Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic

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Climate change is having a dramatic impact on marine animal and plant communities but little is known of its influence on marine prokaryotes, which represent the largest living biomass in the world oceans and play a fundamental role in maintaining life on our planet. In this study, for the first time to our knowledge, experimental evidence is provided on the link between multidecadal climatic variability in the temperate North Atlantic and the presence and spread of an important group of marine prokaryotes, the vibrios, which are responsible for several infections in both humans and animals. Using archived formalin-preserved plankton samples collected by the Continuous Plankton Recorder survey over the past half-century (1958–2011), we assessed retrospectively the relative abundance of vibrios, including human pathogens, in nine areas of the North Atlantic and North Sea and showed correlation with climate and plankton changes. Generalized additive models revealed that long-term increase in *Vibrio* abundance is promoted by increasing sea surface temperatures (up to ∼1.5 °C over the past 54 y) and is positively correlated with the Northern Hemisphere Temperature (NHT) and Atlantic Multidecadal Oscillation (AMO) climatic indices ($P < 0.001$). Such increases are associated with an unprecedented occurrence of environmentally acquired *Vibrio* infections in the human population of Northern Europe and the Atlantic coast of the United States in recent years.

climate | *Vibrio* | prokaryotes | infections | North Atlantic

Convincing evidence is now accumulating that points to a link between human activities and the emission of carbon dioxide in the Earth's atmosphere (1, 2). Globally averaged combined land and ocean surface temperature has risen by nearly 0.85 °C since the late 19th century. In the Northern Hemisphere, the period from 1983 to 2012 is believed to comprise the warmest 30-y period for the past 1,400 y (3). Marine temperate regions have been the most seriously affected; for instance, all European seas have warmed during the past few decades at four- to sevenfold the global rate (2).

These changes in ocean conditions have had a pronounced impact on the abundance, distribution, and phenology of marine organisms, as reported in long-term ecological and paleontological studies of eukaryotic animal and plant populations (4). In contrast, because of a lack of historical data, little is known about potential effects of global warming on marine prokaryotic communities. Prokaryotes comprise the largest living biomass within the ocean and have a significant impact on the life of the planet, by contributing not only to nutrient element cycling but also to the incidence of human and animal diseases (5). In fact, serious concern has been expressed earlier over the impact of ocean warming on the (re)-emergence and spread of environmental microbial pathogens (6).

Bacteria belonging to the genus *Vibrio* represent one of the largest culturable fractions of marine picoplankton, including more than 110 recognized species of which many are known human and animal pathogens (7) and are autochthonous to the marine environment (8). Cholera, a global disease, is caused by the pathogen *Vibrio cholerae* and is responsible for an estimated 3–5 million cases and 100,000–120,000 deaths every year (9). *Vibrio parahaemolyticus* and *Vibrio vulnificus* infections are also associated with high morbidity and mortality throughout the world. Members of these species have become and continue to be formidable pathogens, especially *V. cholerae* O1 and *V. parahaemolyticus* serotype O3:K6, which have been responsible for two of the most significant bacterial pandemics (10, 11).

Recent data have shown that the incidence of *Vibrio*-associated illnesses is increasing worldwide (12, 13). Of particular relevance are an unprecedented number of domestically acquired human infections that occurred in Northern European countries and were associated with swimming/bathing in coastal waters (14–17). Most of these cases were reported during heat waves (e.g., 1994, 1997, 2003, 2006, 2010), and it is expected that, as global warming continues, such events are likely to increase in frequency and intensity (18). Besides human illnesses, evidence has also been gathered linking *Vibrio* infections to increasing mass mortality of marine life in the coastal marine environment (19).

In the aquatic environment, vibrios are found attached to chitin-containing organisms, especially zooplankton, which represent one of the most important environmental reservoirs of these bacteria in nature (20). There is limited understanding of

Significance

Long-term ecological and paleontological data analyses indicate climate change is having an impact on marine eukaryotic communities. However, little is known about effects of global warming on marine prokaryotes, which are, by far, the largest living biomass in world oceans. Here, we report, for the first time to our knowledge, that a warming trend in sea surface temperature is strongly associated with spread of vibrios, an important group of marine prokaryotes, and emergence of human diseases caused by these pathogens. Our results are based on formalin-preserved plankton samples collected in the past half-century from the temperate North Atlantic.

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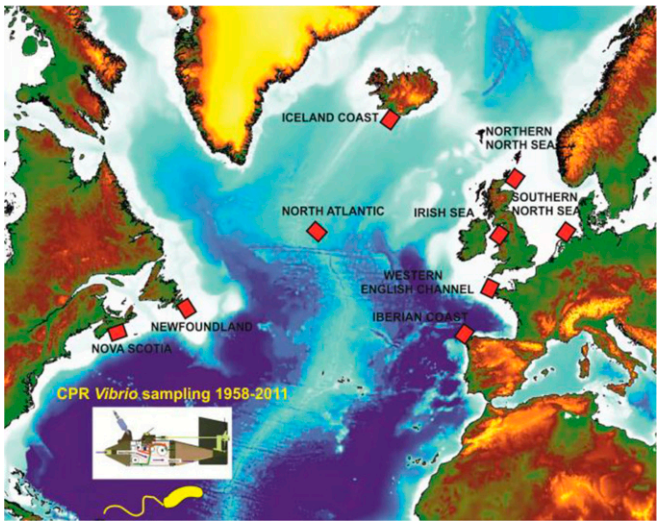

Fig. 1. Sampling areas in the temperate North Atlantic where CPR samples were collected for retrospective molecular studies of *Vibrio* populations over the period 1958–2011 [each area is scaled to an actual size of 1° longitude × 1° latitude (i.e., ~32 × 60 nautical miles)].

factors triggering *Vibrio* outbreaks and epidemics at the worldwide scale, as well as the presence and nature of a causal link between the spread of *Vibrio* illnesses and climate change. This limitation can be explained, at least in part, by lack of mesoscale historical data from microbiological studies mainly because of costs and time constraints to do large scale microbiological monitoring.

