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Temporal and Spatial Distribution Patterns of Potentially Pathogenic *Vibrio* spp. at Recreational Beaches of the German North Sea

Simone I. Böer · Ernst-August Heinemeyer · Katrin Luden · René Erler · Gunnar Gerdts · Frank Janssen · Nicole Brennholt

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Abstract The number of reported *Vibrio*-related wound infections associated with recreational bathing in Northern Europe has increased within the last decades. In order to study the health risk from potentially pathogenic *Vibrio* spp. in the central Wadden Sea, the seasonal and spatial distribution of *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio cholerae* were investigated at ten recreational beaches in this area over a 2-year period. *V. alginolyticus* and *V. parahaemolyticus* were found to be omnipresent all year round in the study area, while *V. vulnificus* occurrence was restricted to summer months in the estuaries of the rivers Ems and Weser. Multiple linear regression models revealed that water temperature is the most important determinant of *Vibrio* spp. occurrence in the area. Differentiated regression models showed a species-specific response to water temperature and revealed a particularly strong effect of even minor temperature increases on the probability of detecting *V. vulnificus* in summer. In sediments, *Vibrio* spp. concentrations were up to three orders of magnitude higher than in water. Also, *V. alginolyticus* and *V. parahaemolyticus* were found to be less susceptible towards winter temperatures in the benthic environment than in the water, indicating an important role of sediments for *Vibrio* ecology. While only a very small percentage of tested *V. parahaemolyticus* proved to be potentially pathogenic, the presence of *V. vulnificus* during the summer months should be regarded with care.

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S. I. Böer (✉) · N. Brennholt
Department G3—Bio-Chemistry, Ecotoxicology, Federal Institute of Hydrology, Am Mainzer Tor 1,
56068 Koblenz, Germany
e-mail: Boeer@bafg.de

E.-A. Heinemeyer · K. Luden
Governmental Institute for Public Health of Lower Saxony,
Lüchtenburger Weg 24,
26603 Aurich, Germany

R. Erler · G. Gerdts
Biological Institute Helgoland, Division of Shelf Seas Systems Ecology, Alfred Wegener Institute for Polar and Marine Research,
Kurpromenade,
27498 Helgoland, Germany

F. Janssen
Federal Maritime and Hydrographic Agency,
Bernhard-Nocht-Str. 78,
20359 Hamburg, Germany

Introduction

Vibrionaceae constitute an important family within the Gammaproteobacteria and are common natural members of marine and estuarine bacterial plankton communities. At least 12 species of the *Vibrio* genus are potentially pathogenic to humans [1]. Particularly *Vibrio parahaemolyticus* and *Vibrio vulnificus*, but also *Vibrio alginolyticus* and *Vibrio cholerae* are known as causative agents of seawater-related illnesses, such as seafood poisoning in association with gastrointestinal infections [2–4] and infections of wounds and mucous membranes [5–11].

A strong link between water temperature and the number of *Vibrio* spp. in seawater or shellfish and the frequency of occurrence of *Vibrio* incidences has been discovered in a vast range of studies in a number of regions (e.g. [12–17]). This link was assigned both to direct temperature effects as

well as to indirect effects by planktonic food–web interactions [18, 19]. For temperate Northern European Waters, an increasing number of seawater-related wound infections have been reported since the mid-1990s, mainly during summer heat waves. The majority of these infections occurred at the Baltic Sea coast such as in Denmark [20, 21], Sweden [5, 22], Finland [23] and Germany [24]; however, sporadic *Vibrio*-related cases were recorded upon contact with North Sea waters in the Netherlands [9, 25] and Britain [26].

An increasing number of studies accumulate evidence of an emerging risk of *Vibrio*-related wound infections in high latitudes as a consequence of climate anomalies such as temporal peaks in sea surface temperatures [27]. Due to its semi-enclosed character, the North Sea is one of the seas most vulnerable towards such ocean warming trends [28]. Mean sea surface temperatures in the North Sea have increased two to four times faster than average, more than 1.3 °C in the last decades [28–30]. The probability of extreme summers and years has more than doubled simultaneously [29], both of which could support the spreading of potentially pathogenic vibrios in this region. Investigations recently showed that *Vibrio* spp. numbers, including potential pathogens, have increased within the plankton-associated bacterial community of the North Sea during the last half century [31].

The increasing number of bathing water-related infections in the Northern European seas in recent years concerned scientists, and a number of studies have been conducted in order to gain a better understanding of *Vibrio* occurrence and ecology in these waters (e.g. [17, 32–37]). Early work has provided an indication of the presence of potentially pathogenic *Vibrio* species at the German North Sea coast [38]; however, the ecology of these organisms has not been studied in depth for these waters. The present study is aimed at elucidating the seasonal and spatial distribution of potentially pathogenic *Vibrio* spp. in the central Wadden Sea and within the estuaries of the rivers Ems and Weser for the first time. *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus* and *V. cholerae* were quantified monthly at ten recreational beaches over a 2-year period in relation to environmental conditions, using a culturing approach. Species identification was verified via molecular biological testing for species-specific gene targets in a number of isolates. The pathogenicity of representative *V. parahaemolyticus* and *V. cholerae* isolates was further investigated via biochemical and molecular biological tests. Since most documented cases in Germany were associated with *V. vulnificus*, special focus was given to this organism. The main research questions were: (1) Do *V. vulnificus* and other potentially pathogenic *Vibrio* species occur in this area and are there species-specific distribution patterns? (2) What are the main environmental drivers shaping the *Vibrio* community in the area? (3) Are there species-specific responses to environmental drivers such as water temperature and salinity? (4) Are virulent strains of *V. parahaemolyticus* and *V. cholerae* part of the *Vibrio* community in this region? Sediment samples were examined in addition to water samples in order to give a more comprehensive insight into *Vibrio* ecology. Although attachment to sediments and particles has been shown to be one of the survival strategies of *Vibrio* spp. (e.g. [15]), few environmental studies have considered the sedimentary matrix (e.g. [39, 40]), thus neglecting an important component with regard to the assessment of possible health risks.

Material and Methods

Study Area and Sampling

Ten beaches (eight designated and two non-designated beaches) along the Central Wadden Sea coast and within the estuaries of the rivers Ems and Weser were tested for the occurrence of potentially pathogenic *Vibrio* species. The sites comprise all types of coastal waters according to the classification of the EU Water Framework Directive with the exception of the euhaline Wadden Sea type (Table 1, Fig. 1).

Water and sediment samples were taken monthly between December 2009 and December 2011 by staff of the local health authorities. Some winter samples could not be taken because of ice formation; one March sample had to be removed from analysis because the sample containers were not adequately labelled (Table 1). Water samples were taken according to ISO 19458 [41]. Surface sediments were sampled aseptically either directly with the sample containers or with sterile sampling devices and decanted afterwards, depending on submergence of the sediments. Water temperature was measured in situ with portable pH meters (Dyksterhusen, Borkum: Hach Lange, type HQ11d; Norderney, Norddeich: Hach Lange, type HQ30d; Duhnen, Dorum, Wremen: WTW, type ProfiLine pH 3110; Dedesdorf, Bremerhaven, Burhave: VOLTCRAFT 300 K). Samples were transported to the laboratory in Aurich within 3–4 h and processed immediately upon arrival. Since cold temperatures impact *V. vulnificus*, while high temperatures may promote growth of the organism, samples were only chilled during transport on hot summer days, avoiding direct contact with cool packs.

