely important with regard to public health—potentially contributing to increasing rates of illness and deaths associated with these microorganisms—very little is known about the magnitude of dermal exposure to these environmental pathogens in recreational settings. Therefore, we investigated the magnitude of non-ingestion, dermal exposures to *V. vulnificus* and *V. parahaemolyticus* among swimmers in select locations of the Chesapeake Bay by testing the prevalence of these microorganisms in handwash samples. Using the handwash data, we also quantified total body dermal exposures that could result from swimming in *Vibrio*-contaminated surface water. Finally, we assessed the efficacy of handwashing to remove *Vibrio* species from the skin surface following dermal exposure, and evaluated surface water conditions that favor

the transmission of these pathogens to humans. To our knowledge, these are the first data of their kind.

2. Materials and methods

2.1. Swimming sites

Recreational beaches on four different rivers in the Chesapeake Bay were chosen for our swimming sites: Choptank River, Chester River, Tred Avon River and Chesapeake mid-Bay (Sandy Point State Park) (Fig. 1). These sites were selected based on differing salinities and geographic locations to ensure a range of surface water *Vibrio* spp. concentrations in order to test associations between *Vibrio* spp. concentrations in water and dermal exposures among swimmers. Swims were conducted approximately 1–2 h post high tide to standardize tidal cycle across swims and best attempts were made to schedule each swim during midday hours, although sampling in the Chester River was completed slightly later in the midafternoon.

2.2. Institutional review board

This study was reviewed and approved by the University of Maryland Institutional Review Board (Protocol: 11-0442).

2.3. Study population

The study population was a convenience sample of individuals recruited from a local academic institution. The initial 2009 swim (Sandy Point State Park) included 19 participants, and subsequent 2011 swims (Choptank River, Tred Avon River, and Chester River) included four participants for each swim, based upon a sample size calculation performed using the 2009 data. Specifically, sample size was calculated for a desired power of 0.90, preferred detection level of 25 CFU and an alpha of 0.05, using standard deviation calculations from the 2009 swim study handwash samples: 4.89 CFU mL^{-1} (between swim), 10.5 CFU mL^{-1} (between swimmer) (*V. vulnificus*); and 3.31 CFU mL^{-1} (between swim), 4.4 CFU mL^{-1} (between swimmer) (*V. parahaemolyticus*). It was determined that three swims were needed

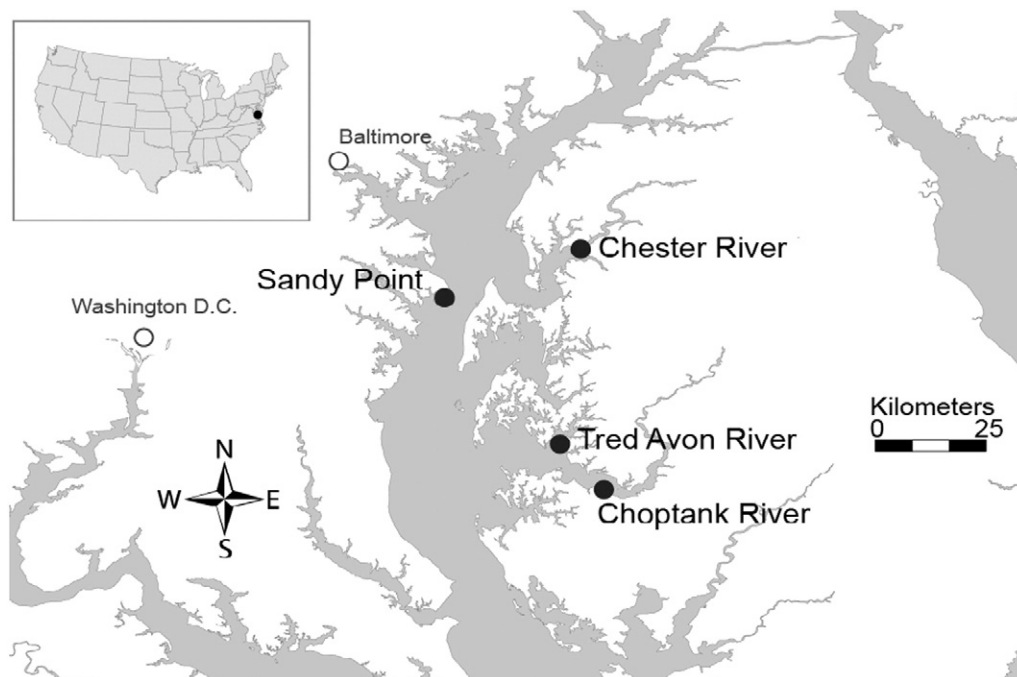


Fig. 1. Map of swimming sites in the Chesapeake Bay that were included in this study. From: Tracey Saxby, Kate Boicourt, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/displayimage-127-5815.html).

per site and three swimmers were needed for each swim. Based on these results, each 2011 study consisted of five swims per location, with four swimmers, to account for any unplanned challenges that might arise during the swim studies.

For each swim, participants were assigned random letters from A to S, which were associated with each of their samples. Subject names were not associated with those sample letters and no identifiable information was recorded to associate samples with swim participants.

2.4. Swim study times and activities

In 2009, a total of ten, independent, timed swims were conducted at each site with the same group of 19 swimmers, ranging from 2 to 20 min, increasing incrementally. In 2011, a standardized swim time of 8 min per swim ($n = 15$) was selected based on the 2009 data, which showed that the average concentration of *Vibrio* spp. in handwash samples stabilized at an approximate exposure duration of 8 min. During the timed swims, swimmers were requested to keep their hands submerged for the full time that they were in the water. Other activity was not restricted. Swimmers were allowed to swim, wade, float, etc., to account for normal swimming behavior. Time between handwash collection (described below) and the subsequent timed swim was approximately 5 min.