To address the challenge, we developed a novel approach to study the long-term ecology of *Vibrio* populations based on molecular analysis of formalin-fixed samples from the historical Continuous Plankton Recorder (CPR) archive (21, 22). The CPR is a long-term survey of phytoplankton and zooplankton in the global ocean, and it has produced one of the longest and most geographically extensive collections of marine biological samples in the world (www.sahfos.ac.uk/). In our pioneering work, we were able to recover environmental DNA from formalin-fixed CPR samples that had been stored for up to ~50 y. The samples of DNA were suitable for molecular analyses of the associated prokaryotic community (21). An index of abundance for *Vibrio* quantification in the CPR samples, termed the "*Vibrio*-relative abundance index" (VAI) was developed. This index measures the proportion of *Vibrio* bacteria relative to total bacteria and was used to evaluate temporal variations of vibrios over a multidecadal period (21). For example, the VAI calculated for CPR samples collected in the Rhine estuary of the North Sea between 1961 and 2005 revealed that the number of *Vibrio* bacteria is increasing (21).

In this study, we have applied the VAI methodology to analyze 133 CPR samples collected during the past half-century at nine locations (northern North Sea, southern North Sea, western English Channel, Iberian coast, Iceland coast, Irish Sea, Newfoundland, Nova Scotia, and North Atlantic) of the temperate North Atlantic (Fig. 1), which is a region that has undergone a rapid warming in the late 20th century (2). By comparing multidecadal changes in *Vibrio* and plankton populations and sea surface temperature (SST), we provide evidence, for the first time to our knowledge, of the important role that ocean warming plays in promoting the spread of *Vibrio* within an extensive area of the North Atlantic. In addition, by compiling epidemiological data on *Vibrio*-related infections that have been recorded in Northern Europe and the Atlantic coast of the United States during the past 54 y, we show that the number of documented human illnesses parallels the observed increase in marine vibrios.

Results and Discussion

Multidecadal Relationship Between *Vibrio* Prokaryote Abundance and Climatic Variability in the Temperate North Atlantic Region. Over most of the North Atlantic, SST has increased over the past few decades (up to ~1.5 °C over the past 54 y), following the average climate warming trend (Fig. 2). This increase generally has resulted in extended summer periods (calculated as the number of months for which the average temperature was equal to or above the top quartile of the yearly means time series for 1958–2011) in the majority of the study areas (Table 1). The increase in SST is sharper from the late 1990s onward (Fig. 3) as a likely consequence of the changes that have taken place in the Subpolar Gyre (23). In the North Sea, this event has been described as a regime shift by Alvarez-Fernandez et al. (24) and Beaugrand et al. (25).

Correlations between the *Vibrio* index and SST over the study period showed a positive relationship in eight of the nine study areas (Fig. 3). The exception to this pattern was observed in the data from off Newfoundland (despite a general increase in regional SST; Fig. 3 and Table 1) and can be related to the remarkably low SST (on average, less than 7 °C) recorded in this area (Fig. 2 and Table 1). Interestingly, in coastal waters characterized by relatively low SST (e.g., 8–10 °C in Iceland and Nova Scotia), a positive *Vibrio*–SST relationship was observed, suggesting that cold-adapted *Vibrio* populations in these areas respond to long-term SST increase at temperature ranges and thresholds lower than the temperature ranges and thresholds affecting warm-water populations. The most marked increase in the *Vibrio* index was observed over the past 10 y in most of the areas, and is related to an abrupt SST rise over the same period. Accordingly, generalized additive model (GAM) regression models revealed a significant positive nonlinear effect of SST on *Vibrio* abundance measured across all areas and time periods (Fig. 4*B*).

The Northern Hemisphere Temperature (NHT) comprises the largest source of variation in North Atlantic SST (26). Other sources of SST variation that might influence the overall climate in this region are intrinsic oceanic modes and dominant atmospheric phenomena, such as the Atlantic Multidecadal Oscillation (AMO) (27, 28), the North Atlantic Oscillation (NAO) (29, 30), and the East Atlantic Pattern (EAP) (31).

On a correlative basis, the main driver of long-term *Vibrio* variability in the North Atlantic and North Sea also appears to be the NHT warming trend and the AMO. The NHT is a measure of atmospheric and ocean temperature over the northern half of the globe, whereas the AMO is considered to be a natural mode of oscillation of the Atlantic Ocean thermohaline circulation (32),

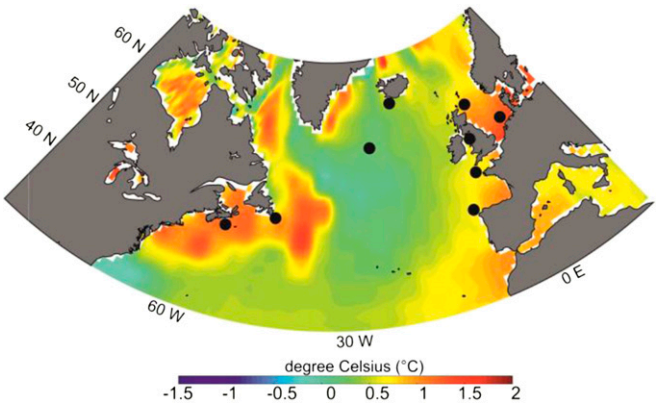

Fig. 2. Change in North Atlantic SST (degrees Celsius) over the study period calculated as delta between SST averaged over the years 2000–2011 and 1890–1958. Hot colors indicate areas of warming. Sampling areas are indicated as black dots on the map.

