

Effect of Climate Change on the Concentration and Associated Risks of *Vibrio* Spp. in Dutch Recreational Waters

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Currently, the number of reported cases of recreational- water-related *Vibrio* illness in the Netherlands is low. However, a notable higher incidence of *Vibrio* infections has been observed in warm summers. In the future, such warm summers are expected to occur more often, resulting in enhanced water temperatures favoring *Vibrio* growth. Quantitative information on the increase in concentration of *Vibrio* spp. in recreational water under climate change scenarios is lacking. In this study, data on occurrence of *Vibrio* spp. at six different bathing sites in the Netherlands (2009–2012) were used to derive an empirical formula to predict the *Vibrio* concentration as a function of temperature, salinity, and pH. This formula was used to predict the effects of increased temperatures in climate change scenarios on *Vibrio* concentrations. For *Vibrio parahaemolyticus*, changes in illness risks associated with the changed concentrations were calculated as well. For an average temperature increase of 3.7 °C, these illness risks were calculated to be two to three times higher than in the current situation. Current illness risks were, varying per location, on average between 10⁻⁴ and 10⁻² per person for an entire summer. In situations where water temperatures reached maximum values, illness risks are estimated to be up to 10⁻² and 10⁻¹. If such extreme situations occur more often during future summers, increased numbers of ill bathers or bathing-water-related illness outbreaks may be expected.

KEY WORDS: Climate change; quantitative microbial risk assessment; recreational water; *Vibrio*; water temperature

1. INTRODUCTION

Worldwide, *Vibrio* is an important agent of water-transmitted illness. The most well-known strain is *Vibrio cholerae* (O1/O139), which causes

acute watery diarrhea and is responsible for a high disease burden in many countries.⁽¹⁾ For the Netherlands, and other northwest European countries, the prevalence of *V. cholerae* (O1/O139) illness is low and related to traveling to endemic countries.⁽²⁾ However, other *Vibrio* species are commonly found in Dutch bathing waters.^(3,4) These species include human pathogens, like *V. vulnificus* and *V. alginolyticus*, which are associated with wound and ear infections after exposure to contaminated waters. Other species such as *V. parahaemolyticus* and *V. fluvialis* may cause gastroenteritis after ingestion of contaminated water or consumption of

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contaminated seafood. In the Netherlands, four cases of illness by *V. alginolyticus* after swimming in the North Sea have been reported,^(3,5) as well as one case in the Binnenschelde, where *V. cholerae* (non-O1/O139) was cultured from a patient's wound.⁽³⁾

The presence and growth of *Vibrio* spp. in water bodies depends on multiple environmental factors. Although the effects of these parameters are highly species dependent, in general, temperature and salinity are considered most important.^(6–11) Other parameters include nutrient and chlorophyll-*a* concentrations and pH.^(10,12,13) Climate change is expected to both directly and indirectly affect these environmental conditions. Higher atmospheric temperatures will lead to higher water temperatures, both in oceans and inland waters.^(14,15) Salinity in fresh waters could be affected by rising sea level and/or changes in precipitation patterns and thereby river discharge.⁽¹⁶⁾ In ocean bays or estuaries, as observed for the Scheldt Estuary by Struyf *et al.*,⁽¹⁷⁾ river discharge could affect salinity as well. Such indirect effects could affect the *Vibrio* concentrations. For example, a study of Constantin de Magny *et al.*⁽¹⁸⁾ showed that *Vibrio* concentrations in the Chesapeake Bay were positively related to river flow. They attributed this to the effect of the river flow on the water salinity because of dilution. Furthermore, climate change could also indirectly affect nutrient concentrations in the water because of changes in input of nutrients, for example through runoff, and dilution caused by changes in precipitation patterns.⁽¹⁹⁾

During the summer of 2006, an increase in the number of bathers with *Vibrio* infections was reported in several countries in northwestern Europe.⁽³⁾ The summer of 2006 was relatively warm and may be a foretaste for future summers in this part of the world. Thus, as a result of climate change, the number of *Vibrio* infections because of contact with recreational water may be expected to increase. In addition, Baker-Austin *et al.*⁽²⁰⁾ suggested that *V. vulnificus* and *V. parahaemolyticus* infections in Europe because of shellfish consumption are already increasing.

As reviewed previously, quantitative predictions on future *Vibrio* concentrations in recreational waters are lacking.⁽²¹⁾ In the literature, several empirical relations between *Vibrio* presence or concentration and environmental factors have been described.^(8,10,12,20) Table I gives an overview of empirical relations between the concentration of *Vibrio* spp. and environmental variables. These

empirical formulas have been derived for different geographical locations with incomparable climate conditions, and include different environmental variables and measurement ranges. It is unclear whether such formulas are interchangeable between locations and if they will result in reliable forecasts of concentrations of *Vibrio* spp. in bathing waters in the Netherlands.

In this study, data on occurrence of *Vibrio* spp. at six different bathing sites in the Netherlands (2009–2012) were used to derive an empirical formula to predict *Vibrio* concentrations as a function of environmental parameters. The goal was to use this formula to predict the effects of climate change on *Vibrio* concentrations in recreational waters in the Netherlands by using Dutch climate scenarios and, in the case of *V. parahaemolyticus*, to evaluate how this may affect the risk of illness.