Vibrio spp. Analyses

For the detection of *Vibrio* spp. in the water samples, two methods were used. Sample volumes of 0.1 mL up to 100 mL were membrane filtered (Whatman, ME 25/21 STL, pore size 0.45 μm), depending on the number of colonies expected based on water temperature and experience from preceding months. Filtration of volumes <10 mL was augmented by the addition of a sterile NaCl solution

Table 1 Overview over the sampling sites and sampling schemes

Site	Coordinates		Classification (coastal waters)	Time of sampling	Bathing water quality[a]	No of samples	
	°N	°E				Sediment	Water
Dyksterhusen	53.2935	7.2291	Transitional waters. fully mixed. mesotidal	Incoming tide	Good	22[b, e, f]	22[b, e, f]
Borkum	53.58765	6.65618	Euhaline exposed. fully mixed	Incoming tide	Excellent	24[f]	24[f]
Norddeich	53.6176	7.1493	Polyhaline. exposed. fully mixed	Outgoing tide	Excellent	24[b]	24[b]
Norderney	53.7017	7.1493	Polyhaline. Wadden Sea type	Outgoing tide	Excellent	25	25
Duhnen	53.8857	8.6352	Polyhaline. Wadden Sea type	Incoming tide	Excellent	25	24[c]
Dorum	53.7416	8.5139	Polyhaline. Wadden Sea type	Incoming tide	Excellent	21[b, c, d, f]	21[b, c, d, f]
Wremen	53.6460	8.4916	Transitional waters. fully mixed. mesotidal	Incoming tide	Excellent	21[b, c, d, f]	22[b, c, f]
Burhave	53.58361	8.37606	Transitional waters. fully mixed. mesotidal	Incoming tide	Excellent	25	25
Bremerhaven	53.3216	8.3435	Transitional waters. fully mixed. mesotidal	Incoming tide	No designated beach	25	25
Dedesdorf	53.44387	8.49837	Transitional waters. fully mixed. mesotidal	Incoming tide	No designated beach	25	25

[a] According to the requirements of the European Bathing Water Directive; results from 2011

[b] December 2009 sample missing

[c] January 2010 sample missing

[d] February 2010 sample missing

[e] March 2010 sample missing

[f] December 2011 sample missing

(Merck). The filters were transferred onto CHROMagar™ Vibrio (CHROMagar, France) and incubated for 18–24 h at 36 °C. Alternatively, sample volumes of 0.1 mL (only in summer) to 1 mL (2×0.5 mL due to the maximum capacity of the plates) were plated onto CHROMagar™ Vibrio directly with a drigalski spatula, and incubated correspondingly.

Two approaches were also used for the detection of *Vibrio* spp. in sediments. Using the most probable number (MPN) technique, the sediments were diluted fourfold in buffered peptone water (Merck, supplemented with additional NaCl giving a final concentration of 1.5 % NaCl). The dilution series were incubated for 18–24 h at 36 °C,

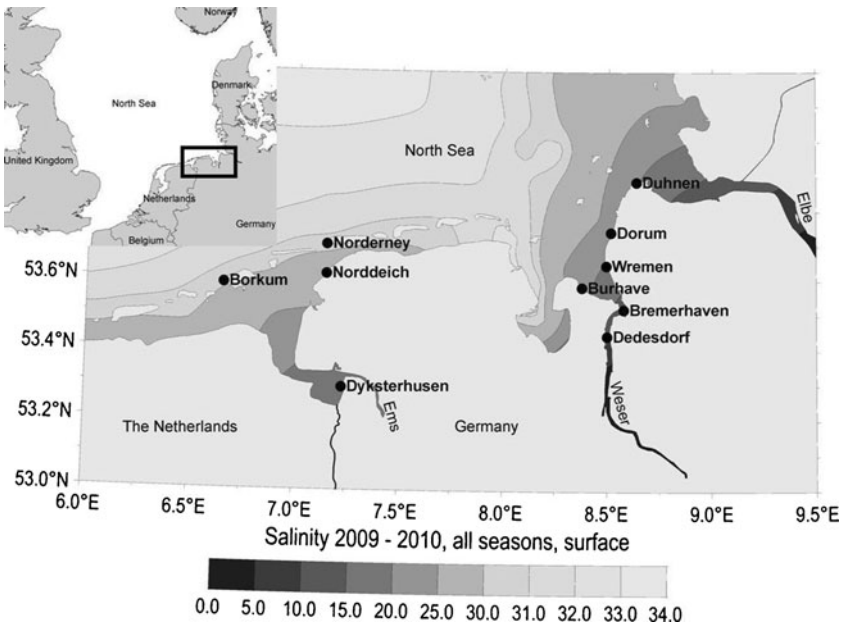

Fig. 1 Overview over the sampling area. Mean salinities for the period January 2009 to December 2010 at the sampling sites are depicted in *grey shading* (Salinity chart courtesy of Dr. Uwe Brockmann and Monika Schütt, University of Hamburg)

followed by sub-cultivation on CHROMagar™ Vibrio and incubation at 36 °C for additional 18–24 h. All MPN series were done in triplicate.

Alternatively, 60 g of the sediment sample was mixed with 60 mL of distilled water and 60 mL of SMD (synthetic sea salt solution, Dr. Brinkmann Floramed GmbH) for 30 min on a magnetic stirrer in order to extract the bacteria from the sediments. The sediment was left to settle, followed by removal of the supernatant. Volumes of 0.1 to 10 mL of the supernatant were membrane filtered and filters transferred onto CHROMagar™ Vibrio. Filtration of volumes <10 mL was augmented by the addition of a sterile NaCl solution. In addition, 1 mL of supernatant was directly plated onto CHROMagar™ Vibrio. All plates were incubated for 18–24 h at 36 °C.

Presumptive *Vibrio* spp. colonies were tested for oxidase activity using Bactident® Oxidase test strips (Merck). Randomly, colonies were microscopically examined for motility and shape. For further differentiation of presumptive *V. vulnificus* and *V. cholerae*, pure cultures of green blue to turquoise blue colonies were grown on thiosulphate citrate bile sucrose agar (TCBS, Merck; green, *V. vulnificus*; yellow, *V. cholerae*). In the case of colonies with multiple shapes and colours (e.g. lighter blue to darker blue), several colonies of each variant were sub-cultivated. In the case of morphological uniformity, single representatives were picked. All colonies destined for further species identification were sub-cultivated on Columbia blood agar (Oxoid) prior to biochemical testing. All presumptive *V. vulnificus* and *V. cholerae* isolates, and randomly chosen representatives of presumptive *V. parahaemolyticus* and *V. alginolyticus* were subjected to the Analytical Profile Index (API) system API20E (BioMérieux, Marcy L'Etoile, France). Verified *V. cholerae* isolates were further examined for O1, O139, Inaba and Ogawa serotypes via agglutination testing (antiserum ZM05 (Murex), O139 "Bengal" antiserum 294487 (Denka Seiken), polyvalent antiserum 293831 (Denka Seiken), antisera No. 3133, 2890 and 3609 of the Robert Koch institute). Based on the species assignment, colony counts were converted to concentrations of colony-forming units (cfu)/100 mL water and cfu/100 g sediment, respectively. According to common microbiological surveillance practice, the highest concentration of *Vibrio* spp. in a sample yielded by any of the approaches was used for further data analyses.

PCR Detection of Species-Specific and Virulence-Associated Gene Targets

In order to check the reliability of the species assignment via culturing and biochemical testing, a number of *Vibrio* strains that were isolated during the study and became part of our strain collection were tested for species-specific and additionally for virulence-associated genes via PCR. *V. vulnificus* as the main agent of *Vibrio*-related wound infections in Germany is primarily represented in this collection. As described previously [42], genomic DNA of 35 *V. parahaemolyticus*, 106 *V. vulnificus* and 22 *V. cholerae* strains was prepared using a lysozyme/SDS lysis followed by a phenol/chloroform extraction and an isopropanol precipitation. All PCR reactions were conducted in triplicates with 10 ng of template DNA for each of the strains. The universal forward primer *UtoxF* was used in combination with species-specific primers for *VvtoxR*, *VptoxR* and *VctoxR*, respectively [43, 44]. For *V. vulnificus* strains, 10 ng of template DNA was used, and the PCR mixture contained 2.5 μL Taq buffer (10×), 5 μL Taq Master PCR Enhancer (5×), 10 pmol of each primer, 10 mM dNTPs and 1. 5 U Taq DNA polymerase (5 Prime). *UtoxR/VvtoxR* fragments were amplified under the following PCR conditions: 4 min at 94 °C, 30 cycles of 94° for 30 s, 61 °C for 30 s, 68 °C for 30 s with a final 68 °C extension of 7 min. All *V. parahaemolyticus* strains were additionally screened for the hemolysin genes *tdh* and *trh* [43, 45]. Parameters used for all *V. parahaemolyticus* PCRs (*VptoxR/tdh/trh*) were the same as for the identification of *toxR* genes in *V. vulnificus* with two exceptions: annealing was performed at 62 °C for 1 min and elongation at 68 °C for 1 min. For *V. cholerae*, a multiplex-PCR was performed with the primer sets *UtoxF/VctoxR*, *O139F/O139R*, *ctxA1/ctxA2* and *O1F/O1R* [43, 46, 47]. Half a micromole of each O1 Primer and 0.125 μmol of every other primer were used. After a denaturation of 4 min at 94 °C, 30 cycles were employed (94 °C–30 s; 59 °C–30 s, 68 °C–30 s) with an extension of 5 min at 68 °C. Resulting PCR products were analysed by agarose gel electrophoresis (2 % agarose; 0.5× TBE). Gels were run at 80 V for 90 min, stained with GelRed and visualized using the ChemiDoc XRS imaging System (Bio-Rad). The following reference strains were used as positive controls: *V. vulnificus* DSM-10143 (*VvtoxR*), *V. parahaemolyticus* RIMD 2210633 (*VptoxR* and *trh*), *V. parahaemolyticus* VN-0088 (*tdh*), *V. cholerae* VN-0147 (*O1*), *V. cholerae* VN-0150 (*O139*) and *V. cholerae* VN-0156 (*ctxA*). *Vibrio harveyi* was used as negative control.