Table 1. Annual means and SDs of environmental data and average *Vibrio* index per decade from 1958 to 2011 in the different geographic areas

Area	SST, °C	Summer length, mo	PCI	TotCop, ind/CPR sample	*Vibrio* index, *Z*-values
Northern North Sea					
1958–1969	9.6 ± 0.3	5.5 ± 0.5	0.8 ± 0.6	799.4 ± 667.8	−0.4 ± 0.4
1970–1979	9.5 ± 0.3	5.2 ± 0.6	0.9 ± 0.3	994.6 ± 365.1	−0.7 ± 0.2
1980–1989	9.5 ± 0.2	5.3 ± 0.5	1.4 ± 0.5	912.6 ± 473.2	−0.1 ± 0.2
1990–1999	9.9 ± 0.3	5.6 ± 0.5	1.5 ± 0.4	742.3 ± 341.3	−0.6 ± 0.8
2000–2011	10.3 ± 0.2	5.9 ± 0.3	1.5 ± 0.3	592.6 ± 270.6	1.2 ± 1.1
Southern North Sea					
1958–1969	10.4 ± 0.6	5.1 ± 0.3	1.6 ± 0.7	945.3 ± 635.5	−0.2 ± 0.3
1970–1979	10.6 ± 0.3	5.2 ± 0.4	1.7 ± 0.5	950.0 ± 633.5	−0.8 ± 0.3
1980–1989	10.5 ± 0.6	5.2 ± 0.4	2.5 ± 0.7	882.1 ± 324.6	−0.9 ± 0.2
1990–1999	11.1 ± 0.7	5.5 ± 0.7	2.1 ± 0.6	1037.9 ± 358.6	−0.3 ± 0.3
2000–2011	11.6 ± 0.4	5.8 ± 0.5	2.3 ± 0.4	413.7 ± 231.5	0.9 ± 0.4
Western English Channel					
1958–1969	13.8 ± 0.3	5.1 ± 0.3	0.8 ± 0.7	839.2 ± 363.3	na
1970–1979	13.6 ± 0.3	4.8 ± 0.6	0.8 ± 0.5	765.9 ± 541.6	−0.8 ± 0.2
1980–1989	13.8 ± 0.4	4.9 ± 0.3	1.3 ± 0.2	783.3 ± 150.4	1.8
1990–1999	14.0 ± 0.3	5.3 ± 0.5	1.0 ± 0.6	575.5 ± 303.7	−0.3 ± 0.5
2000–2011	14.3 ± 0.2	5.5 ± 0.7	1.2 ± 0.4	386.8 ± 390.6	0.4 ± 0.2
Iberian coast					
1958–1969	15.6 ± 0.2	5.0 ± 0.4	1.0 ± 0.5	959.6 ± 451.2	na
1970–1979	15.3 ± 0.2	4.7 ± 0.5	1.2 ± 0.7	853.0 ± 568.5	−0.6 ± 0.3
1980–1989	15.7 ± 0.3	5.2 ± 0.8	0.9 ± 0.4	679.2 ± 585.8	−0.7 ± 0.2
1990–1999	15.9 ± 0.3	5.4 ± 0.5	0.5 ± 0.4	84.7 ± 34.9	−0.3
2000–2011	16.1 ± 0.2	5.5 ± 0.5	1.0 ± 0.4	241.8 ± 96.7	1.3 ± 0.5
Iceland coast					
1958–1969	9.4 ± 0.3	5.1 ± 0.8	0.1 ± 0.2	214.2 ± 318.0	−0.7 ± 0.1
1970–1979	8.8 ± 0.2	3.9 ± 0.9	0.3 ± 0.3	333.3 ± 344.1	−1.2
1980–1989	8.8 ± 0.2	3.8 ± 0.6	0.6 ± 0.7	214.9 ± 211.7	−0.5 ± 0.2
1990–1999	8.9 ± 0.4	4.2 ± 0.9	0.7 ± 0.9	208.5 ± 270.9	0.1
2000–2011	9.5 ± 0.2	4.9 ± 0.3	0.7 ± 0.3	162.8 ± 231.3	1.2 ± 0.4
Irish Sea					
1958–1969	11.2 ± 0.5	5.4 ± 0.8	na	na	na
1970–1979	11.2 ± 0.3	5.5 ± 0.7	1.1 ± 0.4	361.4 ± 249.2	−0.4 ± 0.4
1980–1989	11.0 ± 0.4	5.2 ± 0.6	1.0 ± 0.6	323.6 ± 152.1	−0.7 ± 0.4
1990–1999	11.4 ± 0.3	5.6 ± 0.5	1.8 ± 0.8	289.2 ± 209.1	−1.3 ± 0.2
2000–2011	11.8 ± 0.2	5.9 ± 0.3	1.2 ± 0.3	208.8 ± 107.1	1.0 ± 0.3
Newfoundland					
1958–1969	6.3 ± 0.5	4.8 ± 0.6	0.7 ± 0.5	573.4 ± 288.8	0.1 ± 0.3
1970–1979	6.4 ± 0.4	5.2 ± 0.8	0.7 ± 0.2	370.0 ± 226.9	0.3 ± 0.9
1980–1989	6.6 ± 0.7	5.2 ± 0.8	na	na	na
1990–1999	6.3 ± 0.5	4.7 ± 0.7	2.0 ± 1.2	449.3 ± 286.2	−0.8 ± 0.4
2000–2011	7 ± 0.4	5.4 ± 0.8	2.0 ± 0.7	449.0 ± 262.9	−0.1 ± 0.3
Nova Scotia					
1958–1969	8.0 ± 0.6	5.2 ± 0.8	0.3 ± 0.3	656.9 ± 336.3	−0.8 ± 0.0
1970–1979	8.7 ± 0.3	5.8 ± 0.4	0.6 ± 0.7	436.2 ± 264.0	−1.4 ± 0.0
1980–1989	8.9 ± 0.5	5.6 ± 0.5	na	na	na
1990–1999	9.2 ± 0.5	5.9 ± 0.3	0.8 ± 0.4	615.1 ± 421.6	0.2 ± 0.5
2000–2011	9.6 ± 0.4	6.0 ± 0.0	0.9 ± 0.3	558.3 ± 503.3	0.8 ± 0.6
North Atlantic					
1958–1969	9.7 ± 0.3	4.8 ± 0.6	0.1 ± 0.3	255.5 ± 213.6	−0.4 ± 0.6
1970–1979	9.3 ± 0.4	4.4 ± 0.7	0.5 ± 0.5	94.0 ± 73.8	−0.5 ± 0.0
1980–1989	9.5 ± 0.3	4.7 ± 0.7	0.2 ± 0.2	192.5 ± 1.2	−0.5
1990–1999	9.7 ± 0.7	5.3 ± 0.8	0.2 ± 0.3	5.0 ± 1.3	−0.5
2000–2011	10.1 ± 0.4	5.5 ± 0.8	0.7 ± 0.4	177.8 ± 205.6	0.8 ± 0.5