2. METHODS

2.1. *Vibrio* Data

Water samples were taken at six official bathing sites in the Netherlands.⁽³⁾ The North Sea was sampled at Bergen (N52°39.622', E004°37.577') and Katwijk (N52°12.908', E004°23.975') during 2009–2012, the Binnenschelde (N51°29.145', E004°16.586') was sampled during 2010–2012, Lake IJsselmeer (N52°41.483', E005°16.815') was sampled in 2009 only, the Oosterschelde was sampled at Tholen (N51°30.726', E004°10.501') in 2009 and at Sint-Maartensdijk (N 51°32.036' E004°04.094') during 2010–2012, and the Wadden Sea (N53°1669', E005°04.160') was sampled during 2011–2012. Sampling was done according to ISO 19458⁽²²⁾ every 2–4 weeks during the bathing season (1st of May to 1st of October). The samples were analyzed for the presence of *Vibrio* spp. by using a most probable number cultivation method as described by Schets *et al.*⁽³⁾ Briefly, samples were enriched in alkaline buffered peptone water using three different incubations: (1) at 36 ± 2 °C during 6–8 h, (2) at 36 ± 2 °C during 18–20 h, and (3) at 41.5 ± 1 °C, during 18–20 h. Subsequently, cultures were spread on thiosulphate citrate bile sucrose agar (TCBS) plates, incubated at 36 ± 2 °C for 16–20 h, and *Vibrio* characteristic colonies were confirmed and characterized to the species level. Data on water temperature, pH, and conductivity were collected for each sampling site. Water temperatures were measured on site at the

Table I. Empirical Formulas, Previously Described in the Literature, on the Relation Between the Concentration of *Vibrio* spp. and Environmental Variables

<i>Vibrio</i>	Variables ^a	Range	Location	Model	Ref				
<i>Vibrio</i> spp.	<i>T</i>	^b > 4.7–13.5 °C	Helgoland	$\text{Log } C = 9.575 - 0.183\text{SAL} + 0.054T - 0.839\text{PO}_4^{3-} - 0.33\text{Secchi}$	13				
	SAL	–	Roads						
	Phosphate	–	(North Sea)						
	Secchi	–							
	Silicate	–							
	CHL-A	–							
<i>Vibrio</i> spp.	<i>T</i>	2.9–32.7 °C	Neuse River Estuary in North Carolina	$\text{Log } C = -0.143 + 0.149\text{SAL} + 0.0528T + 0.00118\text{DOC} + 0.00562\text{CHLA} + 0.000544\text{TSS} - 0.0880\text{PH}$	12				
	SAL	0.0–27.3 PSU							
	DOC	–							
	pH	–							
	TSS	–							
	CHL-A	–							
<i>Vibrio</i> spp.	<i>T</i>	27.0 ± 2.6 °C	Neuse River Estuary in North Carolina	$\text{Log } C = 1.685 + 0.126\text{SAL}$	8				
	SAL	0–24 PSU							
	POC	0.3–4.3 mg/L							
	CHL-A	0–30 µg/L							
	FPV	0.5–13 µL/L							
	<i>T</i>	13.3–29.4 °C							
<i>Vibrio</i> spp.	DOC	64.1 – 94.7 µM C	Atlantic Ocean	$\text{Log } C = -1.3 + 0.2T$	10				
	DON	1.8 – 10.3 µM N							
	C/N of DOM	8.8 – 41.7							
	SAL	35.2 – 36.9							
	CHL-A	0.11 --- 2.25 µg/L							
	DIN	0 – 3.86 µM N							
	Silicate	0.40 --- 1.60 µM Si							
	Phosphate	0.05 --- 0.51 µM P							
	PON	0.30 – 1.69 µM N							
	POC	2.00 – 11.8 µM C							
	Iron	0.47 – 5.98 nM							
	<i>V. vulnificus</i>	<i>T</i>				0–29 °C	Barnegat Bay, NJ	$\text{Log } C = -0.280 + 0.085T$	37
		SAL				15–28 ppt			
	<i>V. vulnificus</i>	CHL-A				2–24 µg/L	Charlotte Harbor, Florida	$\text{SAL} \leq 15 \text{ psu:}$ $\text{Log } C = 1.15 + 0.0406T + 0.0793\text{SAL}$	31
<i>T</i>		17.0–31.1 °C							
SAL		3.5–25.9 PSU							
pH		–							
TURB		–							
Rainfall stream flow		–							

^aOnly correlations with variables in bold were significant. *T* = temperature, SAL = salinity, TURB = turbidity, CHL-A = chlorophyll-*a*, DOC = dissolved organic carbon, FPV = total particle suspension volume, POC = particulate organic carbon, PON = particulate organic nitrogen, DON = dissolved organic nitrogen, DOM = dissolved organic matter, TSS = total suspended solids, Secchi = Secchi disk water transparency measurement, DIN = NO₂ + NO₃⁻ + NH₄⁺.

^bRange not mentioned.

time of sampling; salinity (Schott Handylab LF11) and pH (Toledo SG2 51302520) were analyzed in the lab.

2.2. Data Analysis

The maximum likelihood method was used to estimate the most probable numbers of total *Vibrio* spp. in the water samples by using Mathematica 9.0.1 (Wolfram Research Inc., Champaign, IL, USA), under the assumptions that bacteria are Poisson distributed throughout the sample and growth occurs when the sample volume contains one or more bacteria.

To estimate the concentration of each *Vibrio* species, the percentage of the screened colonies on TCBS identified as a specific species was calculated. These percentages were then applied to the total concentration of *Vibrio* spp., yielding concentrations of specific *Vibrio* species.

Conductivity values were transformed to salinity using UNESCO International Equation of State (IES 80) as described in Fofonoff,⁽²³⁾ and scaled to salinity values for the North Sea, Oosterschelde, and IJsselmeer provided by the Dutch Ministry of Infrastructure and the Environment (RWS, Helpdeskwater). Because records of pH and conductivity were not complete, average values of salinity and pH were used per site.