2.5. Handwash stations and handwash collection

Handwash stations were assembled on rectangular, plastic resin folding tables and shaded completely by a tent. Sterile phosphate buffered saline (PBS, pH 7.4, 500 mL) (FDA, 1998) was aliquoted into Ziploc freezer bags (1 gal size) and stored at 4 °C until use (<24 h storage). Bags corresponding to each swim participant were clipped to a central holding apparatus and opened approximately 1 min before each discrete swim time was completed. After each swim, each participant completely submerged their hands in the bag of PBS and rubbed them together in a vigorous manner for 60 s following the guidance of Larson et al. (1998), Brouwer et al. (2000) and Chen et al. (2001). During the Choptank River 2011 swim study, an additional handwash sample was taken after each initial handwash to estimate the efficiency of handwashing in the reduction of *Vibrio* concentrations on hands. All bags were immediately sealed upon handwash completion. Samples were either filtered in the field or frozen and filtered in the lab using sterile 0.22 µm Sterivex-GP polyethersulfone filters (Millipore, Billerica, MA), wrapped in Parafilm M laboratory wrapping film (Bemis Flexible Packaging, Oshkosh, WI), sealed in a labeled 7 oz Whirlpak bag (Nasco, Fort Atkinson, WI) and stored at –20 °C.

Control handwash samples were collected (one per person) before individuals entered the water for the first time to account for any background *Vibrio* spp. on their hands. Also, control sample bags ($n = 2$ at each time point) of sterile PBS were clipped onto the board and opened at the same time as each handwash collection bag to account for any potential airborne contamination or prior contamination of collection bags.

2.6. Surface water collection

Surface water samples were collected at each sampling location in sterile wide mouth polypropylene 1 L bottles (Nalgene Thermo Scientific, Waltham, MA). At the location nearest to swimmers, bottles were rinsed three times with surface water and then dipped below the surface for a final 1 L collection volume. Surface water (200 mL) was filtered in the field through sterile 0.22 µm Sterivex-GP polyethersulfone filters (Millipore, Billerica, MA) using a 60 mL BD Luer-Lok syringe (BD, Franklin Lakes, NJ). Air was pushed through the filter to remove as much water as possible, and then filters were wrapped in Parafilm M laboratory wrapping film (Bemis Flexible Packaging, Oshkosh, WI) and sealed in a labeled Whirlpak bag (Nasco, Fort Atkinson, WI). Filters were stored on ice until return to the laboratory (approximately 1 h), where they were stored at –20 °C.

2.7. Fecal indicator measurements

Fecal indicator measurements were made following standard methods for enumerating *Enterococci* (Eaton et al., 1998). Briefly, surface water samples were filtered in triplicate volumes onto sterile 0.45 µm pore size, 47 mm diameter, nitrocellulose Fisherbrand water-testing membrane filters (Fisher Scientific, Pittsburgh, PA), plated onto Difco™ m *Enterococcus* (BD, Franklin Lakes, NJ) agar, and incubated for 48 h at 35 °C. All light to dark red colonies were recorded as presumptive *Enterococci*.

2.8. DNA extraction, detection and quantification

DNA was extracted from all filters following a modified MO BIO PowerSoil extraction protocol (Jacobs et al., 2009) and stored at –80 °C. A Bio-Rad CFX96 Touch™ real-time PCR detection system (Bio-Rad, Hercules, CA, USA) was then used to detect total *V. vulnificus* (Panicker and Bej, 2005) and total *V. parahaemolyticus* (Nordstrom et al., 2007) in each sample using TaqMan chemistry. Samples testing positive for either species were subjected to further qPCR testing for virulence-associated genes (*V. vulnificus*: virulence correlated gene, *vcgC* allele (Baker-Austin et al., 2010); *V. parahaemolyticus*: thermostable direct hemolysin (*tdh*) and thermostable related hemolysin (*trh*) (Nordstrom et al., 2007)). Quantitative PCR was performed by using 2.50 µL of 10× PCR buffer (Qiagen, Valencia, CA), 1.25 µL of 25 mM MgCl₂ (Qiagen), 0.50 µL of 10 mM dNTP's solution (Qiagen), 5 µL 1× Q solution (Qiagen), 0.45 µL of 5 U/µL⁻¹ TopTaq DNA polymerase (Qiagen), 0.188 µL of 10 µM internal control primers (each), 0.375 µL of 10 µM internal control probe, 2 µL internal control DNA, 0.50 µL of 10 µM primer (each), 0.188 µL of 10 µM probe and 3 µL DNA template per reaction, with the exception of the *vcgC* assay, in which 5 µL of DNA template was used. DNase/RNase free water was added to bring the total reaction volume to 25 µL. Two-stage qPCR cycling parameters have been described previously (Shaw et al., 2014).

A unique internal control assay, including a primer set, probe with unique fluorochrome, and internal control DNA, was added to each tube, excluding the *vcgC* analyses, to test for inhibition (Nordstrom et al., 2007). Positive controls were also run in a separate well of each qPCR assay plate: *V. parahaemolyticus* USDA TX2103 and *V. vulnificus* ATCC 27562. Standard curves were constructed as reported in Jacobs et al. (2010) from spiked environmental matrices and used during each qPCR analysis with the appropriate qPCR parameters. Cycle threshold (Ct) value was plotted against standards of known concentrations to determine PCR unit quantities of CFUs.