Summer length is expressed as the number of months for which the average temperature was equal to or above the top quartile of the yearly means time series, 1958–2011. ind, individuals; na, not available.

showing a periodicity (oscillation) of about 60–80 y (27). The NHT is also subject to an oscillatory effect, which has a period of around 60 y, meaning it is not orthogonal to the AMO (33). Both indices are known to affect SST variance significantly in the North Atlantic, and it is known that the AMO behavior is very similar to the NHT signal in the long term (26). The GAM regression models showed that these two climatic indices had a significant increasing effect on *Vibrio* abundance (Fig. 4 *A* and *C*) and explained 11.3% and 19.5% of the total deviance for the NHT and AMO, respectively. Interestingly, if fitted together,

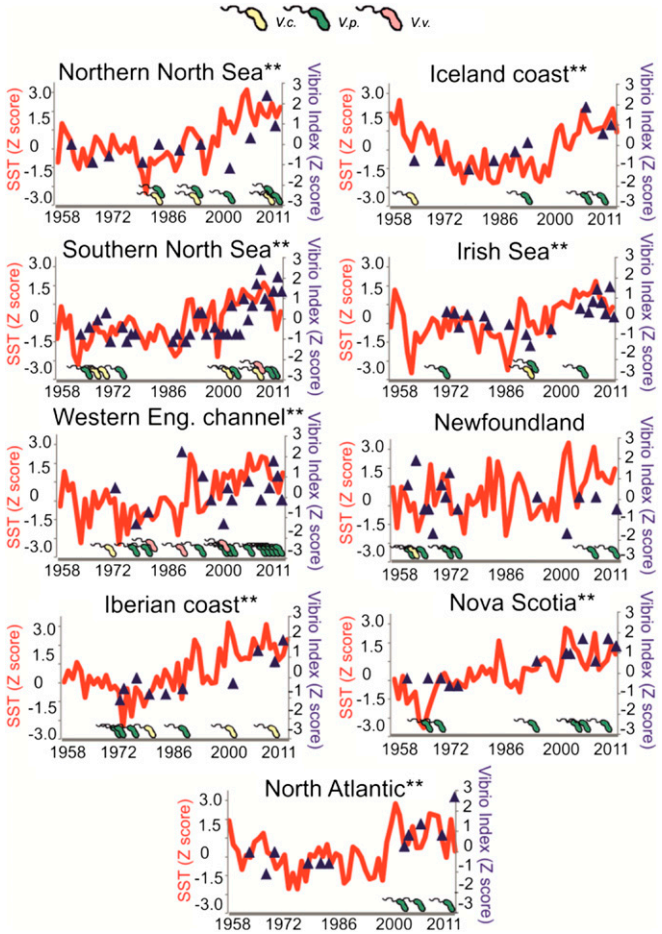

Fig. 3. Multidecadal relationship between *Vibrio* prokaryote abundance and SST in the temperate North Atlantic and North Sea. Standardized (*Z*) VAI data (blue triangles) are superimposed to standardized (*Z*) SST monthly means time series data (red line) for nine geographic areas in the temperate North Atlantic. The presence of the human pathogenic species *V. cholerae* (V.c., yellow bacterial cell), *V. parahaemolyticus* (V.p., green bacterial cell), and *V. vulnificus* (V.v., pink bacterial cell) is shown. *Z*-values were obtained by subtracting the population mean and dividing the difference by the SD. $^{**}P < 0.05$, Pearson correlation analysis.

SST and the AMO explained 30% of the total deviance in the GAM.

Although not significant, a less marked positive nonlinear relationship was observed between the *Vibrio* index and the EAP, whereas no significant relationship was found with the NAO index (Fig. 4*E*). The NAO is a meridional seesaw in atmospheric pressure between the Icelandic Low (IL) and the Azores High (AH) (34, 35), and is the dominant, recurrent atmospheric phenomenon in the North East Atlantic. It accounts for over one-third of the total variance in sea-level pressure (36). The EAP is the second leading climate mode in the North Atlantic sector and consists of a well-defined monopole in the sea-level pressure field to the south of Iceland and west of the United Kingdom, near 52.5°N, 22.5°W (37). Both atmospheric phenomena are believed to play a role in driving SST across the entire North Atlantic (26). The NAO is known to have a dipole in its spatial influence, and has a positive influence on SST in the North Sea with a negative influence in the Subpolar Gyre. This factor might help to explain the lack of correlation observed between the *Vibrio* index and the NAO in the overall dataset in our study (Fig. 4*E*). In contrast, although the EAP behaves similar to the NAO, the anomaly centers of this pattern are displaced southeastward to the approximate nodal lines of the NAO and a positive phase of the EAP is associated with above-average surface temperatures in a larger area of the East Atlantic in all months (26). This observation is consistent with the weak positive relationship observed between the *Vibrio* index and EAP in the North East Atlantic sector (Fig. 4*D*). Large-scale oceanographic patterns, such as the latitudinal displacements of the Gulf Stream, are also related to changes in temperature in the Northeast Atlantic (38), albeit no correlation was found between *Vibrio* and the Gulf Stream north wall (GSNW) indices (Fig. 4*F*).