Kanagawa Test

The 35 *V. parahaemolyticus* isolates and reference strain *V. parahaemolyticus* DSZM 11058 were tested for the Kanagawa phenomenon as described by Oberbeckmann et al. [42].

Environmental Parameters

Weather data were provided by the National Meteorological Service (DWD). The salinity of the water samples was determined according to Mohr [48] as defined in DIN 38405–1 [49] using an automatic titrator (Mettler Toledo DL55). Salinities were calculated according to Knudsen [50].

Sediments were characterized as follows: sediments were freeze-dried, homogenized and particles >2 mm separated from the rest of the sediment by dry sieving. The <2-mm fraction was split on a rotor sampler and one to two parts of the sediment grinded using a planetary mill with zirconium vessels and beads. This sub-sample was subjected to TOC analysis according to DIN EN 12137 [51]. Two to three parts of the remaining sediment were used to determine grain sizes via ultrasonic sieving as described elsewhere [52]. For each season (21/06–20/09=summer, 21/09–20/12=fall, 21/12–20/03=winter, 21/03–20/06=spring) a representative sediment sample was analysed for grain size distribution with the KVS software (author: Dr. Johann Buss, Braunschweig/Germany, version 4.01, 20/02/1997). The sediment classification was carried out according to Figge et al. [53]. In case of seasonal variations in the sediment classifications, additional sediment samples were analysed and averages used for data processing. The percentage of clay and silt was used for statistical analyses.

Statistical Analyses

The open-source program *R* (R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; version 2.15.1) was used for all statistical calculations. All bacterial parameters were $\log_{10}(x+1)$-transformed prior to the analyses. In cases were bacterial levels were below the detection limit of the available method, a value of zero was assigned prior to logarithmic conversion. Occasional values above detection limits were set to the detection limit value plus one in order to allow differentiation for rank tests. TOC values below detection limit were assigned half the value of the limit. Weather data were selected as follows: global solar irradiance as the sum of 3 days before sampling, sunshine duration as the sum of 1 week before sampling as well as cloud cover, rainfall, wind direction and wind speed as a weekly mean.

Variations in *Vibrio* abundance between seasons and sampling sites were tested for significance using the Kruskal–Wallis test. Pairwise comparisons between samples were conducted using the *kruskalmc* function for Kruskal–Wallis post hoc tests on dependent variables as implemented in the *R* package *pgirmess*.

Multiple correlations between *Vibrio* species and all environmental parameters were calculated by using Spearman's rank correlation coefficient, and significances were adjusted for multiple comparisons by the Bonferroni method [$p<0.000292$ (0.05/171)]. Environmental parameters were tested for their impact on the occurrence and abundance of individual *Vibrio* species via stepwise multiple logistic regression analyses and stepwise multiple linear regression analyses, respectively. *Vibrio* spp. in water and sediment were regarded separately. Regressions were considered significant when the p value was <0.05. Additionally, probabilities of the presence of individual *Vibrio* species as a function of water temperature were visualized using simple logistic regression models.

Particle Transport Model

A possible drift of *V. vulnificus* from the Ems estuary to the island of Borkum in summer 2010 was checked using a particle transport model, which is developed and applied for drift simulations at the German Federal Maritime and Hydrographic Agency (BSH). The 3D, baroclinic regional ocean circulation model BSHcmod [54] calculates the three-dimensional current field as well as water level, temperature, salinity and ice cover in the North Sea and the Baltic Sea with an overall horizontal resolution of 5.5 km and 900 m resolution in the German Bight and the western Baltic Sea. The model includes tidal and meteorological forcing, as well as baroclinic effects due to temperature changes and varying river discharge.

Based on archived results from BSHcmod, the drift of particles is calculated by the particle transport model BSHdmod.L [55]. Both model components make use of meteorological forcing data provided by the weather prediction models of the DWD.

Results

Seasonal and Spatial Distribution of *Vibrio* spp.

PCR testing for species-specific *toxR* genes verified that 35 *V. parahaemolyticus*, 106 *V. vulnificus* and 21 out of 22 tested *V. cholerae* isolates belonged to the supposed species assigned in API testing, thus proving the reliability of our culturing approach. All four potentially pathogenic *Vibrio* species were detected during the study period. *V. alginolyticus* was by far the most frequently occurring species and could be detected in 79 % of water samples and 94 % of sediment samples, respectively. The second most frequent species was *V. parahaemolyticus* with 44 and 67 % of positively tested water and sediment samples, respectively. Five percent of water and sediment samples contained *V. vulnificus*, while *V. cholerae* were detected in 2 % of water samples and 4 % of sediment samples, respectively, with all isolates belonging to the non-O1/O139 type. *Vibrio* spp. were not only present more often in sediments than in water but benthic *V. alginolyticus*, *V. parahaemolyticus*, and *V. vulnificus* were also one to three times more abundant. Mean *V. alginolyticus* concentrations ranged from 1.5×10^3 to 2.9×10^5 cfu/100 g in sediments and from 6×10^1 to 8.4×10^4 cfu/100 mL in water, mean *V. parahaemolyticus* concentrations ranged from 7.6×10^2 to 1.6×10^5 cfu/100 g in sediments and from 3.6×10^1 to 6.3×10^3 cfu/100 mL in water, and *V. vulnificus* concentrations ranged from 0 to 4.8×10^3 cfu/100 g in sediments and from

0 to 4.6×10^1 cfu/100 mL in water samples (Table S3). *V. cholerae* was detected only in very low numbers (0–5 cfu/100 mL; 0–7 cfu/100 g) both in sediment and water.

A strong correspondence between water temperature and the presence and abundance of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* was observed in this study. Water temperature values ranged from 0 °C in winter to 26.5 °C in summer (Table S1), with the highest water temperatures occurring primarily in July or August. Correspondingly, the number of samples positively tested for *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* was highest in these months. Furthermore, the abundance of these three species significantly increased with increasing temperature, while *V. cholerae* did not show a significant seasonal pattern (Fig. 2, for results of nonparametric post hoc tests see Table S2). Figure 2 shows clearly that, despite the general trend towards elevated presence and abundance at high water temperatures, *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* had a distinct species-specific response towards temperature changes. While *V. alginolyticus* and *V. parahaemolyticus* were present the whole year, *V. vulnificus* was only detected at temperatures between 14 and 26.5 °C. Presence of *V. vulnificus* at water temperatures <20 °C appears to be uncommon compared to what other studies have shown. However, this was only true for fall samples (Fig. 3); in most samples, a threshold of 20 °C had to be reached before culturable *V. vulnificus* cells could be detected. Once reached, *V. vulnificus* appeared suddenly, and could be found for several months even at decreasing temperatures (Figs. 2 and 3).

V. alginolyticus and *V. parahaemolyticus* were both present even at temperatures around freezing point. *V. alginolyticus*, however, was overall less sensitive towards cold temperatures than *V. parahaemolyticus*, and was more or less frequently present at all sites throughout May until November (Fig. S1). Presence of *V. parahaemolyticus*, in contrast, followed the seasonal temperature changes with a lag of 1 to 2 months, such that the number of positively tested samples was highest in August (2010) and September (2011), respectively, while decreasing with decreasing water temperature. Interestingly, the impact of water temperature on both organisms was found to be more pronounced in water than in sediment samples, all the more for *V. parahaemolyticus*, indicating a protective effect of the sediments at low temperatures.