To investigate the relationship between log-transformed concentrations of *Vibrio* spp. and environmental factors (temperature, pH, and salinity), multiple step-wise regression analyses were carried out using R (R version 3.0.1). Concentration of *Vibrio* spp. was treated as the dependent variable with the environmental parameters as independent variables. To examine the effect of the method, enrichment method was added as a categorical variable. Similarly, season was included as a categorical variable, with spring from March to May, summer from June to August, fall from September to November, and winter from December to February. The effects of the environmental factors and all two-way interactions were considered. The best model was selected using step-wise model selection by Akaike's information criterion (AIC), with the multiple of the number of degrees of freedom used for the penalty (k) equal to 3.84. This value for k responds to the χ^2 with 95% confidence and one degree of freedom.

Multiple step-wise regression analysis was carried out for each *Vibrio* species individually.

Table II. Climate Change Scenarios Evaluated Based upon KNMI Scenarios⁽²⁴⁾

	G_L	W_H
2050	+1 °C	+2.3 °C
2085	+1.3 °C	+3.7 °C

2.3. Climate Change Scenarios

The empirical formulas resulting from the multiple step-wise regression analyses were used to predict *Vibrio* concentrations in Dutch bathing waters during future summers (Mathematica 9.0.1, Wolfram Research Inc.). The measured concentrations in 2009–2012 were used for a reference. For the future scenarios, the most conservative (G_L) and most extreme (W_H) scenarios of the Royal Netherlands Meteorological Institute (KNMI) were adopted.⁽²⁴⁾ These scenarios are based on global temperature changes and changes in atmospheric circulation over western Europe, resulting from predictions by global climate models (GCMs). They predict changes in air temperature, precipitation, wind, and sea level in 2050 and 2085 compared to the period 1981–2010, but do not give data for water temperature, salinity, or pH at the specific locations. In general, water temperature is expected to rise gradually with the expected rise in atmospheric temperature.^(14,15) Therefore, the assumption has been made that water temperatures increase proportional to the expected increase in daily mean air summer temperatures, namely, 1–1.3 °C and 2.3–3.7 °C for 2050 and 2085, respectively (Table II). Salinity in river deltas, such as the Scheldt, could be affected by the rising sea level, aggravated by lower discharge during summers.⁽¹⁶⁾ Also, elevated CO₂ concentrations in the atmosphere are expected to decrease the pH of oceans.⁽²⁵⁾ Based on model predictions, a pH reduction in the ocean ranging from 0.3 to 0.5 units over the next 100 years was estimated. However, quantitative predictions of changes in salinity and pH for the surface waters of the Netherlands are not available. Consequently, salinity and pH were kept unchanged in the 2050 and 2085 scenarios.

To evaluate effects of changes in salinity and pH, an alternative scenario was evaluated as well (see Table III). In this scenario, the summer of 2006 was used as a proxy for future warm summers. Water temperature, salinity, and pH data from this summer, as well as for the reference period (2009–2012), were available from the Dutch Ministry of Infrastructure

Table III. Overview Average Conditions in Reference Period (2009–2012) Compared to 2006; Maximum Temperatures Between Parentheses

	2009–2012	2006
North Sea		
Temperature (°C)	15.4 (19.4 °C)	+0.1 (+0.7)
Salinity	29.6	–
pH	8.0	+0.1
Oosterschelde		
Temperature (°C)	15.4 (20.2 °C)	+0.8 (+2.3)
Salinity	32.1	–
pH	8.1	+0.1
Wadden Sea		
Temperature (°C)	15.5 (20.7 °C)	+0.6 (+0.3)
Salinity	26.5	–1.8
pH	8.1	+0.2

and the Environment (Rijkswaterstaat, Helpdesk water) for three locations (North Sea, Wadden Sea, and Oosterschelde). These sample locations are located offshore and are not officially designated for bathing, so their values may vary from the bathing water locations. Nevertheless, because data at these locations are useful to make comparisons between summers, predictions for *Vibrio* concentrations were made for both the reference period and the warm summer of 2006.

2.4. Quantitative Microbial Risk Assessment

Effects of climate change on the risk of illness were evaluated using QMRA. For pathogenic *Vibrio* spp. causing wound or ear infections, no dose-response relationships are available. However, for *V. parahaemolyticus* causing gastroenteritis, a dose-response relationships is available for the risk of illness.⁽²⁶⁾ Hence, changes in the risk of illness were evaluated for this specific species only. For the other *Vibrio* species only concentrations to which people may be exposed, could be compared.

Concentrations of *V. parahaemolyticus* (enrichment method 36 °C, 18–20 h) during a certain bathing event were determined using the associated empirical formula. Uncertainty in this prediction was included by random sampling from the error term that represents the residuals. For this calculation, it was assumed that bathing only takes place during the bathing season (1st of May to 1st of October) and when water temperatures exceed 17 °C. In addition, risks of illness during a bathing event on a day when water temperatures reach their

maximum (as measured during the reference period) were evaluated.

Ingested dose of *V. parahaemolyticus* D was calculated using:

$$D = CV, \quad (1)$$

where $C[L^{-1}]$ is the predicted pathogen concentration under the scenario examined and $V[L]$ is the individual volume of water that was consumed. Distributions of ingested volumes of water during swimming in seawater were based on random sampling from the gamma distribution of Schets *et al.*⁽²⁷⁾ Illness risks were calculated for both men (>15 years) and children (0–14 years).