2.9. Physical and chemical measurements

Physical and chemical measurements were taken before and during each swim. Measurements were taken with a YSI 556 Multiprobe System (YSI Incorporated, Yellow Springs, OH). Due to probe malfunction during the Choptank swim, salinity measurements from July 10, 2011 at the Choptank River were retrieved from the Maryland Department of Natural Resources monthly sampling on July 13, 2011, collected 1.23 nautical miles from the swim study site. According to almanac data records (<http://www.wunderground.com/history/airport/KSBY/2011/7/10/DailyHistory.html>), there was no precipitation between July 10 and July 13, so it can be deduced that the salinity was likely similar on July 10.

2.10. Data analysis

Quantitative PCR data were exported to Excel (Microsoft Word, Redmond, WA) using Bio-Rad CFX Manager™ Software (Bio-Rad, Hercules, CA, USA). Statistical analysis was completed using Intercooled Stata 9.1 for Macintosh statistical software (StataCorp LP, College Station, TX). Descriptive statistics included means, standard deviations and ranges (min to max) of *Vibrio* spp. concentrations. Analysis of variance

(ANOVA) was conducted to determine if any individual participant contributed to significant variance in handwash data. Linear regression was completed to evaluate associations between handwash concentrations by swim length, surface water concentration, salinity and temperature. Handwash concentrations were then divided by the corresponding surface water concentration to normalize data before additional linear regression analyses evaluating associations with exposure time. Logistic regression was then conducted to evaluate associations between the presence of virulence-associated genes in handwash samples, *Vibrio* surface water density and environmental conditions.

2.11. Conversion of handwash qPCR results to CFU cm⁻²

Previously calculated total body surface area (TBSA) averages for adults and children, including the ratio of hand and palm surface area to TBSA, were used to quantify total body dermal exposures from the data collected in this study. Measurements of patient hands are routinely employed by physicians to estimate the area of a burn injury (Amirshaybani et al., 2001). The average adult hand (distal wrist to finger tips) is ~1% of total body surface area (TBSA) and the average adult palm (wrist to base of fingertips) is ~0.5% (Mosteller, 1987). A rough, and likely conservative, estimate of the entire area of the average adult hand (palm, fingertips and back of hand) would therefore be approximately double the average percentage of TBSA for a hand, equaling ~2% of TBSA. The average TBSA for adult males and females is 1.9 m⁻² and 1.6 m⁻², respectively, with a combined average of 1.73 m⁻² (Mosteller, 1987). If average handwash densities of each *Vibrio* species are interpreted as CFUs per hand area, an estimate of density for total body surface area can be calculated by dividing the PCR unit quantity by average hand area such that CFU cm⁻² = CFU/(0.04 * 17,300 cm²).

3. Results

3.1. Environmental conditions

Average salinity and water temperature (±standard deviation) for each of the four swim sites were as follows: 9.9 ppt (±0.01), 27.7 °C (±0.22) (Sandy Point State Park); 6.1 ppt (±0.00), 31.4 °C (±0.26) (Choptank River); 7.5 ppt (±0.48), 31.0 °C (±0.59) (Tred Avon

River); and 5.5 ppt (±0.05), 30.9 °C (±0.21) (Chester River). Each site experienced small changes in salinity (0–1 ppt) and temperature (0.5–1 °C) over the course of each swim study.

3.2. Enterococci counts

Enterococci counts confirmed that all swim study sites were appropriately open for recreational swimming according to Maryland's single sample maximum allowable density at a recreational beach (COMAR, 2013), which is less than 104 CFU 100 mL⁻¹. The geometric mean (±standard deviation) of the *Enterococci* counts (CFU 100 mL⁻¹) for each swim study site was as follows: 22.2 (±1.3) (Sandy Point); 9.9 (±13.7) (Choptank); 8.8 (±9.1) (Tred Avon); and 22.5 (±7.9) (Chester).

3.3. Surface and handwash concentrations

Average concentrations (±standard deviation) of *Vibrio* CFU mL⁻¹ in surface water and handwash samples are presented in Table 1. Mean surface water *V. vulnificus* and *V. parahaemolyticus* concentrations were 1128 (95% CI: 665.6, 1591.4) CFU mL⁻¹ and 18 (95% CI: 9.8, 26.1) CFU mL⁻¹, respectively, across all sampling locations. Mean *Vibrio* concentrations in handwash samples were 180 (95% CI: 136.6, 222.5) CFU cm⁻² (*V. vulnificus*) and 3 (95% CI: 2.4, 3.7) CFU cm⁻² (*V. parahaemolyticus*).

During the Choptank River swim study we observed that handwashing resulted in an overall average reduction of 89.4% (95% CI: 80.1%, 98.7%) for *V. vulnificus* and 93.9% (95% CI: 86.5%, 101.3%) for *V. parahaemolyticus* concentrations in handwash samples.

Data were log transformed (log₁₀) to equalize variance before regression analyses. Linear regression analysis demonstrated a significant positive association between *V. vulnificus* handwash concentrations and surface water concentrations, predicting the log handwash CFU cm⁻² as $y = 0.808 * (\text{surface water cells CFU mL}^{-1}) - 0.4192$ (adjusted R² = 0.6139; $p < 0.001$) (Fig. 2). When a similar regression model was fit for *V. parahaemolyticus*, a significant positive association was also found (adjusted R² = 0.3071; $p < 0.002$) and log handwash CFU cm⁻² were predicted as $y = 0.3563 * (\log \text{ surface water CFU mL}^{-1}) - 0.0896$ (Fig. 2). The average ratio of CFU cm⁻² in handwash samples to

Table 1
Average concentrations of *V. vulnificus* (Vv) and *V. parahaemolyticus* (Vp) observed in surface water and handwash samples.