V. alginolyticus, *V. parahaemolyticus* and *V. vulnificus* did not only show species-specific responses to water temperature, but exhibited species-specific spatial distribution patterns as well. Although mean salinities in the study area ranged from 4.1 to 27.4 psu, *V. alginolyticus* and *V. parahaemolyticus* were ubiquitously distributed over the entire region. However, their abundance varied significantly between sites in both sediment and water for *V. alginolyticus* (K=37.4 and K=49.3, respectively; $p<0.001$) as well as in sediments for *V. parahaemolyticus* (K=31.2; $p<0.001$; Fig. S2; for results of the nonparametric post hoc tests see Table S4). Highest mean *V. alginolyticus* and *V. parahaemolyticus* abundances occurred at mean salinities of approximately 15–17 psu, while mean cell numbers were generally lower in brackish waters or at higher salinities. In contrast, *V. vulnificus* occurrence was, with one exception, restricted to sites within the Ems and Weser estuaries where the lowest mean salinities of 4.1 to 17.2 psu were measured, and its abundance did not vary significantly between these sites. Figure 4 shows the range of salinities (4.1–17.2 psu) and water temperatures (14–26.5 °C) at which *V. vulnificus* was present. In contrast to *V. alginolyticus* and *V. parahaemolyticus*, the sediments did not have an effect on the tolerance of *V. vulnificus* towards cold water temperatures nor did they change the acceptable salinity range. The findings presume, however, that high temperatures could broaden the salinity tolerance of the organism because positive *V. vulnificus* detections at high temperatures were often related to high salinities, while *V. vulnificus* presence at lower temperatures was connected to low salinities. However, enhanced salinity in the Weser estuary due to reduced precipitation and riverine freshwater input in summer usually coincides with particularly high water temperatures due to the high thermal load of the river, which could cause this trend. Figure 4 shows that despite its preference for brackish waters, *V. vulnificus* may occasionally occur at salinities that are far beyond the salinity range that is usually tolerated by this organism: *V. vulnificus* occurred in Borkum sediment in September 2010, although Borkum is strongly influenced by the open North Sea and has the highest mean salinity of all sites (Table S1). Since *V. vulnificus* occurred at the Dyksterhusen site in July and August 2010 prior to its detection on Borkum 1 month later, we hypothesized that *V. vulnificus* may have drifted towards Borkum with freshwater currents deriving from the Ems estuary. This was tested with a computational simulation. The particle transport model, which considered current and wind regimes in this area during late summer 2010, showed that *V. vulnificus* in Borkum may have had its origin in the Ems estuary (Fig. 5), suggesting that it can be transported over longer distances.

Impact of Environmental Variables on Bacterial Parameters

In a first step, interactions between environmental variables and *Vibrio* spp. were analysed using the Spearman's rank correlation test. Figure 6 (see also Table S5) shows a summary of all statistically significant correlations. *V. cholerae* was the only species that was not significantly correlated to any of the parameters and is thus not represented in the figure. The scheme clearly demonstrates that *Vibrio* abundance in sediment and water was strongly positively correlated, suggesting an intense link between the *Vibrio* communities

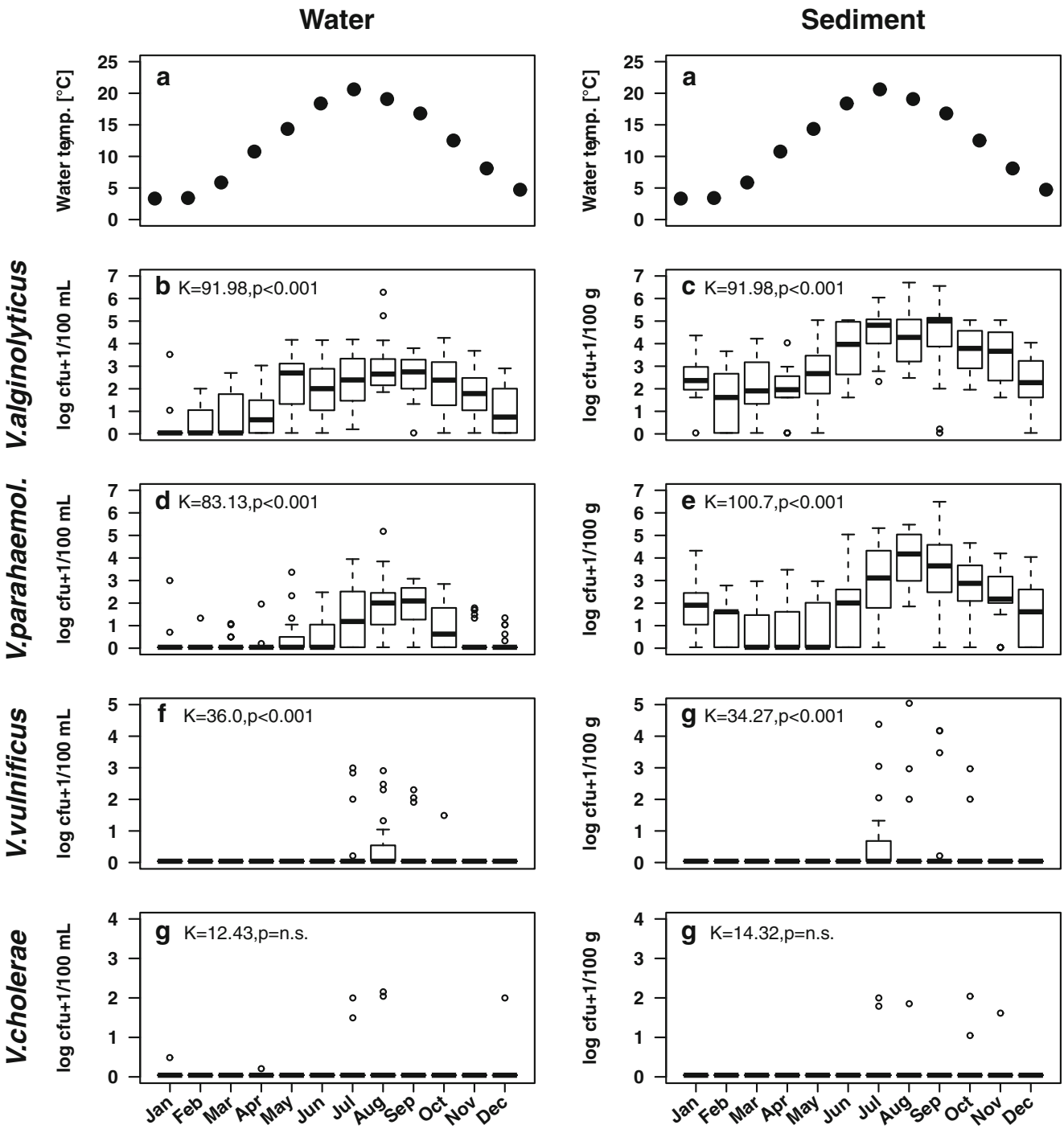

Fig. 2 Figure visualizing the seasonal variations in *Vibrio* abundances in water (*left column*) and sediment (*right column*). The *upper* two plots (**a**) show the mean water temperatures that were measured each month while sampling. *Boxplots* represent the seasonal variations in abundances of *V. alginolyticus* (**b**, **c**), *V. parahaemolyticus* (**d**, **e**), *V. vulnificus* (**f**, **g**), and *V. cholerae* (**h**, **i**), respectively. *Thick bars in the boxes* represent the sample median, *boxes* themselves show the upper and lower quartiles, *whiskers* show the range and *circles* represent outliers. Outliers are defined as data points that fall below the first quartile or exceed the third quartile by 1.5 times the interquartile range. Kruskal–Wallis test statistics (*K)* and significance levels (*p*) for comparison of abundances between seasons are given in the *top left corner* of each sub-plot

in both compartments. Water temperature was the most important factor that was significantly and positively correlated to *V. vulnificus*, *V. parahaemolyticus* and *V. alginolyticus* abundance in sediment and water, thus mirroring the observed seasonal distribution patterns of these three species. The weather data exhibited various interdependences. As expected, global solar irradiance and sunshine duration were strongly correlated with each other and showed significant negative correlations with