Next, the risk of illness per person per exposure event was calculated using the following dose-response relationship:⁽²⁸⁾

$$P_{\text{event}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}, \quad (2)$$

where α and β are the parameters of the Beta-distributed dose-response relationship. The best estimates of parameters α and β for *V. parahaemolyticus* are, based on outbreak data from oyster consumption, 0.6 and $1.3E^6$, respectively.⁽²⁶⁾ These dose-response parameters are applied here for the swallowing of bathing water. Because $\beta > 1$ and $\alpha \ll \beta$, this formula was used instead of a hypergeometric function that would require too much computational time in this case.⁽²⁸⁾

Illness risks were calculated not only for a single bathing event, but also for an entire summer, using a negative binomial distribution that describes the number of bathing events (N):⁽²⁷⁾

$$P_{\text{summer}} = 1 - \prod_{i=1}^N (1 - P_i). \quad (3)$$

Risks were calculated (Mathematica 9.0.1, Wolfram Research Inc.) using Monte Carlo simulations with random sampling of 10,000 values. Calculations have been done for both the current and future summers under the different G_L and W_H scenarios. To evaluate effects of climate change, a fixed seed number was used in the Monte Carlo simulations.

3. RESULTS

3.1. Description of Input Data

The measured concentrations showed a seasonal fluctuation in the concentration of *Vibrio* spp. at the

Table IV. Number of Samples Positive for *Vibrio* spp., Range of *Vibrio* Concentration, Temperature Measurements, and Average Values for pH and Salinity for each of the Locations

	North Sea (Bergen)	North Sea (Katwijk)	Binnenschelde	Oosterschelde	IJsselmeer	Wadden Sea
Positive samples	44/53	46/53	40/42	58/61	4/19	25/28
<i>Vibrio</i> spp. (MPN/L)	0–4.3 × 10 ⁴	0–2.3 × 10 ⁴	0–4.3 × 10 ⁶	0–2.4 × 10 ⁵	0–2.3 × 10 ⁴	0–2.4 × 10 ⁶
Temperature (°C)	11.7–21.1	9.6–21	15.2–25.2	9.7–27.3	11.2–20.8	13–22.1
pH	8	8	9.2	7.7	8.3	7.6
Salinity	29.4	27.6	1.6	30.6	0.4	17.5

different bathing locations. Concentrations ranged from undetectable to 10⁷ cells/L with a median of 9 × 10² cells/L, with concentrations and number of positive samples varying between locations (Table IV). The cultivated species mainly consisted of *V. alginolyticus* (39%) and *V. cholerae* non-O1/O139 (37%), followed by *V. parahaemolyticus* (11%), but dominant species varied between locations. *V. fluvialis*, *V. mimicus*, and *V. vulnificus* were detected in a few samples only.

Water temperatures were similar for all sampling locations, and showed a clear seasonal variation. Water temperatures were on average 12 °C in April and peaked at around 23 °C in August. Measured pH and conductivity varied between sites, but showed less variation over time than water temperature. See Table IV for the average values of pH and salinity and the range of temperature measurements.

3.2. Multiple Regression Analysis

From the 256 measured concentrations, 39 data points were excluded from the multiple regression analyses because the concentration was below the detection limit (nondetects cannot be log transformed). The regression analyses showed that *Vibrio* spp. concentration was significantly determined by the temperature, salinity, pH, and enrichment method. Season was not a significant factor. Full details on the results of the regression analysis can be found in Supporting Information S1.

Best model fitted resulted in the following formula:

$$\text{Log } C = C_0 + M + 0.23T - 0.06\text{Sal} - 1.11\text{pH} \\ (R^2 = 0.50; df = 211), \quad (4)$$

where C is the concentration, the intercept (C_0) equals 8.66, and M is a factor representing the enrichment method and equals 0 for enrichment at 36 °C for 6–8 h, 1.31 for enrichment at 36 °C for

18–20 h, and 0.74 for enrichment at 41.5 °C for 18–20 h (Table V). Fig. 1 shows the predicted *Vibrio* spp. concentrations per location as a function of temperature. To show the course of the concentrations over time, the predictions for the North Sea at Bergen have been plotted as an example against time (Fig. 2).

Regression analysis has also been done for *V. parahaemolyticus*, *V. alginolyticus*, and *V. cholerae* (non-O1/O139). Occurrence of the other species was too sporadic to allow data analysis. Results of these analyses do differ between the species and are not similar to the results obtained for total *Vibrio* spp. The analyses lead to the following formulas.

V. alginolyticus ($R^2 = 0.55; df = 157$):

$$\text{Log } C = C_0 + M + 0.22T - 0.08\text{Sal} - 1.33\text{pH} \quad (5)$$

V. parahaemolyticus ($R^2 = 0.64; df = 46$):

$$\text{Log } C = C_0 + M + 0.16T - 0.05\text{Sal} - 2.5\text{pH} \quad (6)$$

V. cholerae (non-O1/O139) ($R^2 = 0.28; df = 53$):

$$\text{Log } C = C_0 + M + 0.24T \quad (7)$$

Again, the intercept depends on the enrichment method (Table V).

Temperature appears to affect concentrations of *V. parahaemolyticus* less than those of *V. alginolyticus* and *V. cholerae*. Dependence on salinity and pH was different between species; concentrations of *V. cholerae* (non-O1/O139) do not appear to be dependent on salinity and pH.