Taken as a whole, despite limitations associated with the low number of samples in some areas, these data suggest that the SST warming trend linked with the NHT and AMO climatic indices is a global driver of long-term variability in *Vibrio* abundance in the temperate North Atlantic. Besides SST, environmental parameters strongly influencing occurrence of vibrios in aquatic environments include salinity [with the highest populations generally occurring in waters of intermediate salinity (e.g., ~23 parts per thousand)], water trophic status (with highest *Vibrio* counts generally found in eutrophic water), and association with plankton (39). With the exception of sea surface salinity (Fig. S1), such conditions appear to vary significantly among and between different areas and also have an

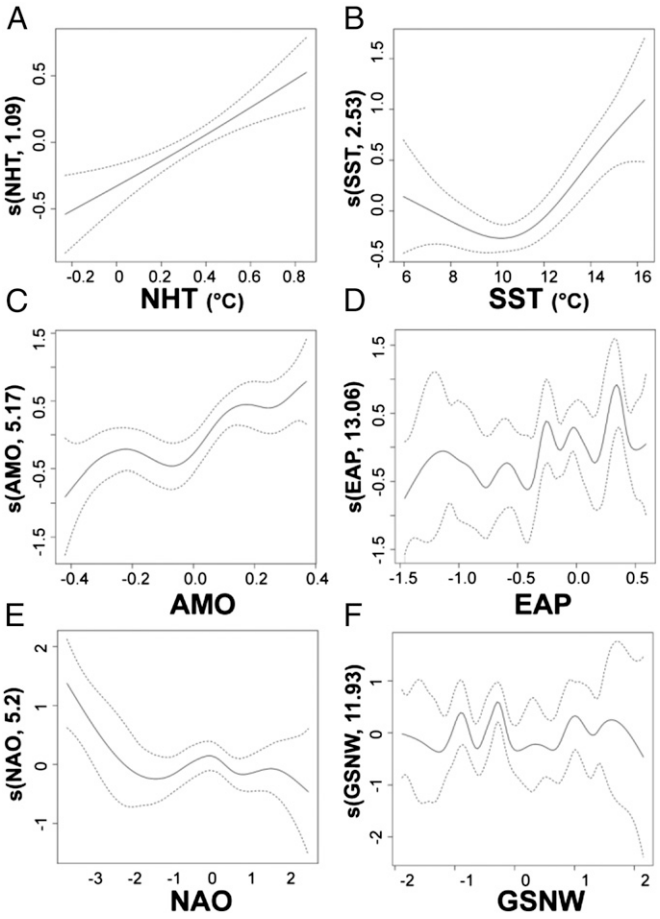

Fig. 4. Effects of climate variables in the GAM explaining *Vibrio* abundance (*Vibrio* index) in the North Atlantic region. Solid lines represent the estimated smooth function, and dashed lines represent the 95% confidence bounds. The *y*-axis captions are the effective degrees of freedom for each term. (*A*) Northern Hemisphere mean land–ocean temperature (NHT) index (deviance explained = 11.3%; $^{**}P < 0.001$). (*B*) SST (deviance explained = 15.7%; $^{**}P < 0.001$). (*C*) AMO index (deviance explained = 19.5%; $^{**}P < 0.001$). (*D*) EAP (deviance explained = 19.4%; $P > 0.05$). (*E*) NAO index (deviance explained = 13.2%; $P > 0.05$). (*F*) GSNW index (deviance explained = 17.4%; $P > 0.05$).

impact on long-term trends observed for *Vibrio* populations (Table 1). This finding particularly holds true for coastal areas, which are generally more supportive of growth of *Vibrio* spp. than oceanic waters.

The influence of natural oscillations may also play a role in driving *Vibrio* abundance and spread, as suggested by the positive correlation with the AMO index. Although this large-scale climate phenomenon is known to influence SST variability in the North Atlantic sector significantly, it also has a more general influence on the marine ecosystem by affecting precipitation, winds, storm activity, and atmospheric pressure, which, in turn, influence ocean circulation and the physiological processes in many marine organisms. As a consequence, although the general warming trend in SST is unequivocally of major importance for the long-term ecology of vibrios, the influence of natural oscillations on other chemical and physical properties of the ocean may also be relevant and warrant further investigation.

Long-Term Variation in *Vibrio* Abundance Relative to Multidecadal Changes in the Plankton Community. Planktonic crustaceans, and especially copepods, are known to represent one of the most important environmental reservoirs for *Vibrio* bacteria in brackish and marine environments (20). Vibrios attach to both the exoskeleton and molts of zooplankton organisms by specific cell ligands, such as pili and membrane proteins, and they can grow and multiply on chitin surfaces via formation of biofilms (40). Phytoplankton and aquatic plants are also potential carriers of vibrios (41, 42) and a source of dissolved organic carbon for microbial metabolism (43). Ultimately, they are food for zooplankton grazers, and their presence and concentration in seawater promote zooplankton productivity, which, in turn, encourages *Vibrio* growth and persistence (44).