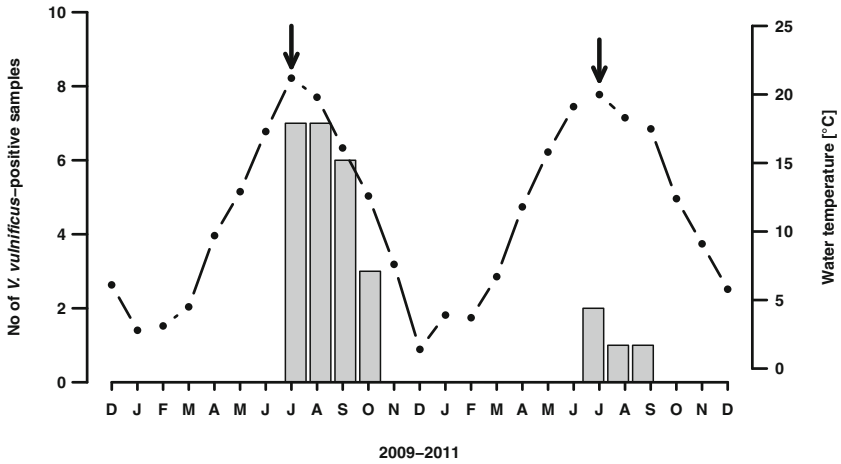

Fig. 3 Seasonal plot showing the number of *V. vulnificus*-positive samples per month in relation to water temperature. *Bars* represent the sum of positively tested samples (water and sediment combined); for line and scatters, the mean water temperature of all sampling sites was taken on a monthly basis. *Arrows* mark the months in which a temperature threshold of 20 °C was exceeded

cloud cover, rainfall and wind speed, while being strongly positively correlated with water temperature. High wind speed in the North Sea is usually related to westerlies which often bring high amounts of rain to the region, a relationship that is reflected in the significant positive correlations between wind speed, wind direction and rainfall in Fig. 6. The positive relationship between water temperature and westerlies in combination with the negative relationship between water temperature and wind speed appears to be contradictory; however, westerlies predominate during the warmer seasons, while easterly winds occur mainly at winter time. The described relationships reflect the strong seasonal dynamics typical for the study area, where sunny weather and warm temperatures prevail in summer, while rainy and cloudy weather predominate in winter. Although individual positive correlations existed between high *V. alginolyticus* and *V. parahaemolyticus* abundance, and high global solar irradiance and long sunshine duration, the strongest effect of sunshine on *Vibrio* spp. was mainly indirect due to the effect of sunshine on water temperature. TOC contents were higher in fine sediments compared to coarse sediments, as reflected in the strong correlation between TOC and the clay and silt content of the sediments. Figure 6 shows that the abundance of benthic *V. alginolyticus* and *V. parahaemolyticus* was positively linked to these nutrient-richer sediments. Fine-grained sediments predominated in the estuaries, while the coastal sites were mainly characterized by a sandy sediment type, which was reflected by the significant negative correlation between salinity and the clay and silt content of the sediments. Salinity itself was strongly dependent on the position of the sites and affected by dry weather periods as reflected in its positive link with global solar irradiance. *Vibrio* spp. and salinity were not significantly correlated.

In order to further investigate the impact of environmental parameters on the occurrence and abundance of *Vibrio* spp., stepwise multiple logistic and linear regression models were developed for each *Vibrio* species (except *V. cholerae*) in water and sediment individually. Those environmental parameters that exhibited a strong direct or indirect interdependence with water temperature (global solar irradiation, sunshine duration, cloud cover and wind speed) and salinity (space) in the correlation analysis were removed from further statistical steps. TOC was strongly connected to the clay and silt content of the sediments; thus, the latter was additionally excluded. Water temperature, salinity, TOC, wind direction and rainfall were kept as independent variables in the models.

The multiple stepwise logistic regression analyses showed that high water temperature was the crucial factor for the occurrence of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* in water and sediment ($p<0.001$, except *V. alginolyticus* in sediment with $p<0.01$; Table 2), thus underlining the strong seasonality of *Vibrio* spp. occurrence observed in this study. Additionally, high salinity had a negative influence on the presence of *V. parahaemolyticus* in water and sediment ($p<0.01$), and a positive effect on the presence of *V. alginolyticus* in sediment alone ($p<0.05$), which indicates certain species-specific preferences towards salinity. Furthermore, the presence of benthic *V. vulnificus* and *V. alginolyticus* was negatively affected by westerlies ($p<0.01$).

The results of the predictive models greatly reflected these patterns. High water temperature was not only related to the mere presence of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* but had a strong positive effect on the abundance of all three species as well, and was the dominant factor in all models ($R^2=0.03$ to 0.13; Table 3). High salinity was an additional significant factor related to low abundance of *V. vulnificus* in water and sediment ($R^2=0.01$), in contrast to high concentrations of *V. alginolyticus* in sediment ($R^2=0.03$). Benthic *V. alginolyticus* and *V. parahaemolyticus* concentrations were significantly and positively affected by the nutrient contents of the sediments as represented by TOC ($R^2=0.42$ and 0.45, respectively), as well as with rainfall ($R^2=0.01$), which is likely linked to freshwater-related nutrient inputs in the area. Rainfall also positively affected *V. alginolyticus* abundance in the water ($R^2=0.01$). Furthermore, westerlies

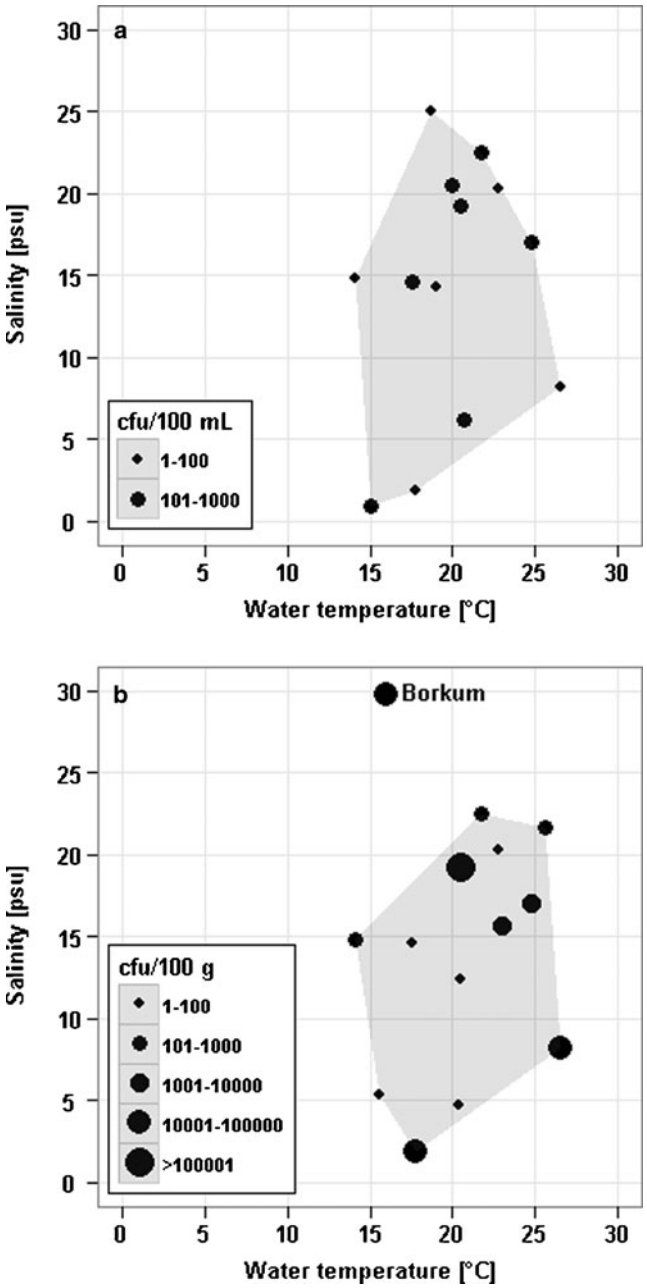

Fig. 4 Bubble plots showing the range of salinities and water temperatures at which *V. vulnificus* was detected in water (**a**) and sediment (**b**) in the study area. Bubble sizes depict size classes of *V. vulnificus* concentrations as described in the legends. A posteriori *shaded areas* visualize the water temperature and salinity ranges that favour *V. vulnificus* occurrence (Borkum presented as outlier)

had a strong negative impact on *Vibrio* spp. concentrations in the sediments (R^2=0.02 to 0.07) and on the abundance of *V. vulnificus* in water (R^2=0.02), presumably due to the combined effect of pressing higher salinity water from the open North Sea into the study area, while reducing the share of nutrient-rich riverine waters.