3.3. Predictions of *Vibrio* Concentrations Under Future Climate Change Scenarios

The empirical formulas were used to predict *Vibrio* spp. concentrations under different scenarios. Calculations were done by using the intercept for the enrichment method of 36 °C for 18–20 h because this method had the highest yield. Predictions for the North Sea, Wadden Sea, Oosterschelde, and

Table V. Values of Intercept (C_0) and Enrichment Method Factor (M) for Different Enrichment Methods and Species

	<i>Vibrio</i> spp.	<i>V. alginolyticus</i>	<i>V. parahaemolyticus</i>	<i>V. cholerae</i> (non-O1/O139)
C_0	8.66	10.87	20.05	-2.00
M (36 °C for 6–8 h)	0	0	0	0
M (36 °C for 18–20 h)	1.31	1.14	1.40	1.52
M (41.5 °C for 18–20 h)	0.74	0.58	0.75	0.99

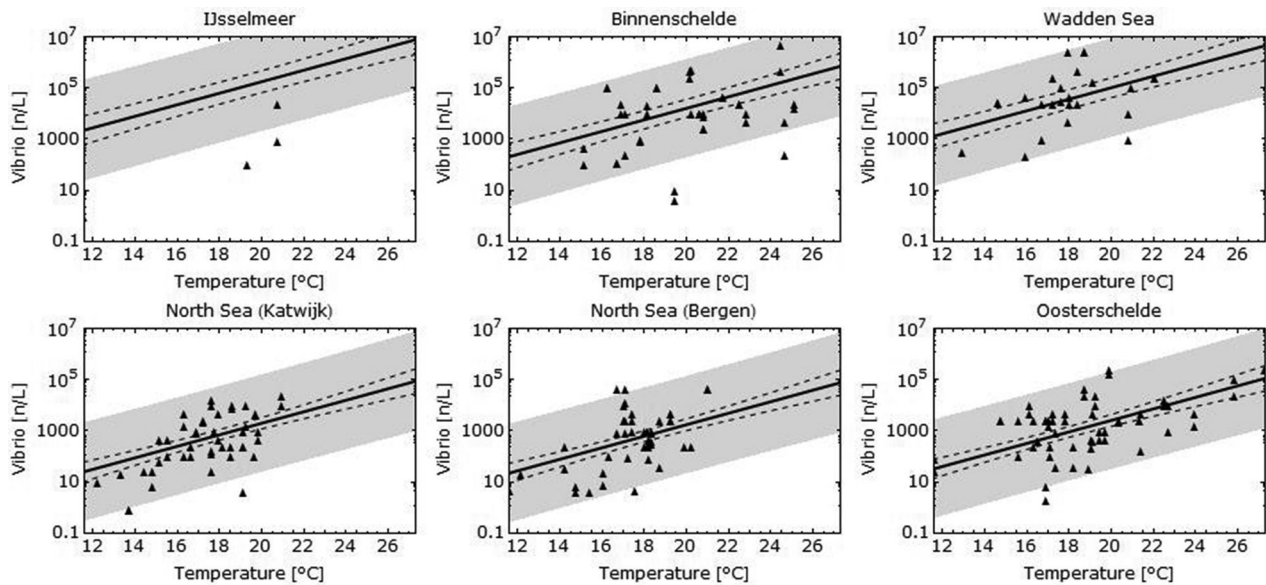


Fig. 1. Results of regression analysis for *Vibrio* spp., enrichment at 36 °C for 18–20 h. Triangles show measured concentrations, dashed lines encompass the 95% confidence bands for mean predictions; the gray-shaded area encompasses the prediction interval for individual observations.

Binnenschelde are shown in Fig. 3. Predictions for the IJsselmeer were not included because only few samples were positive for *Vibrio* spp. at this location. Because the concentration of *Vibrio* spp. at the Binnenschelde was dominated by *V. cholerae* (non-O1/O139), predictions for *V. alginolyticus* and *V. parahaemolyticus* at this location were not included either.

Predicted increase in the average concentration differs per scenario; comparing the current situation and the 2085 W_H (+3.7 °C) scenarios showed a maximum increase of approximately 0.65 \log_{10} for *Vibrio* spp. and *V. alginolyticus* (four to five times). Maximum increase of the average *V. parahaemolyticus* concentration is approximately 0.5 \log_{10} (three times).

The alternative scenario (results shown in Supporting Information S2), comparing the summer of 2006 to the reference period based on the environ-

mental data of RWS, showed that for the Oosterschelde average concentrations of *Vibrio* spp. would be approximately 0.2 \log_{10} higher (1.6 times) than in the current situation; however, for both the North Sea and Wadden Sea, predictions showed a minor decrease (<0.1 \log_{10}).

3.4. Evaluation of Changes in the Risks of Illness

Calculations showed that the risk of illness for ingestion of *V. parahaemolyticus* differs between locations (Tables VI and VII). For the current situation, average illness risks for ingestion of *V. parahaemolyticus* are on the order of 10^{-5} (95th percentile 10^{-4}) per person per bathing event for the North Sea locations for both adults and children and 10^{-4} for an entire summer. For the Oosterschelde, these risks were estimated to be approximately 10 times higher. The highest average risks, 10^{-3} (95th