Although short-term changes (e.g., seasonal changes) in abundance and composition of the plankton community are known to affect *Vibrio* concentration significantly in the aquatic environment

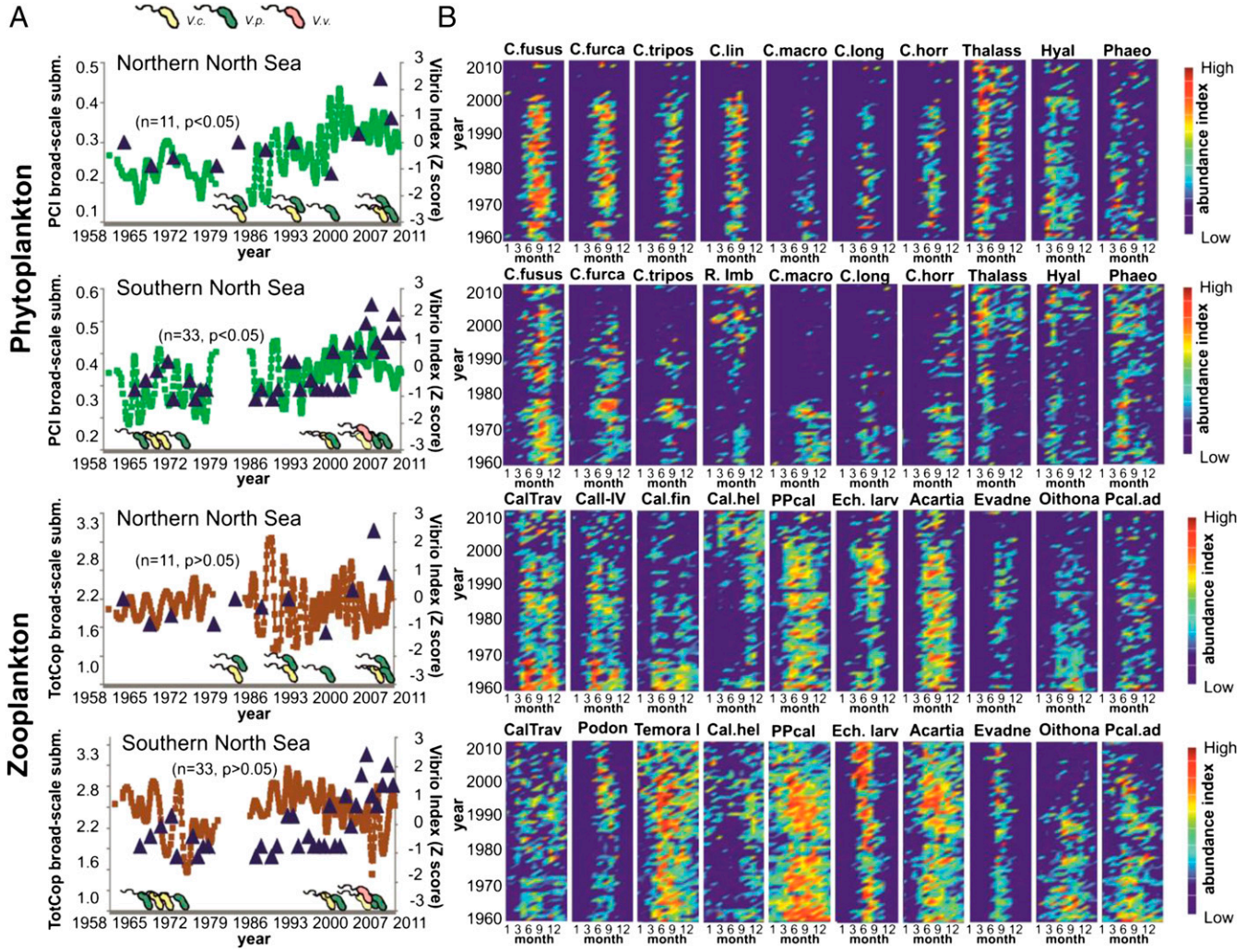

Fig. 5. Multidecadal relationship between *Vibrio* prokaryote abundance, plankton abundance, and plankton community structure in the North Sea for 1958–2011. (*A*) Standardized (*Z*) *Vibrio* index (VAI) data (blue triangles) are superimposed to broad-scale submodel monthly time series for the PCI (green line) and TotCop (brown line) data for the northern and southern North Sea. The presence of human pathogenic species *V. cholerae* (V.c., yellow bacterial cell), *V. parahaemolyticus* (V.p., green bacterial cell), and *V. vulnificus* (V.v., pink bacterial cell) is shown. Pearson correlation analysis was performed between the VAI and corresponding plankton submodel data for the month of August 1958–2011. (*B*) Month-by-year contour plots of plankton abundance for the 10 most abundant phytoplankton and zooplankton species in the northern and southern North Sea, according to Johns and Reid (80). The phytoplankton species are as follows: *Ceratium furca* (C.furca), *Ceratium fusus* (C.fusus), *Ceratium horridum* (C.horr), *Ceratium lineatum* (C.lin), *Ceratium longipes* (C.long), *Ceratium macroceros* (C.macro), *Ceratium tripos* (C.tripos), *Chaetoceros*(*Hyalochaete*) spp. (Hyal), *Chaetoceros*(*Phaeoceros*) spp. (Phaeo), *Rhizosolenia imbricata* (R.Imb), and *Thalassiosira* spp. (Thalass). The zooplankton species are as follows: *Acartia* spp. (Acartia), *Calanus finmarchicus* (Cal.fin), *Calanus helgolandicus* (Cal.hel), *Calanus* I–IV (Call-IV), *Calanus* traverse (CalTrav), Echinoderm larvae (Ech.larv), *Evadne* spp. (Evadne), *Oithona* spp. (Oithona), *Para-Pseudocalanus* spp. (PPcal), *Podon* spp. (Podon), *Pseudocalanus* adult (Pcal.ad), and *Temora longicornis* (Temora I).

[e.g., by affecting *Vibrio* seasonality (45)], not much is known about the impact that long-term changes in the plankton community may have on *Vibrio* populations. To address this issue, multidecadal changes in the phytoplankton and zooplankton communities were investigated in two model areas of the northern and southern North Sea and were correlated with the *Vibrio* data. Phytoplankton color index (PCI), an index of the total phytoplankton standing stock, increased in the two selected areas from the late 1980s (Fig. 5*A*), concurrent with an increase in the *Vibrio* index (Fig. 5*A*). However, whether the observed correlation can be ascribed to a direct association between *Vibrio* and their phytoplankton carriers/food sources or to an indirect influence of rising SST is difficult to determine. A significant positive correlation ($P < 0.01$) was found between PCI and SST, suggesting that increasing temperatures have a positive effect on phytoplankton abundance. These results are in agreement with PCI data recorded in the other study areas (Table 1) and with previous studies reporting that total phytoplankton abundance has increased over the past three decades across the entire North East Atlantic, most likely related to an increase in light intensity and/or longer seasonal growth periods (26, 46).