Site	Swim #	Swim time (min)	Surface water Vp CFU mL ⁻¹	Vp CFU cm ⁻² handwash (standard deviation)	Surface water Vv CFU mL ⁻¹	Vv CFU cm ⁻² handwash (standard deviation)
Sandy Point	1	2	15.9	3.9 (5.7)	631.9	18.7 (26.9)
Sandy Point	2	4	61.8	2.2 (1.8)	2411.4	86.9 (83.6)
Sandy Point	3	6	70.3	8.7 (10.1)	4699.6	159.4 (195.5)
Sandy Point	4	8	56.6	4.7 (4.0)	2544.7	170.9 (302.3)
Sandy Point	5	10	10.8	0.9 (0.7)	1546.7	161.4 (162.7)
Sandy Point	6	12	14.3	1.4 (2.0)	1792.3	218.5 (204.6)
Sandy Point	7	14	32.5	2.9 (8.1)	2373.9	529.6 (814.1)
Sandy Point	8	16	17.5	1.9 (4.3)	2839.7	406.4 (574.6)
Sandy Point	9	18	15.4	2.4 (3.7)	1742.4	216.0 (248.9)
Sandy Point	10	20	28.8	4.7 (3.2)	982.3	193.1 (196.5)
Choptank	1	8	19.4	2.3 (1.3)	644.4	88.2 (69.6)
Choptank	2	8	29.3	1.4 (1.6)	153.2	17.9 (12.2)
Choptank	3	8	26.1	2.9 (2.9)	703.1	79.2 (63.8)
Choptank	4	8	12.3	1.3 (1.0)	297.6	26.8 (21.9)
Choptank	5	8	10.5	0.6 (1.2)	477.2	19.5 (26.2)
Tred Avon	1	8	7.1	3.7 (3.2)	316.4	51.9 (49.4)
Tred Avon	2	8	3.0	1.1 (1.6)	164.7	13.2 (14.6)
Tred Avon	3	8	10.1	8.2 (10.9)	988.1	133.5 (41.4)
Tred Avon	4	8	6.7	0.4 (0.8)	330.7	104.4 (196.2)
Tred Avon	5	8	0.0	0.8 (0.8)	123.1	15.3 (7.2)
Chester	1	8	0.0	1.5 (2.1)	874.1	70.6 (72.5)
Chester	2	8	0.0	2.1 (3.2)	621.5	62.6 (38.9)
Chester	3	8	0.0	1.7 (3.4)	239.5	109.5 (104.2)
Chester	4	8	0.0	0.3 (0.6)	327.6	52.2 (35.0)
Chester	5	8	0.0	0.5 (1.0)	387.0	40.0 (26.6)

CFU mL⁻¹ in surface water, was 13.1% (95% CI: 9.3%, 17.0%) (*V. vulnificus*) and 17.8% (95% CI: 8.5%, 27.2%) (*V. parahaemolyticus*) CFU cm⁻²: CFU mL⁻¹. Linear regression indicated no significant relationship between handwash concentration and salinity or temperature for either *Vibrio* species (*V. vulnificus* adjusted R² = 0.26, *p* = 0.52, *p* = 0.17; *V. parahaemolyticus*, adjusted R² = 0.11, *p* = 0.82, *p* = 0.06).

Handwash *Vibrio* concentrations tended to increase until approximately the third swim of the day and remained fairly constant (*V. vulnificus*) or decreased (*V. parahaemolyticus*) for subsequent, longer-timed swims during the 2009 swim study (Supp. Fig. 1). During each of the four swim studies (Supp. Fig. 2), there was an appreciable, although statistically non-significant, increase in surface water concentrations of both *Vibrio* species by the third swim. However, time was not found to be a significant predictor of exposure when 2009 swim study data were analyzed with regression analysis. *Vibrio* handwash concentrations, normalized to surface water concentrations, were plotted against time and demonstrated low to minimal regression coefficients for *V. vulnificus* (adjusted R² = 0.207, *p* ≤ 0.001) and *V. parahaemolyticus* (adjusted R² = 0.003, *p* ≤ 0.01) (Supp. Fig. 3). ANOVA tests showed that individual swimmers did not contribute to the variance in the data (*p* = 0.282 (*V. vulnificus*), *p* = 0.134 (*V. parahaemolyticus*)).

3.4. Virulence associated genes

V. vulnificus *vcgC* was not detected in any of the handwash samples or surface water samples. *tdh*-Positive strains of *V. parahaemolyticus* were detected in 4.1% of handwash samples (10/243) and 7% of surface water samples (2/28). Of the 10 handwash samples positive for *tdh*,

nine handwash samples were from Sandy Point State Park and one was from the Choptank River. Sandy Point State Park and the Choptank River each had one *tdh*-positive surface water sample. *tdh* presence was not statistically significantly associated with salinity, temperature or surface water concentrations (*p* = 0.134). No *trh*-positive strains were detected.

3.5. TBSA exposures

Based on the range of *Vibrio* concentrations seen in handwash samples, the highest estimated TBSA exposure was 3060 CFU cm⁻² for *V. vulnificus* and 43 CFU cm⁻² for *V. parahaemolyticus*. The average estimated TBSA exposure was 180 CFU cm⁻² for *V. vulnificus* (95% CI: 136.6, 222.5) and 3 CFU cm⁻² for *V. parahaemolyticus* (95% CI: 2.4, 3.7) (Table 2).