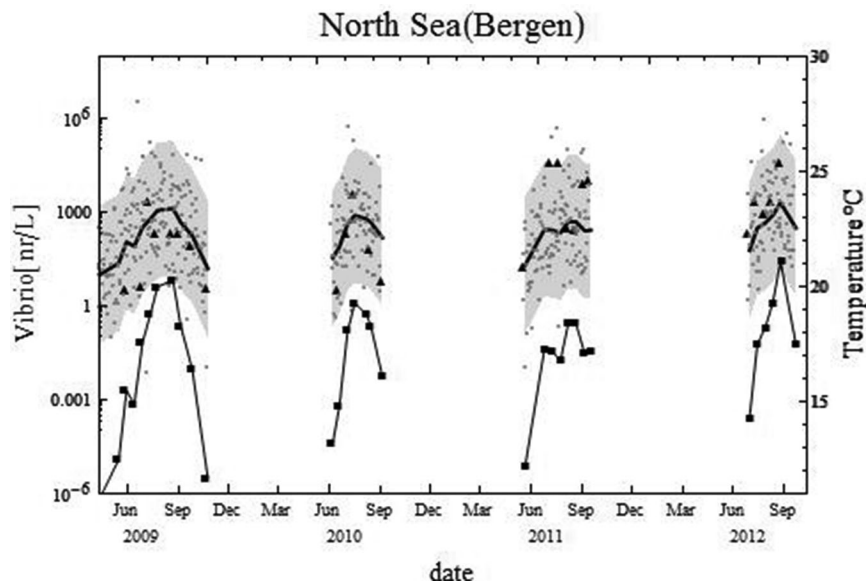


Fig. 2. Measured, predicted, and sampled concentrations for *Vibrio* spp. at the North Sea (Bergen), for enrichment at 36 °C for 6–8 (2009–2010) or 18–20 h (2011–2012). Black line shows mean prediction for *Vibrio* concentration; gray-shaded area encompasses the prediction interval for individual observations. Triangles represent the measured concentrations and dots represent random samples, as used for the calculations of illness risks. Squares represent temperature measurements.

Table VI. Average Risk of Illness per Person per Bathing Event for *V. parahaemolyticus* for the Reference Scenario; 95th Percentile Between Parentheses

P_{event}	Average ^a	Maximum ^b
North Sea (Bergen)		
Man	7.3E-05 (2.6E-04)	6.0E-02 (3.1E-01)
Child	7.8E-05 (1.8E-03)	6.8E-02 (3.5E-01)
North Sea (Katwijk)		
Man	6.6E-05 (2.7E-04)	6.4E-02 (3.3E-01)
Child	8.6E-05 (1.8E-03)	7.8E-02 (4.0E-01)
Oosterschelde		
Man	5.3E-04 (1.7E-03)	3.7E-01 (9.0E-01)
Child	5.9E-04 (1.3E-02)	4.2E-01 (9.1E-01)
Wadden Sea		
Man	3.1E-03 (1.2E-02)	3.8E-01 (9.1E-01)
Child	3.9E-03 (8.0E-02)	4.3E-01 (9.1E-01)

^aOn a random day during the bathing season when water temperature >17 °C.

^bBathing on a day when water temperature has reached the maximum.

percentile 10^{-2}) and 10^{-2} per person per bathing event and per summer, respectively, were estimated for the Wadden Sea.

These risks are based on the assumption that bathing takes place randomly over the summer (within bathing season and when water temperature exceeded 17 °C). Illness risks calculated for a

single bathing event under conditions where water temperature reached its maximum are between 10^{-2} and 10^{-1} depending on the location (Table VI).

Predictions of the infection for the climate change scenarios also differed slightly per location. There is only a 50% increase in 2085 for the most conservative G_L scenario (+1.3 °C). For the W_H scenarios in 2085 (+3.7 °C), illness risks per person per summer are calculated to be about two to three times higher than in the current situation (Table VII).

4. DISCUSSION

According to multiple regression analyses, *Vibrio* spp. concentrations in Dutch recreational waters are significantly determined by temperature, salinity, and pH, as well as enrichment method. The empirical formula for *Vibrio* spp. derived here shows a temperature effect that is the same as was derived for the Atlantic Ocean by Neogi *et al.*⁽¹⁰⁾ Other formulas from the literature (Table I) did not show any correspondence. The differences between these statistical formulas imply that caution needs to be taken when extrapolating such formulas to other locations. First, differences may arise from factors not included in the formulas, such as climatological differences, flow velocity, water depth, or chemical composition of the