The increase in total phytoplankton biomass is also coupled with a marked change in phytoplankton phenology (e.g., timing and intensity of seasonal blooms) and community structure (47) (Fig. 5*B*). By analyzing long-term seasonal changes of the 10 most abundance phytoplankton species collected by the CPR survey in the North Sea, it was found that diatoms and dinoflagellates dominate this community of larger phytoplankton, with some species increasing in abundance and others experiencing a decline (Fig. 5*B*). The sharp increase in *Vibrio* abundance observed in recent years (e.g., 2000 onward) is concomitant with an increase of certain phytoplankton species, notably diatoms (e.g., *Thalassiosira* spp., *Rhizosolenia imbricata*), and a decline in abundance of other species, especially dinoflagellates (e.g., mainly *Ceratium* species). These results agree with recent work that demonstrated a significant correlation between *Vibrio* abundance in seawater and phytoplankton, with higher correlations with diatoms compared with dinoflagellates (48). One possible explanation for this finding is that diatoms genera, such as *Thalassiosira*, use chitin as a structural component of the silica cell wall, and it has been recently shown that some *Vibrio* species interact with diatom-derived chitin to foster their environmental persistence (42).

In contrast, long-term change in the absolute abundance of copepods did not appear to play a significant role with respect to temporal variation in the *Vibrio* population. Interestingly, a lack of association between *V. parahaemolyticus* and copepods was reported for offshore waters by Martinez-Urtaza et al. (13).

This result may be a consequence of the efficiency of bacterial attachment to their copepod hosts (e.g., the number of bacteria attached to a single copepod) under various environmental conditions. For example, Stauder et al. (49) have previously shown that increased SST promotes *V. cholerae* O1 El Tor attachment to chitin particles via enhanced expression of chitin-targeting colonization factors, such as *N*-acetylglucosamine-binding protein A (GbpA) and mannose-sensitive hemagglutinin.

Another potential explanation lays in species-specific interaction between *Vibrio* and plankton species. It has been demonstrated that as a response to global warming, the overall change in zooplankton abundance over time is paralleled by a significant change in community structure (50). For example, despite a decline in total copepods in the North Sea, the dominant warm-temperate copepod species, such as *Calanus helgolandicus*, are increasing with rising temperature (Fig. 5*B*) to the detriment of cold-temperate species, such as *Calanus finmarchicus* (51). Cold-water species may have a slightly different O_2 demand, which may be affected by temperature in a quantifiable way (52, 53). A replacement of large-sized copepods (e.g., total *Calanus*) with small copepods (e.g., *Para pseudocalanus*, *Temora longicornis*, *Acartia* spp.) has also been observed (Fig. 5*B*) to be a result of global warming (54).

Different species and/or life stages of zooplankton may provide alternative colonization niches for bacteria in terms of space, chitin surface, wax coverage, molt frequency, and nutrients (55). Previous studies have shown that some plankton species, such as the copepod *Acartia tonsa*, play a key role in the occurrence and distribution of *V. cholerae* in coastal areas (56–58). The presence of other planktonic crustaceans, such as cladocerans, was also found to be correlated with occurrence of *V. cholerae* O1 and the incidence of cholera cases in endemic cholera regions (59). Evidence from CPR samples on the abundance of planktonic species belonging to these groups (e.g., the copepod *Acartia* spp., the cladocerans *Podon* spp. and *Evadne* spp.) reinforces this linkage; these species have increased in abundance over the past 54 y in the North Sea simultaneously with the *Vibrio* increase (Fig. 5*B*).

Overall, the impact that a long-term change in plankton community structure may have on the temporal variation and spread of *Vibrio* remains poorly understood. As an intriguing hypothesis, the registered cross-oceanic migration and northward shift of plankton in response to ocean warming (50) may have a significant impact on *Vibrio* assemblages and redistribution of *Vibrio* populations. From this perspective, a poleward transport of *Vibrio* species mediated by zooplankton will occur as a result of global warming. Therefore, additional studies will be needed to decipher species-specific interactions between these bacteria and plankton.

Long-Term Relationship Between *Vibrio* Abundance and Human Disease. Substantial evidence has now been gathered showing *Vibrio*-associated diseases are increasing worldwide with climate warming (6). This increased incidence is particularly evident in rapidly warming areas of the world, such as Northern European countries (18) and the Atlantic coast of the United States (60), where the number of reported *Vibrio*-related infections (e.g., human infections associated with recreational bathing and foodborne infections) has significantly increased in recent decades, coinciding especially with heat waves. Possible reasons behind this trend include changes in disease transmission patterns, such as pathways of exposure and susceptibility of the host populations, and/or as a direct consequence of the increased number and spread of *Vibrio* bacteria in the aquatic environment (22).

To provide evidence for this hypothesis, the numbers of *Vibrio* infections recorded for Northern European countries (including the Baltic Sea, where a number of such cases occurred in recent years) and the US Atlantic coast were analyzed with data on potentially pathogenic *Vibrio* species found in CPR samples across the same macroregions (e.g., northern/southern North Sea areas for Northern Europe and Nova Scotia/Newfoundland for the US Atlantic coast) during 1973–2011 (Fig. 6).

It should be noted that the incidence of *Vibrio* diseases has been recorded in the United States since 1997 (www.cdc.gov/nationalsurveillance/cholera-vibrio-surveillance.html), whereas in Europe, with the exception of toxigenic *V. cholerae*, vibriosis is not a notifiable disease. For this reason and because of the incomplete surveillance of *Vibrio* infections in European countries, the epidemiological data that are available for Europe must be considered as indicative, but not conclusive, of the actual situation (18).