Water temperature was the most important explanatory variable in all *Vibrio* models; however, the seasonal distribution of the individual *Vibrio* species suggested certain species-specific responses to changes in water temperature (Fig. 2). In order to take a closer look at these individual relationships, a simple logistic regression model was created for each of the three *Vibrio* species in order to predict the probability of their detection as a function of water temperature. Figure 7 is based on presence/absence data from both the sediment and water, and clearly demonstrates that the responses of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* to temperature increases were in fact individually different. The simple logistic regression model of *V. alginolyticus* strongly reflected the good adaptation of this organism to cold temperatures, with a 85 % probability of detecting *V. alginolyticus* even at water temperatures around freezing point. Below 10 °C, even minor increases in water temperature strongly affected *V. alginolyticus* presence in the area, and at 10 °C, the probability of detecting *V. alginolyticus* was already 95 %, which reflects the (almost) year-round presence of *V. alginolyticus* in the study area. Above 10 °C, the effect of water temperature on *V. alginolyticus* presence decreases substantially. At a temperature of 25 °C however, 100 % of samples will probably contain culturable *V. alginolyticus*. In comparison, *V. parahaemolyticus* is quite well adapted to cold temperatures, however not as good as *V. alginolyticus*. At 0 °C, the probability of detecting this species in the study area is 60 %, thus 25 % lower than the probability of detecting *V. alginolyticus*. Below 15 °C, an increment of 1 °C enhances the probability of *V. parahaemolyticus* detection by ~1.5 %; above 15 °C this trend slows down slightly. At a temperature of 25 °C, the probability of *V. parahaemolyticus* presence in the study area is more than 90 %, indicating that many sites, particularly those in the Weser estuary where highest summer temperatures occur, will harbour *V. parahaemolyticus* during summer time.

In contrast, *V. vulnificus* presence is strongly dependent on warm temperatures as demonstrated in Fig. 7c, and the species is not likely to be present at water temperatures below 15 °C. Above 15 °C, the probability of *V. vulnificus* presence rapidly increases. In a temperature range of 15–20 °C, an increment in 1 °C of water temperature increases the probability of *V. vulnificus* presence by ~4 %. At water temperatures above 20 °C, every 1 °C increment even leads to a tenfold increase in the probability of *V. vulnificus* presence, indicating that water temperatures >20 °C strongly promote this species with substantial effects of even minor temperature changes.

Pathogenic Potential of *Vibrio* Isolates

V. parahaemolyticus and *V. cholerae* strains were further tested for presence of virulence-associated gene targets. None of the tested *V. cholerae* isolates contained the *O1*, *O139* and *ctxA* genes, corresponding to the results of the agglutination tests, thus indicating that all strains were non-virulent representatives. Two *V. parahaemolyticus* isolates

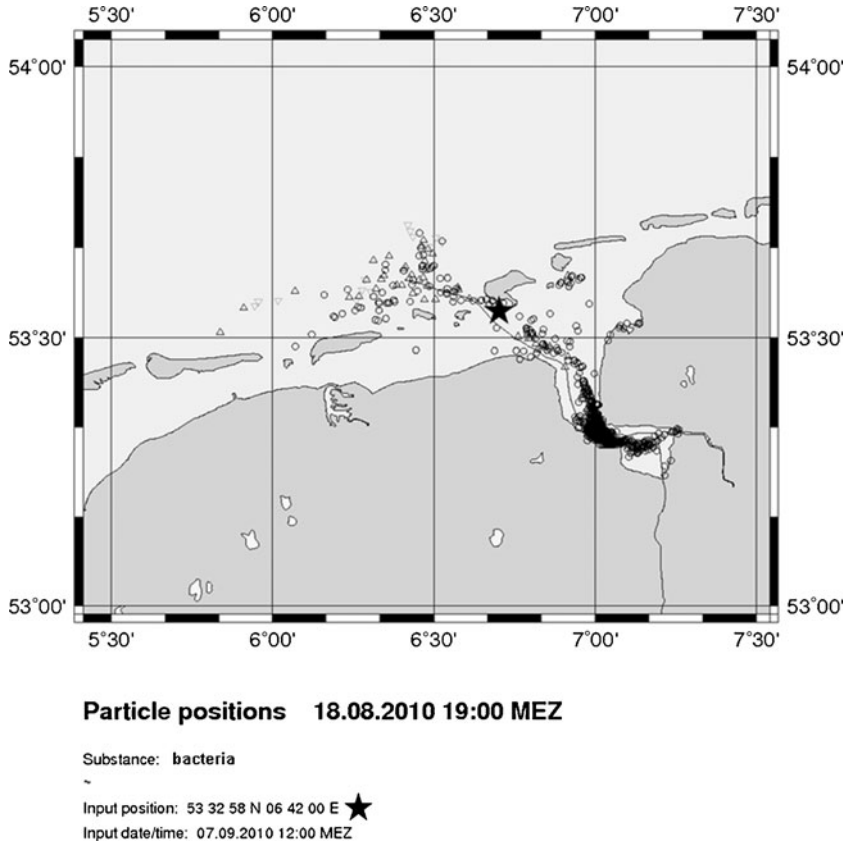

Fig. 5 Graphic showing a potential *V. vulnificus* drift from the Ems estuary (positive proof in July and August 2010) to Borkum (positive proof in September 2010), based on modeling of 1,000 hypothetical particles. Each symbol represents one particle and stands for a hypothetical *V. vulnificus* cell. The *star* marks the starting position of all particles at the beginning of the model calculations, which was set to the date of *V. vulnficus* detection on Borkum (September 08, 2012). Calculations were run backwards until the date of *V. vulnificus* detection at Dyksterhusen (August 18, 2012), and the most probable position of particles during this time is shown

proved to have the *trh* gene; however, none had the *tdh* gene that is supposed to be associated with haemolytic capability. In contradiction, two additional isolates that had neither the *trh* nor the *tdh* gene showed haemolytic abilities in the Kanagawa test, suggesting the presence of additional haemolysis genes.

Discussion

The results of this study showed that *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* occurred in water and sediment of recreational beaches in the central Wadden Sea and within the estuaries of the rivers Ems and Weser. The ecology of these bacteria was found to be very complex in this particularly dynamic ecosystem and characterized by distinct species-specific responses to environmental determinants, such as water temperature and salinity. *V. alginolyticus* was by far the most prevalent species in water and sediment, followed by *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* non-O1/non-O139. Earlier environmental studies in other parts of the North Sea region revealed comparable *Vibrio* community compositions [32, 35–37, 39, 56–58], suggesting that this distribution pattern could be a common feature of *Vibrio* communities along the North Sea coast.

V. cholerae non-O1/non-O139 occurred only sporadically and exhibited neither apparent seasonal nor spatial distribution patterns in this study. Furthermore, we could not identify any significant environmental drivers of *V. cholerae* occurrence. Since salinities were generally in a range where *V. cholerae* may occur [59], viability of this species must be controlled by some other determinants.

In contrast, *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* strongly responded to increases in water temperature, and followed distinct seasonal cycles in terms of isolation frequency and abundance, corresponding to earlier observations on *Vibrio* ecology [19, 60, 61]. Water temperature ranged between 0 and 26.5 °C throughout the study, and was shown to be the crucial factor governing the occurrence and abundance of these three species in the study area, both in correlation analyses and regression models. The frequency of occurrence of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* increased with increasing temperature, and the abundance of all three species was significantly higher in summer than in winter. Nevertheless, simple logistic regression models revealed a distinct species-specific response of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* to water temperature. *V. alginolyticus* and *V. parahaemolyticus* persisted perennially in the study area; however, *V. alginolyticus* was found to be much better adapted to cold water temperatures than *V. parahaemolyticus* with a