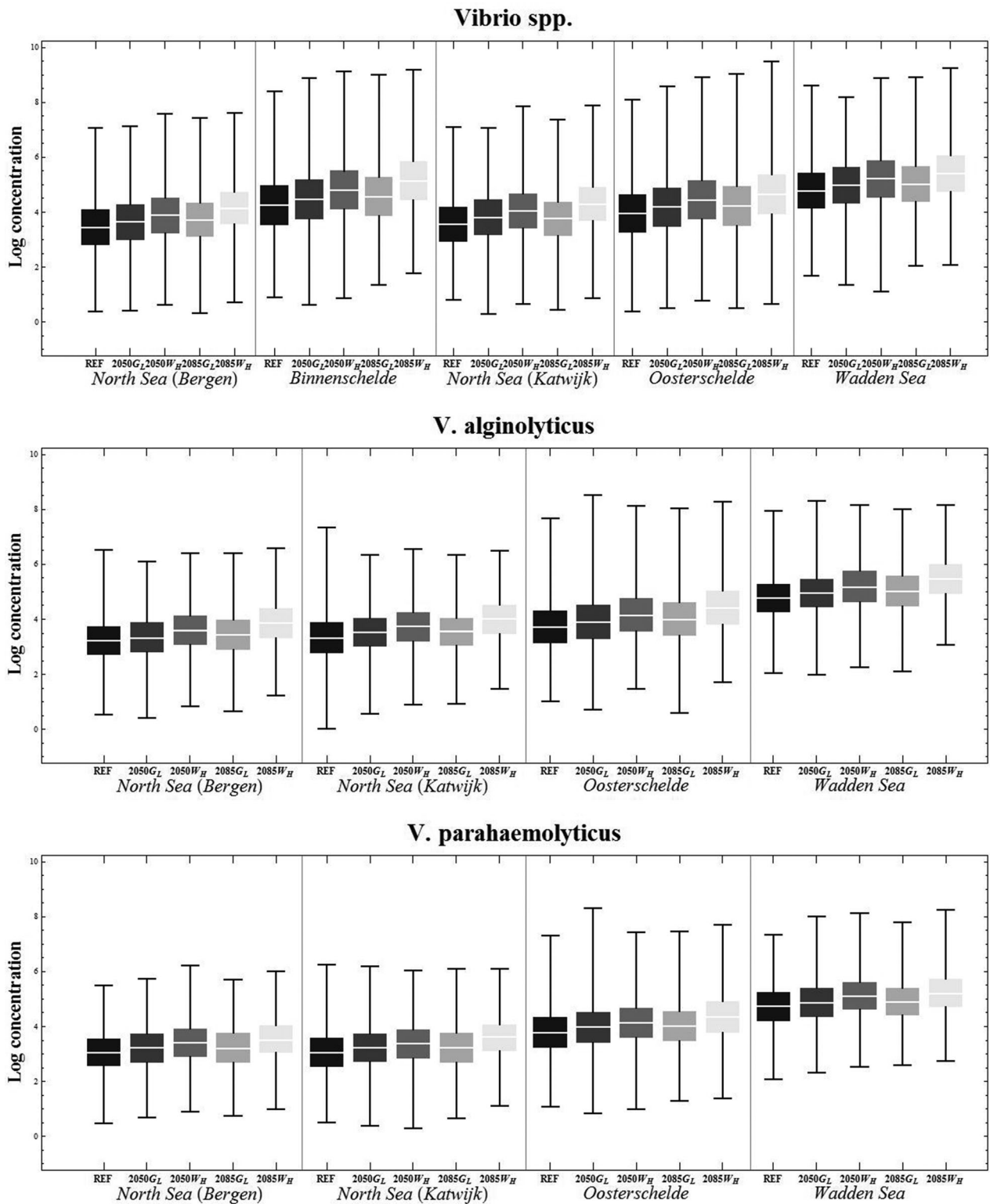


Fig. 3. Box whisker plots for predictions of future *Vibrio* LOG10 concentrations during summer under the different scenario's. The bottom of the box represents the 25th percentile and the top the 75th, the divider in the box shows the median and the whiskers extend to the highest and lowest observation. Variation includes both variation per season and uncertainty in the predictions.

water. Differences in the range of variation of the included variables may also play a role. In this study, *V. cholerae* (non-O1/O139) was detected mostly at the freshwater locations (Binnenschelde and IJsselmeer), which likely results from this species' preference for low saline conditions.⁽²⁹⁾ Therefore, only data for a small range of salinities could be included and this may explain why salinity was not a significant factor in the empirical equation. Second, different methods have been used to determine the *Vibrio* concentrations. As seen in the regression analyses of this study, the use of different enumeration methods has a significant effect on the concentration determined. Finally, composition of the *Vibrio* spp. population likely varies between studies. This study shows that the different *Vibrio* species depend differently on temperature, pH, and salinity; therefore when one species dominates total concentrations of *Vibrio* spp. this will affect the empirical formulas determined.

Because the multiple regression analysis explains only 50% of the variation, predictions for future *Vibrio* concentrations have a large uncertainty (Fig. 1). Inclusion of variability for salinity and pH in time per location would improve the model. Predictions may also be improved by inclusion of additional parameters such as nutrient concentrations^(10,12) or interaction with sediments.^(30,31) However, this may not directly lead to better predictions under climate change because quantitative predictions of the climate change effect on such parameters are still unavailable. Even though it was clear from the multiple regression analysis that pH and salinity significantly affect the concentration of *Vibrio* spp., changes of pH and salinity could not be included in the 2050 and 2085 scenarios. Based upon the formulas, the expected acidification of the oceans⁽²⁵⁾ would contribute to the increase of *Vibrio* concentrations. Direction of change in salinity, which depends on the location and variable factors such as river discharge and precipitation, could have both a positive and negative effect on *Vibrio* concentrations. The necessity of inclusion of other environmental factors is illustrated by the alternative scenario for the summer of 2006. Comparing this scenario to the reference period (2009–2012) did not show a clear increase in concentration for all locations. For the North Sea and Wadden Sea, even though the water temperature is higher, there is a small decrease in concentration. This effect is because of differences in salinity and pH measurements between the two periods, counteracting the effect of increased temperatures.

Under current climate conditions (2009–2012), concentrations of *Vibrio* spp. in Dutch surface waters ranged from undetectable to 10^7 cells/L with an average on the order of 10^4 , and varied between bathing sites. The predictions of *Vibrio* spp. concentrations under future climate conditions showed a maximum increase in concentration for the W_H scenarios (+2.3 °C / +3.7 °C). This increase was slightly higher for total *Vibrio* spp. concentration and *V. alginolyticus* compared to *V. parahaemolyticus* because of their different sensitivity to temperature changes. An increase of the *Vibrio* concentration in recreational water increases the dose to which bathers are exposed and consequently the risk of illness.