4. Discussion

Handwash samples collected during this study suggest that swimmers in the Chesapeake Bay are dermally exposed to *Vibrio* spp. while recreating in waters where such bacteria naturally occur. In addition, our handwash efficiency experiment confirmed that the handwash methods employed in this study resulted in ~1 log removal of *V. vulnificus* and *V. parahaemolyticus* from swimmers' hands. The positive correlation between surface water concentrations and handwash samples that we observed provides a quantitative model to assess the degree of dermal exposure to *Vibrio* while swimming in waters harboring these bacteria. Moreover, virulent strains of *V. parahaemolyticus* were detected in surface waters and handwash samples, indicating that virulent species are present and swimmers could be exposed. While data regarding virulent strains was not quantitative, the presence of such strains raises additional concerns regarding the risk of infection from recreating in waters harboring *Vibrio*, especially given that the infectious dose of non-virulent strains—let alone virulence-associated strains—is largely unknown for dermal exposures.

Recently, a number of *Vibrio* spp. wound infections in Europe resulted from contact with Baltic and North Sea waters. In 2003, implicated water bodies associated with two cases of *V. vulnificus* wound infections, including one fatality, had *V. vulnificus* concentrations up to 10³ CFU mL⁻¹ (Ruppert et al., 2004). In 2006, three *V. vulnificus* cases prompted biweekly sampling at beaches in Germany, resulting in 9 out of 10 samples testing positive for *V. vulnificus* (Frank et al., 2006). Additionally, a surface water survey in The Netherlands showed a maximum of 10² CFU mL⁻¹ total *Vibrio*, with one *Vibrio cholerae* wound infection occurring during the sampling period (Schets et al., 2011b).

In this study, we observed a maximum of 4.7 × 10³ CFU mL⁻¹ *V. vulnificus* and 70 CFU mL⁻¹ *V. parahaemolyticus* in Chesapeake Bay water samples. A recent quantitative microbial risk assessment of *Vibrio*, using modeled *V. parahaemolyticus* water concentrations up to 10 CFU mL⁻¹, estimated that, under these conditions, thirteen surfer and nine child illnesses per 1000 recreationists (surfer or swimmer) could occur, falling below the Environmental Protection Agency's benchmark for recreational exposure illnesses of 19 per 1000 for either group (Dickinson et al., 2013). Given these estimates, *Vibrio* concentrations measured in water during our study could compromise swimmer safety, particularly swimmers with open wounds, resulting in a number of illnesses above this benchmark level.

With the use of our handwash data, it is possible to quantify *Vibrio* exposure in units of a predicted dose. By estimating the size of a typical wound for an adult or a child, the relative exposure dose can be evaluated. For instance, if an adult experiences the average *V. vulnificus* handwash concentration from this study of 180 CFU cm⁻² and a wound is 2 cm², the person's wound would be exposed to an estimated dose of 360 CFU. While it is possible that the exposure dose may be impacted by a swimmer's position in the water column (e.g., an upright swimmer's legs being closer than their arms to the sediment layer), a

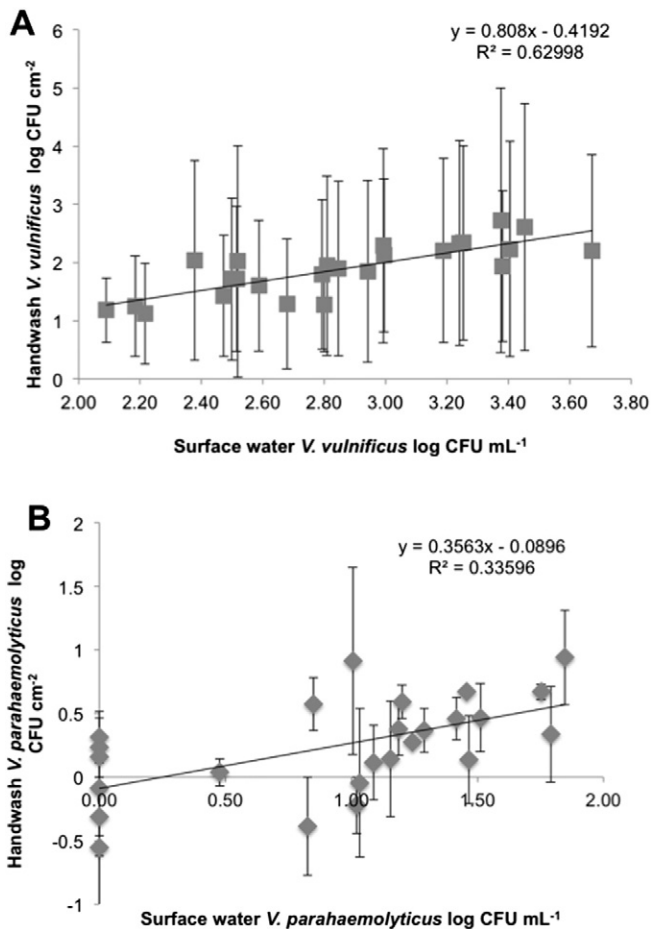


Fig. 2. *V. vulnificus* (panel A) and *V. parahaemolyticus* (panel B) average handwash concentrations in relation to surface water concentrations for all swim studies.