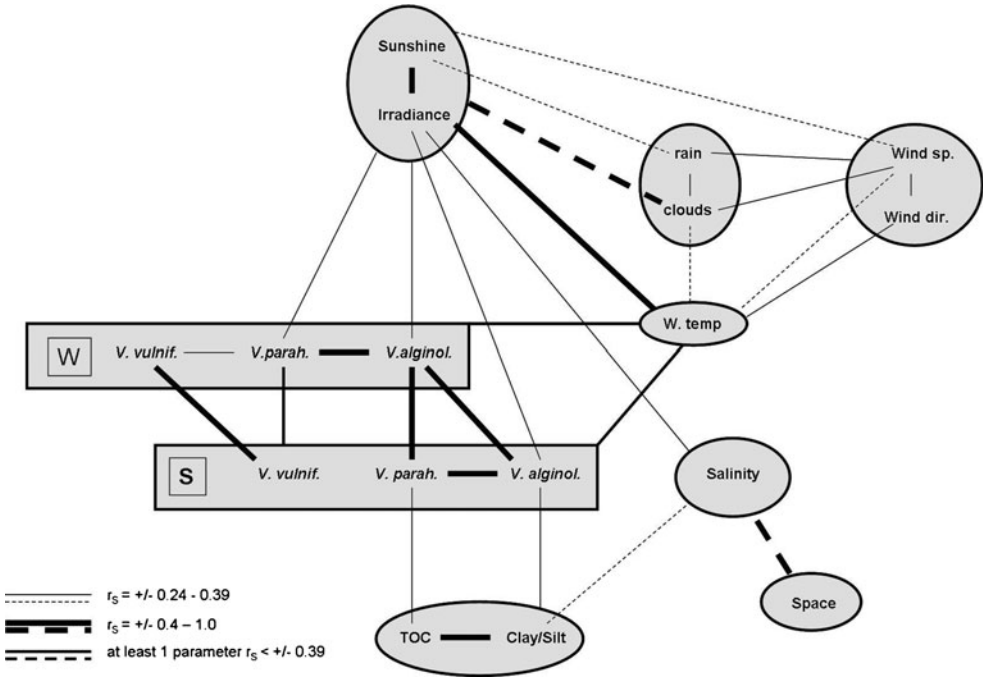

Fig. 6 Spearman's rank correlations between *Vibrio* species in sediment (*S*) and water (*W*), and the environmental variables. Data of the complete study are covered, and variables were partly grouped for better overview. Only significant correlations at the Bonferroni-corrected level $p \leq 0.000292$ (0.05/171) are depicted. *Thin and thick connecting lines* represent significant correlations with coefficients of 0.24–0.39 and 0.4–1.0, respectively. *Medium-sized lines* represent connections where the correlation of at least two variables within connected groups yielded a coefficient <0.39, but where the rest was more strongly connected. *Lines connecting to a whole group of variables* show that all variables within this group were correlated with the respective parameter. Individual correlations are represented by *lines directly connected to single variables*. *Connecting lines* are correlations with at least one of these two parameters. *Continuous and dashed lines* represent positive and negative coefficients, respectively

probability of the presence of the two species at freezing temperatures of 85 and 60 %, respectively. Increases in water temperature were accompanied by an increase in the probability of the presence of *V. alginolyticus* and *V. parahaemolyticus* in the study area. However, while the effect of temperature on *V. alginolyticus* presence diminishes substantially at temperatures >10 °C (presumably because of other environmental factors becoming restrictive), *V. parahaemolyticus* profits considerably from further temperature increases. While the highest probability of the presence of *V. alginolyticus* corresponds to the highest water temperatures, maximum *V. parahaemolyticus* occurrence was shown to appear with a time lag of approximately 1 to 2 months, suggesting that interactions with other organisms could play an important role in *V. parahaemolyticus* ecology in the study area.

In contrast, presence of culturable *V. vulnificus* was found to be strongly dependent on water temperatures >14 °C. This species could be exclusively isolated at water temperatures of 14 to 26.5 °C, supporting earlier studies that reported isolation of *V. vulnificus* only at water temperatures between 15 and 32 °C [4]. Interestingly, the isolation of *V. vulnificus* at water temperatures <20 °C succeeded only in autumn samples, while comparable spring samples remained negative. Our results clearly show that a threshold of 20 °C has to be reached in order to establish *V. vulnificus* viability in the study area. Once present, the bacterium can remain culturable for several months even at lower temperatures without significant diminution in cell numbers, before vanishing abruptly. *V. vulnificus* responds particularly to strong to minor temperature increases when water temperature is overall high. Above 20 °C, every 1 °C increment causes a tenfold increase in the probability of *V. vulnificus* presence.

Since *V. vulnificus* could not be detected at water temperatures <14 °C, the question remains as to from which sources the species is recruited at summer time. Previous studies suggested that *V. vulnificus* can withdraw into the sediments and remain in a viable but non-culturable state, when environmental conditions become unfavourable [62]; however, investigations on this topic were not within the scope of this study.

In addition to the species-specific responses of the three *Vibrio* species to water temperature, *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* showed diverging spatial distribution patterns that uncovered individual trends in terms of salinity preferences. A range of earlier studies in different regions described a strong relationship between salinity and he spatial distribution of *Vibrio* spp. [14–16]. Although salinity in

Table 2 Results of the stepwise multiple logistic regression models

			Estimate	Std. error	*z* value	Pr(>|*z*|)	Significance	Null deviance	Residual deviance	AIC
Water	*V. alginolyticus*	(Intercept)	–0.86	0.30	–2.90	0.00	**	282.32	229.76	233.76
		Water temperature	0.19	0.03	6.26	0.00	***			
	V. parahaemolyticus	(Intercept)	–1.85	0.41	–4.50	0.00	***	315.44	262.45	268.45
		Water temperature	0.17	0.03	6.29	0.00	***			
		Salinity	–0.05	0.02	–2.70	0.01	**			
	V. vulnificus	(Intercept)	–8.06	1.64	–4.92	0.00	***	101.09	74.14	78.14
		Water temperature	0.32	0.08	3.81	0.00	***			
Sediment	*V. alginolyticus*	(Intercept)	2.97	1.29	2.31	0.02	*	127.86	110.38	118.38
		Water temperature	0.14	0.05	2.73	0.01	**			
		Salinity	0.07	0.03	2.22	0.03	*			
		Wind direction	–0.14	0.07	–2.10	0.01	*			
	V. parahaemolyticus	(Intercept)	–1.85	0.41	–4.50	0.00	***	315.44	262.45	268.45
		Water temperature	0.17	0.03	6.29	0.00	***			
		Salinity	–0.05	0.02	–2.70	0.01	**			
	V. vulnificus	(Intercept)	–6.47	2.20	–2.93	0.00	**	101.09	60.53	66.53
		Water temperature	0.49	0.12	4.10	0.00	***			
		Wind direction	–0.25	0.11	–2.32	0.02	*			

*$p<0.05$; **$p<0.01$; and ***$p<0.001$, significant

the study was spatially and temporally variable covering a broad range from 0.6 to 33.5 psu, the overall effect of salinity on the distribution of *Vibrio* spp. in the study area was less important than the effect of water temperature. Particularly *V. alginolyticus* and *V. parahaemolyticus* tolerated the entire range of mean salinities between ~4 and 27 psu and were ubiquitously distributed at all sites in the study area. Nevertheless, the regression models revealed that the presence of *V. alginolyticus* and *V. parahaemolyticus* at individual time points was linked to species-specific salinity preferences. While high salinity values were shown to be significantly negatively correlated to occurrence of *V. parahaemolyticus*, they exhibited a significant positive influence on the occurrence and abundance of *V. alginolyticus*. In contrast, presence of *V. vulnificus* was mainly restricted to sites within the Ems and Weser estuaries with mean salinities of ~4 to 17 psu, and *V. vulnificus* abundance in the study area was significantly related to low salinity. These findings reveal a preference of *V. vulnificus* for brackish waters, supporting earlier descriptions as an estuarine bacterium [11]. The present study showed, however, that *V. vulnificus* can occasionally occur at sites with high mean salinities that are usually not expected to be within the range tolerated by this bacterium. *V. vulnificus* was detected in sediments at Borkum beach in September 2010, a site highly influenced by the open North Sea. Results of a particle transport model showed that wind and current regimes at that time could have caused a drift of *V. vulnificus* from the Ems estuary to Borkum, suggesting that certain environmental conditions can favour short-term presence of *V. vulnificus* outside the estuaries.

Wind direction was determined as a significant determinant of *Vibrio* spp. in the regression models. Particularly in

Table 3 Full predictive multiple linear regression models for $\log_{10}$ *V. alginolyticus*, $\log_{10}$ *V. parahaemolyticus* and $\log_{10}$ *V. vulnificus* in sediment and water, respectively

Predictable variable ($\log_{10}$)	Full multiple linear regression model	R^2	*p*
$V.\ alg._{\text{Water}}$	=(0.10×water temp.)+(0.01×rain)+0.40	0.24	<0.001
$V.\ alg._{\text{Sediment}}$	=(0.13×water temp.)+(0.03×salinity)–(0.06×wind direction)+(0.42×TOC)+(0.01×rain)+2.14	0.30	<0.001
$V.\ parah._{\text{Water}}$	=(0.07×water temp.)–0.08	0.19	<0.001
$V.\ parah._{\text{Sediment}}$	=(0.11×water temp.)+(0.45×TOC)–(0.07×wind direction)+(0.01×rain)+1.73	0.19	<0.001
$V.\ vuln._{\text{Water}}$	=(0.03×water temp.)–(0.02×wind direction)–(0.01×salinity)+0.35	0.13	<0.001
$V.\ vuln._{\text{Sediment}}$	= (0.04×water temp.) – (0.02×wind direction) – (0.01×salinity) + 0.30	0.12	<0.001

Significant explanatory variables determined following a stepwise forward selection procedure