The calculations for the effect of climate change on *Vibrio* concentrations in bathing water in the Netherlands obviously only included species that are already present in Dutch surface waters. The formulas suggest that *V. alginolyticus* and *V. cholerae* (non-O1/O139) may become even more dominant because they are more temperature sensitive. Results of the analyses do not reveal what will happen when conditions shift from unfavorable to favorable for species that are now present only sporadically, like *V. vulnificus*, which prefers warm estuarine or coastal waters and can cause serious infections especially in immunocompromised people.^(32,33)

Computation of the risks of illness showed that the current risks of bathing associated with *V. parahaemolyticus* vary between locations, and range from 10^{-4} and 10^{-2} for an entire summer. Risks of illness for a single bathing event at the North Sea are, remarkably because geographical locations are completely different, similar to risks for two southern California recreational waters, namely, on the order of 10^{-5} .⁽³⁴⁾ Risks for the Oosterschelde and Wadden Sea were predicted to be one and two \log_{10} higher, respectively, mainly because of higher water temperatures at these locations. Illness risks calculated for a bathing event on a day when water temperatures have reached their maximum were much higher and resulted in risks up to 10^{-1} for the Wadden Sea.

Under the W_H scenario, estimations of average illness risks from exposure to *V. parahaemolyticus* would approximately be twice as high for 2050 (+2.3 °C) and three times higher for 2085 (+3.7 °C). Because current average illness risks are quite low, the average risk of *Vibrio* illness in bathing waters in the Netherlands will remain low even under the W_H scenarios. However, such an increase on days where water temperatures reach their maximum and risks are already high would result in a considerable

Table VII. Current Average Risk of Disease per Person per Summer for *V. parahaemolyticus* and Relative Increase of Average Risk for Future Scenarios; 95th Percentile Between Parentheses

P_{summer}	Current situation	2050 (G_L)	2050 (W_H)	2085 (G_L)	2085 (W_H)
North Sea (Bergen)					
Man	4.2E-04 (1.6E-03)	1.3	2.0	1.5	3.0
Child	5.7E-04 (2.3E-03)	1.3	2.0	1.5	3.0
North Sea (Katwijk)					
Man	4.6E-04 (1.6E-03)	1.3	2.0	1.5	3.0
Child	6.4E-04 (2.3E-03)	1.3	2.0	1.5	3.0
Oosterschelde					
Man	3.2E-03 (1.3E-02)	1.4	2.0	1.5	3.0
Child	4.5E-03 (1.9E-02)	1.3	2.0	1.5	2.9
Wadden Sea					
Man	1.8E-02 (8.5E-02)	1.3	1.7	1.4	2.3
Child	2.5E-02 (1.2E-01)	1.3	1.7	1.3	2.3

absolute risk increase. This indicates that expressing climate change impacts in terms of relative risks is not always useful to guide climate change adaptation strategies when quantification of the current risks is absent.

In this study, average risks were calculated for a bathing event on a random day within the bathing season, under the conditions that water temperatures are above 17 °C. However, a better approach could be to use actual numbers of bathers per day to calculate the illness risks. Numbers of Statistics Netherlands show that recreation takes place more often during the weekends than on a week day.⁽³⁵⁾ Also, water temperature is most likely not the only factor that determines if people will be bathing; probably air temperatures, cloudiness, and economic factors like amount of leisure time will largely determine decisions to go to the beach. When high risks of illness coincide with a day on which the probability of bathing is high, this could lead to more illness cases than expected based on the average. Therefore, also future changes in factors related to human behavior with respect to recreational water activities may have a large influence on the degree of the effect of climate change.

Illness risks calculated for ingestion of *V. parahaemolyticus* were based on dose-response data for oyster consumption. It is unknown if these dose-response data are suitable for swallowing of bathing water. Furthermore, not all strains of *V. parahaemolyticus* cause illness in humans; the dose-response data were determined defining pathogenic *V. parahaemolyticus* strains as those that can produce thermostable direct hemolysin.⁽²⁶⁾ In

this study, *V. parahaemolyticus* strains were not specified and consequently risks may be overestimated.

V. parahaemolyticus is, like *V. alginolyticus* or *V. cholerae* (non-O1/O139), capable of causing ear or wound infections. However, because estimates of exposure and dose-response relations are not available for such infections, it is not possible to calculate these illness risks. Because only 11% of the total *Vibrio* concentrations found in Dutch bathing waters consists of *V. parahaemolyticus*, inclusion of illness risks for wound and ear infections is necessary to examine the full effects of climate change on illness risks from exposure to *Vibrio* spp. Roser *et al.*⁽³⁶⁾ give an extensive overview of all the constraints associated with development of a dermal dose-response model for *P. aeruginosa*, including factors like exposure duration and frequency, epidemiological data, and individual susceptibility. Presumably, development of dose-response relations for skin and ear infections because of *Vibrio* bacteria will be similarly challenging.

Even though illness risks for total *Vibrio* spp. (dominated by *V. alginolyticus*) could not be determined based upon expected increase in concentrations, and assuming an approximately linear relation between concentration and illness risks, risks of illness could become four to five times higher than current risks. However, to determine the impact of this increase for public health, it is necessary to first define current risks. Because *Vibrio* is a natural occurring pathogen, its abundance cannot be controlled. Consequently, mitigation of potential increases in risk of exposure and illness under climate change conditions will be difficult. Regularly

monitoring *Vibrio* concentrations in bathing waters and educating bathers on the risks could be an option to reduce risks.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Regression Analysis Parameter Table for *Vibrio* spp.

Table S2. Regression Analysis Parameter Table for *V. alginolyticus*.

Table S3. Regression Analysis Parameter Table for *V. parahaemolyticus*.

Table S4. Regression Analysis Parameter Table for *V. cholerae* (non-O1/O139).

Figure S1. Box whisker plots for predictions of *Vibrio* LOG₁₀ concentrations at the RWS locations for the summer of 2006 compared to the reference period.