Table 2
Estimated *V. vulnificus* (Vv) and *V. parahaemolyticus* (Vp) CFU by swim site, for hands (HW), for total body surface area (TBSA) and per cm² body surface area.

Swim site	CFU per	Overall Vp average	Overall Vp standard deviation	Highest HW Vp	Overall Vv average	Overall Vv standard deviation	Highest HW Vv
Choptank	HW	1315	480	4844	32,843	34,957	116,795
	TBSA	32,881	11,988	121,104	823,914	575,401	2,919,865
	cm ²	1.9	0.7	7.0	47.5	50.5	169
Tred Avon	HW	2286	1902	16,909	44,044	66,393	275,770
	TBSA	57,152	47,552	422,736	1,032,290	828,803	6,894,238
	cm ²	3.3	2.8	24.4	59.7	47.9	398.5
Chester	TBSA	2346	1541	4731.56	46,343	41,962	179,643
	cm ²	58,649	38,514	118,289	1,158,582	457,061	4,491,080
	cm ²	3.4	2.2	6.8	67.0	60.6	259.6
Sandy Point	HW	2633	1590	29,816	125,290	176,781	1,675,186
	TBSA	65,818	39,754	745,403	1,474,215	1,585,427	52,943,859
	cm ²	3.8	2.30	43.1	218.5	382.6	3060.3

previous study showed no significant difference in *Vibrio* concentrations relative to water column depth, although the study was conducted in conditions with an undisturbed sediment layer (Rhodes et al., 2013).

Oral ingestion rates of surface water during swimming have been estimated by (Dufour et al., 2006) and are used in the Environmental Protection Agencies Exposure Factors Handbook (U.S. EPA, 2011). Based on these rates, the ingestion of *V. vulnificus* and *V. parahaemolyticus* among Chesapeake Bay swimmers can be estimated using the average bacterial levels found in surface water samples in this study. According to these estimates, a child (<18 years of age) may ingest an average of 42,000 *V. vulnificus* CFU per swimming event or 55,000 CFU h⁻¹ (Table 3).

Due to limited data from human exposure studies, the dose-response mechanism for *V. vulnificus* and *V. parahaemolyticus* is poorly understood and creates an obstacle in estimating true overall risk associated with recreational exposures. Additionally, genetic virulence markers and overall mechanisms of virulence for each *Vibrio* species are debated within the scientific community, resulting in a level of uncertainty when depending only on virulence markers to estimate overall risk of illness (Jones et al., 2012; Staley and Harwood, 2010; Thiaville et al., 2011). Because of this, it is unknown whether the estimated dermal and oral doses from this study would result in an infection in an immuno-competent individual, much less someone with compromised immune function or a pre-existing condition known to increase susceptibility to *Vibrio* illness (e.g., liver disease).

While overtly immuno-compromised populations (e.g., HIV-positive individuals, cancer patients, organ-transplant recipients) could be particularly susceptible to *Vibrio* infection, there are emerging populations in rising numbers that should be considered immuno-compromised, including diabetics (CDC, 2012) and those taking steroidal medications (e.g., to control asthma, rheumatoid arthritis and inflammatory bowel disease) (Akinbami et al., 2012; Molodecky et al., 2012; Myasoedova et al., 2010). With immuno-compromised populations growing, it is conceivable that a greater proportion of the population could be susceptible to *Vibrio* infections at lower levels of dermal and/or oral exposures. Moreover, given the increasing sub-population of children diagnosed with asthma and diabetes (Akinbami et al., 2009; CDC, 2012), it is prudent to consider these most sensitive sub-populations when formulating recommendations for recreational water use based upon surface water *Vibrio* spp. concentrations. A recent swimming exposure assessment of children highlighted the fact that children swim more often, tend to stay in the water longer, submerge their heads more often and swallow more water than adults while swimming (Schets et al., 2011a). Additionally, non-intact skin conditions (i.e., cuts, scrapes)

that are common among children may lead to increased susceptibility to *Vibrio* infection. While pediatric *Vibrio* case reports resulting from ingestion exposures are limited, perhaps due to limited ingestion of raw or undercooked seafood by children or underreporting, future wound infections and otitis (ear inflammation or infection) cases may be anticipated to increase as recreational water temperatures rise.

Predictive models of surface water *V. vulnificus* and *V. parahaemolyticus* concentrations have been developed for the Chesapeake Bay, using the variables of salinity and temperature as the key determinants of surface water bacterial presence and abundance (Jacobs et al., 2010). Other studies have also shown that these are important environmental variables when modeling *V. vulnificus* and *V. parahaemolyticus* surface water concentrations in other geographical areas (Baker-Austin et al., 2013; Johnson et al., 2010; Johnson et al., 2012; Zimmerman et al., 2007). Due to the warm, uniform water temperatures and small range of salinity (5–9 ppt) at the studied beaches, salinity and temperature could not be properly tested as correlates of *Vibrio* exposure. However, we did observe that surface water samples from Sandy Point State Park had higher concentrations of *V. vulnificus* and *V. parahaemolyticus*, likely due to the favorable salinity of ~10 ppt (Banakar et al., 2011; Jacobs et al., 2010). In the absence of broad salinity and temperature ranges, surface water predictive models can be coupled with regression models of dermal exposure for these *Vibrio* species to estimate an individual's level of dermal exposure when encountering water with known *Vibrio* concentrations. These models may provide a powerful predictor of overall dermal exposures for use by public health managers to protect public health.