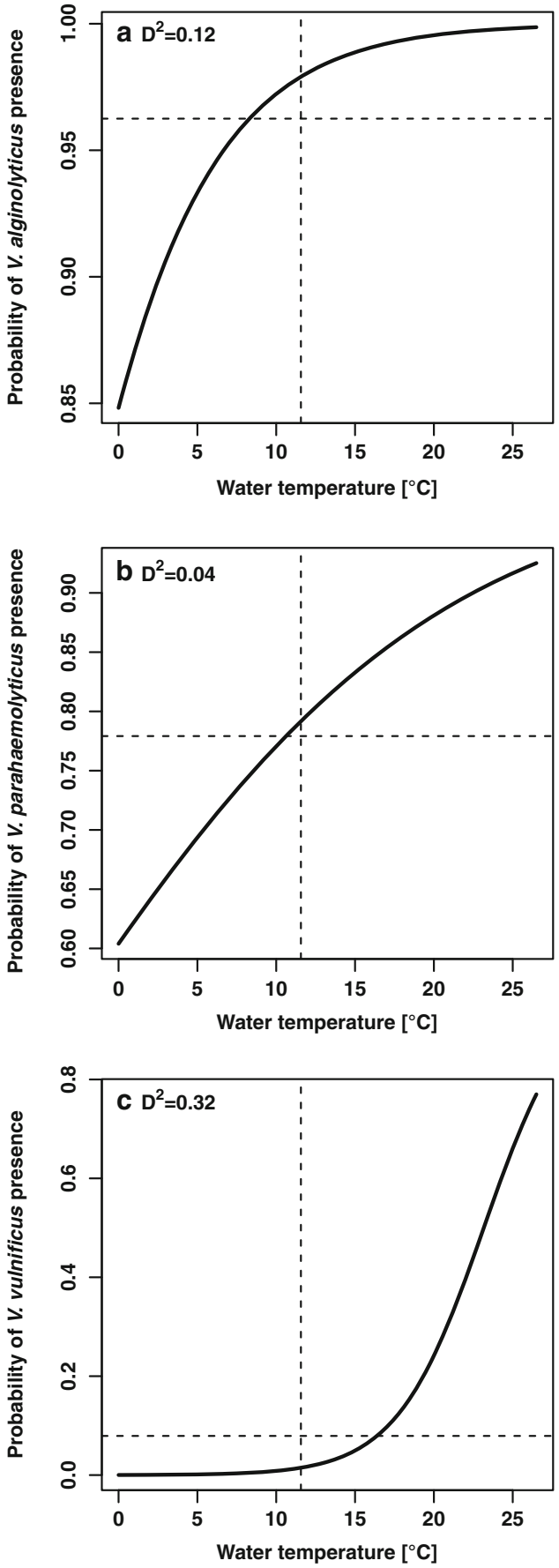

Fig. 7 Simple logistic regression models presenting the predicted probabilities for the presence of *V. alginolyticus* (**a**), *V. parahaemolyticus* (**b**) and *V. vulnificus* (**c**) in the study area as a function of water temperature. Combined data from sediment and water samples were considered for the analyses. The test statistic D^2 describes the overall model performance and is given in the *upper left corner* of each sub-plot

the sediments, wind direction significantly influenced the occurrence of *V. vulnificus* and *V. alginolyticus*, and the abundance of all three species, respectively. Low abundance of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* was significantly connected to westerly, which usually presses high salinity water from the open North Sea into the study area, while reducing the share of nutrient-rich riverine waters. In contrast, rainfall had a minor positive effect on *V. alginolyticus* and *V. parahaemolyticus* abundance which can likely be linked to a higher share of nutrient-rich freshwater in the area.

Results of this study further show that sediments play a very important role for *Vibrio* ecology in this temperate environment. Culturable estimates of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* were generally one to three orders of magnitude higher in sediments than in water and were ~10^5 cfu/g wet sediment in summer. Although previous studies in different regions showed that sediments can harbour high amounts of *Vibrio* spp. [12, 40, 63, 64], such a high difference in the number of viable cells between sediment and water seems to be extraordinary. The possibility of an active benthic lifestyle of *Vibrio* spp. has not been seriously discussed [12]. The sediments in general are mainly regarded as a retreat for *Vibrio* under unfavourable environmental conditions, for example, low temperatures. Our results indicate that sediments in fact may exhibit a protective effect on *V. alginolyticus* and particularly *V. parahaemolyticus* at winter time. However, with regard to the generally high numbers of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* in sediments reported here, it seems likely that *Vibrio* spp. are active members of the benthic bacterial community. *Vibrio* spp. can use a large variety of different carbon sources [65, 66], and a significant positive relationship between TOC and the abundance of *V. alginolyticus* and *V. parahaemolyticus* in sediments observed in this study suggests that these species could potentially gain energy from benthic organic carbon mineralization.

In order to get a first idea of the pathogenic potential of *Vibrio* spp. occurring in the study area, a limited number of *V. parahaemolyticus* and *V. cholerae* strains that were isolated during the study were checked for the presence of virulence-related genes. Thirty-five *V. parahaemolyticus* strains were tested for the presence of *tdh* and *trh*, 22 *V. cholerae* strains for the presence of *ctxA*, *O1* and *O139*, respectively. None of the *V. cholerae* strains was pathogenic, supporting the results of our agglutination tests. Only two *V. parahaemolyticus* isolates tested positive for presence of the

trh gene, but not for *tdh*. Comparisons with other studies show that generally between 3 and 5 % of environmental *V. parahaemolyticus* isolates bear either of the two virulence gene markers [67, 68]. This suggests that the ratio observed here is realistic, although more strains need to be checked for more reliable evidence. Presence of the *trh* gene alone seems to be a common characteristic of *V. parahaemolyticus* communities in Northern European waters and has been shown for several studies in this region [39, 69, 70]. Up to now, only *tdh*-positive strains were associated with *V. parahaemolyticus* infections in Europe, however [68], and *tdh* is the gene that has been mainly attributed to haemolysis [71]. Despite this, two strains that were identified as being *tdh*- and *trh*-negative exhibited haemolytic capacity in the Kanagawa test. This phenomenon has been described earlier [16], and additional genes than *trh* and *tdh* have been suggested to be involved in *V. parahaemolyticus* haemolysis [67, 72]. The small percentage of putatively pathogenic *V. parahaemolyticus* and *V. cholerae* isolates detected here indicates that these species do not constitute a significant health risk at recreational beaches of the German North Sea. Interestingly, however, all *trh*- and Kanagawa-positive strains were isolated from sediment samples. Organisms that are able to persist in sediment biofilms will more likely be able to colonize other tissues such as skin or the intestinal tract [73]; thus, the role of sediments in putting forth pathogenic clones could be an interesting aspect for future investigations.

Although putative virulence genes of *V. vulnificus* were not considered in this study, the mere presence of *V. vulnificus* should be viewed with concern. The potential health risk by this organism needs to be properly determined, especially during the summertime. The haemolysis gene *vvh* has been associated with a large proportion of *V. vulnificus* isolates in Northern European Waters [37, 39] and was even used as a species-specific marker [74], suggesting that environmental *V. vulnificus* isolates inherently carry haemolysis genes. Despite this, *V. vulnificus* is generally not very virulent; however, it has an exceptional toxicity with 30 % of wound infections ending in fatalities [4]. Common surveillance practices cannot accurately monitor the potential health risk emanating from *V. vulnificus* because the species does not correlate with faecal indicators (data not shown) and can occur at sites with excellent water quality [75].

In conclusion, non-virulent *V. alginolyticus* and *V. parahaemolyticus* are ubiquitously and perennially distributed in water and sediment at recreational beaches of the German North Sea, while the putative pathogen *V. vulnificus* frequently occurs in the estuaries of the rivers Ems and Weser at summertime. *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* exhibited distinct seasonal cycles, with water temperature being the crucial factor governing the presence and abundance of all three species. Minor additional effects of salinity, wind direction and rainfall were detected. The response of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* to water temperature was shown to be species-specific with a particularly good adaptation of *V. alginolyticus* to temperatures around the freezing point, and an enormous increase in the probability of *V. vulnificus* presence with minor temperature increments at water temperatures >20 °C. *Vibrio* spp. were not only more frequently isolated from sediments than from water, but also their abundance was generally one to three orders of magnitude higher in the benthic environment. In addition, the sediments were shown to have a protective effect on *V. alginolyticus* and particularly *V. parahaemolyticus* during the wintertime, suggesting an important role of sediments for *Vibrio* ecology in this dynamic temperate environment. Future studies will need to clarify the extent to which climate change may alter the composition of the *Vibrio* community in the Central Wadden Sea (particularly with regard to *V. vulnificus*), and whether climate change could favour the emergence and spreading of virulent representatives.

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