5. Conclusions

This study is the first of its kind to show that swimmers could experience significant dermal exposures to *V. vulnificus* and *V. parahaemolyticus*. Due to a lack of information regarding non-ingestion dose–response for *V. vulnificus* and *V. parahaemolyticus*, it is unknown whether current levels of recreational dermal exposures in the Chesapeake Bay are likely to cause illness. However, based on our findings, swimmers are potentially being exposed to *V. vulnificus* and *V. parahaemolyticus* at concentrations for which infections are conceivable. In addition, we report that washing ones' hands following exposure to Chesapeake Bay water is effective in reducing the number of *Vibrio* on a person's skin by one log. These data support present recommendations by the Maryland Department of Health and Mental Hygiene (DHMH, 2010) to wash skin with soap and water after exposure to marine/estuarine water. Moreover, avoidance of surface waters harboring elevated concentrations of *Vibrio* by

Table 3
Mean (97% upper percentile) of estimated oral ingestion of surface water and *Vibrio* during swimming activity based on exposure estimates from EPA 2011.

	Surface water ingestion		<i>V. vulnificus</i> ingestion		<i>V. parahaemolyticus</i> ingestion	
	mL event ⁻¹	mL hour ⁻¹	CFU event ⁻¹	CFU h ⁻¹	CFU event ⁻¹	CFU h ⁻¹
Children	37 (90)	49 (120)	41,754 (101,565)	55,296 (135,421)	663 (1613)	878 (2151)
Adult	16 (53)	21 (71)	18,056 (59,811)	23,698 (80,124)	286 (950)	376 (1273)

immunocompromised individuals and those with open wounds is recommended to avoid risk of infection. In order to better protect human health, estimates of non-ingestion dose–response would be helpful in completing a quantitative microbial risk assessment to calculate relative risk of swimming in waters known to harbor *Vibrio* bacteria. Finally, these data could be paired with models of surface water *Vibrio* concentrations to predict exposure at local and regional scales.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.envint.2014.09.016>.

References

- Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. NCHS data brief; 2012. p. 1–8.
- Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of childhood asthma in the United States, 1980–2007. *Pediatrics* 2009;123:S131–45.
- Amirshaybani HR, Crecelius GM, Timothy NH, Pfeiffer M, Siggers GC, Manders EK. The natural history of the growth of the hand: I. Hand area as a percentage of body surface area. *Plast Reconstr Surg* 2001;107:726–33.
- Baker-Austin C, Gore Anthony, Oliver James D, Rangdale Rachel, McArthur J Vaun, Lees David N. Rapid in situ detection of virulent *Vibrio vulnificus* strains in raw oyster matrices using real-time PCR. *Environ Microbiol Rep* 2010;2:76–80.
- Baker-Austin C, Trinanes JA, Taylor NGH, Hartnell R, Siitonen A, Martinez-Ustaza J. Emerging *Vibrio* risk at high latitudes in response to ocean warming. *Nat Clim Chang* 2013;3:73–7.
- Banakar V, Constantin de Magny G, Jacobs J, Murtugudde R, Huq A, Wood RJ, et al. Temporal and spatial variability in the distribution of *Vibrio vulnificus* in the Chesapeake Bay: a hindcast study. *Ecohealth* 2011;8:456–67.
- Brouwer Derk H, Boeniger Mark F, van Hemmen Joop. Hand wash and manual skin wipes. *Annals of Occupational Hygiene* 2000;44(7):501–10.
- CDC. Increasing prevalence of diagnosed diabetes—United States and Puerto Rico, 1995–2010. *MMWR Morb Mortal Wkly Rep* 2012;61:918–21.
- CDC. Incidence and trends of infection with pathogens transmitted commonly through food — foodborne Diseases Active Surveillance Network, 10 U.S. sites, 1996–2012. *MMWR Morb Mortal Wkly Rep* 2013a;62:283–7.
- CDC. National Enteric Disease Surveillance: COVIS annual summary, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC; 2013b.
- CDC. *Vibrio parahaemolyticus*: general information. National Center for Emerging and Zoonotic Infectious Diseases D.o.F. Waterborne, and Environmental Disease; 2013c.
- CDC. *Vibrio vulnificus*: general information. National Center for Emerging and Zoonotic Infectious Diseases D.o.F. Waterborne, and Environmental Disease; 2013d.
- Chen Y, Jackson KM, Chea FP, Schaffner DW. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J. Food Prot* 2001; 64(1):72–80.
- COMAR. Maryland Code of Regulations: 26.08.02.03-3 water quality criteria specific to designated uses. In: Environment D.o.t, editor; 2013. Annapolis, MD.
- Dechet AM, Yu PA, Koram N, Painter J. Nonfoodborne *Vibrio* infections: an important cause of morbidity and mortality in the United States, 1997–2006. *Clin Infect Dis* 2008;46:970–6.
- DHMH. M. Vibriosis (non-cholera) fact sheet. In: Hygiene M.D.o.H.a.M., editor; 2010.
- Dickinson G, Lim KY, Jiang SC. Quantitative microbial risk assessment of pathogenic vibrios in marine recreational waters of southern California. *Appl Environ Microbiol* 2013;79:294–302.
- Dufour AP, Evans O, Behymer TD, Cantu R. Water ingestion during swimming activities in a pool: a pilot study. *J Water Health* 2006;4:425–30.
- Dziuban EJ, Liang JL, Craun GF, Hill V, Yu PA, Painter J, et al. Surveillance for waterborne disease and outbreaks associated with recreational water — United States, 2003–2004. Morbidity and mortality weekly report. Surveillance summaries 2006;55(12):1–30.
- Eaton AD, Clasceri LS, Greenberg AE, Franson MAH. Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association; 1998.
- FDA. R59: phosphate-buffered saline (PBS), pH 7.4. Bacteriological analytical methods; 1998.
- FDA. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. In: U.S. Department of Health and Human Services, editor. Center for Food Safety and Applied Nutrition U.S.F.a.D.A. U.S. Food and Drug Administration; 2005.
- FDA. Bad bug book: foodborne pathogenic microorganisms and natural toxins handbook. DC: Vibrio Washington; 2012.
- Frank C, Littman M, Alpers K, Hallauer J. *Vibrio vulnificus* wound infections after contact with the Baltic Sea, Germany. Euro surveillance: bulletin European sur les maladies transmissibles = European communicable disease bulletin; 2006 [11:E060817 060811].
- Hlavsa MC, Roberts VA, Anderson AR, Hill VR, Kahler AM, Orr M, et al. Surveillance for waterborne disease outbreaks and other health events associated with recreational water — United States, 2007–2008. *MMWR Surveill Summ* 2011;60:1–32.
- Jacobs J, Rhodes M, Sturgis B, Wood B. Influence of environmental gradients on the abundance and distribution of *Mycobacterium* spp. in a coastal lagoon estuary. *Appl Environ Microbiol* 2009;75:7378–84.
- Jacobs JM, Rhodes MR, Brown CW, Hood RR, Leight AK, Long W, Wood RJ. Predicting the distribution of *Vibrio vulnificus* in Chesapeake Bay. NOAA Technical Memorandum NOS NCOOS 112. Oxford, MD: NOAA National Centers for Coastal Ocean Science, Center for Coastal Environmental Health and Biomolecular Research, Cooperative Oxford Laboratory; 2010.
- Johnson CN, Bowers JC, Griffitt KJ, Molina V, Clostio RW, Pei S, et al. Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington, United States. *Appl Environ Microbiol* 2012;78:7249–57.
- Johnson CN, Flowers AR, Noriea 3rd NF, Zimmerman AM, Bowers JC, DePaola A, et al. Relationships between environmental factors and pathogenic vibrios in the northern Gulf of Mexico. *Appl Environ Microbiol* 2010;76:7076–84.
- Jones JL, Ludeke CH, Bowers JC, Garrett N, Fischer M, Parsons MB, et al. Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. *J Clin Microbiol* 2012;50:2343–52.
- Larson EL, Hughes CA, Pyrek JD, Sparks SM, Cagatay EU, Bartkus JM. Changes in bacterial flora associated with skin damage on hands of health care personnel. *Am J Infect Control* 1998;26(5):513–21.
- Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;142:46–54. [e42].
- Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med* 1987;317:1098.
- Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955–2007. *Arthritis Rheum* 2010;62:1576–82.
- Nordstrom JL, Vickery MC, Blackstone GM, Murray SL, DePaola A. Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters. *Appl Environ Microbiol* 2007;73:5840–7.
- Oliver JD. Wound infections caused by *Vibrio vulnificus* and other marine bacteria. *Epidemiol Infect* 2005;133:383–91.
- Panicker G, Bej AK. Real-time PCR detection of *Vibrio vulnificus* in oysters: comparison of oligonucleotide primers and probes targeting vvhA. *Appl Environ Microbiol* 2005;71: 5702–9.
- Rhodes M, Bhaskaran H, Jacobs J. Short-term variation in the abundance of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in a tidal estuary. *Mar Biol Oceanogr* 2013;2. 2:2.
- Ruppert J, Panzig B, Guertler L, Hinz P, Schwesinger G, Felix SB, et al. Two cases of severe sepsis due to *Vibrio vulnificus* wound infection acquired in the Baltic Sea. *Eur J Clin Microbiol Infect Dis* 2004;23:912–5.
- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;17:7–15.
- Schets FM, Schijven JF, de Roda Husman AM. Exposure assessment for swimmers in bathing waters and swimming pools. *Water Res* 2011a;45:2392–400.
- Schets FM, van den Berg HH, Marchese A, Garbom S, de Roda Husman AM. Potentially human pathogenic vibrios in marine and fresh bathing waters related to environmental conditions and disease outcome. *Int J Hyg Environ Health* 2011b;214:399–406.
- Shaw KS, Rosenberg Goldstein RE, He X, Jacobs JM, Crump BC, Sapkota AR. Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. *PLoS One* 2014;9:e89616.
- Staley C, Harwood VJ. The use of genetic typing methods to discriminate among strains of *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. *J AOAC Int* 2010;93:1553–69.
- Thiaville PC, Bourdage KL, Wright AC, Farrell-Evans M, Garvan CW, Gulig PA. Genotype is correlated with but does not predict virulence of *Vibrio vulnificus* biotype 1 in subcutaneously inoculated, iron dextran-treated mice. *Infect Immun* 2011;79:1194–207.
- WHO. Risk assessment of *Vibrio vulnificus* in raw oysters: interpretative summary and technical report. Microbiological risk assessment series. Geneva; Rome: World Health Organization; Food and Agriculture Organization of the United Nations; 2005.
- Yoder JS, Hlavsa MC, Craun GF, Hill V, Roberts V, Yu PA, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events—United States, 2005–2006. *MMWR Surveill Summ* 2008;57:1–29.
- Zimmerman AM, DePaola A, Bowers JC, Krantz JA, Nordstrom JL, Johnson CN, et al. Variability of total and pathogenic *Vibrio parahaemolyticus* densities in northern Gulf of Mexico water and oysters. *Appl Environ Microbiol* 2007;73:7589–96.