It can be observed that the incidence of *Vibrio* illness in recent years (e.g., from the 2000s to the present) has been matching the increase in the *Vibrio* index during the same time period (Fig. 3). The years showing the highest number of *Vibrio* illnesses are correlated with the presence of potentially pathogenic species, such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, detected in the CPR samples (Fig. 6). An intriguing example is 2006, when more than 60 cases of human infection associated with *Vibrio* spp. were reported in the North Sea and Baltic Sea (18). That year, the *Vibrio* index calculated for a CPR sample collected in the southern North Sea was among the highest

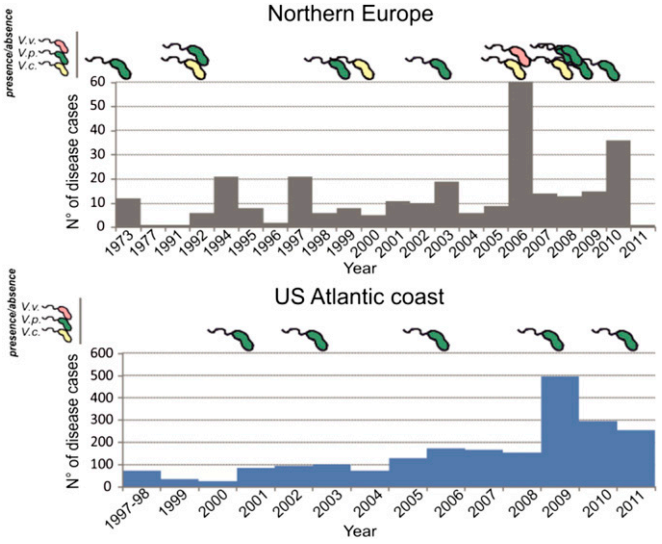

Fig. 6. Cases of *Vibrio* infections reported for Northern European countries (including the Baltic Sea) and Atlantic coast of the United States, 1958–2011. The presence of potential human pathogenic species in CPR samples collected in the same macroareas is also shown. The presence of human pathogenic species *V. cholerae* (V.c., yellow bacterial cell), *V. parahaemolyticus* (V.p., green bacterial cell), and *V. vulnificus* (V.v., pink bacterial cell) is shown.

recorded for the entire sample set of this study (Fig. 3). Interestingly, *V. cholerae* and *V. vulnificus* were detected in this sample that had been collected in an area and period (summer 2006) when human infections associated with these pathogens were reported (14). The presence of *V. vulnificus* is noteworthy because this pathogen was identified as the causative agent of an unprecedented number of human infections reported in 2006, a time of the most intense heat wave experienced in Northern Europe (18).

Similar to the phenomenon observed for Northern Europe and the US Atlantic coast, a large number of *Vibrio* outbreaks in northwest Spain were reported over the past two decades (e.g., a *V. parahaemolyticus* outbreak in 1999 and 2004) (61) that can also be linked to increasing SST and *Vibrio* abundance off the Iberian coast (Fig. 3).

Hence, an increased *Vibrio* concentration in seawater as a result of ocean warming can be concluded to be linked with increased incidence of environmentally acquired human infections. The connection is strong in those coastal environments highly favorable for *Vibrio* growth, such as the Baltic Sea, where warming is exacerbated by the occurrence of low-salinity water (18).

Conclusive evidence of linkage between the presence of pathogenic *Vibrio* in CPR samples and reports of human disease will require molecular epidemiological studies (e.g., comparative genomics studies based on bacterial identification at the strain level). Development of next-generation sequencing methods for direct genotyping of *Vibrio* strains in CPR samples is currently in progress in our laboratory, and such methods will eventually be used for this purpose.

Conclusion

The evidence is strong that ongoing climate change is influencing outbreaks of *Vibrio* infections on a worldwide scale. Whether this pattern is linked to increased spread of these bacteria in the aquatic environment remains a matter of debate. The lack of long-term ecological studies incorporating historical data, such as those studies gathered for eukaryotic communities, is the main obstacle in obtaining conclusive outcomes of such investigations.

To our knowledge, this study is the first to provide experimental evidence of linkage between multidecadal climatic variability, *Vibrio* prokaryote abundance, and *Vibrio*-associated human disease in the temperate North Atlantic. The main driver of long-term *Vibrio* variability in this large oceanic area is concluded to be the SST warming trend, which is likely responsible for a long-term increase in *Vibrio* concentration in the ocean over the past 54 y. This increase is, in turn, associated with a recent increase in *Vibrio*-associated disease, observed to be occurring at an unprecedented rate in Northern Europe and along the US Atlantic coast. Pronounced changes observed in the plankton community over the same temporal scale, such as an increase in total phytoplankton abundance and shift in zooplankton community structure, may also have contributed to these events, the mechanisms of which remain unclear.

CPR is proving a promising technology for the study of the macroecology of *Vibrio* species. Its utility and performance can be improved by extracting DNA immediately after sampling and/or avoiding/improving fixation of the sample, and it is a procedure that can be used in other geographical areas of interest for *Vibrio* studies, such as the Mediterranean Sea, Black Sea, Canadian coast, and Gulf of Mexico. Unfortunately, CPR samples and associated data are not available for those geographic areas with significant rates of warming, such as some parts of the Baltic Sea (warming at ∼1 °C per decade), the White Sea between Finland and Russia (warming at ∼1.5 °C per decade) (62), and the east coast of the United States (63), which are all considered at very high risk for *Vibrio* infections (18). Sentinel CPR studies in such areas, coupled to epidemiology, oceanography, and climate science, can provide unambiguous confirmation of the role of climate in mediating the spread of infectious disease caused by *Vibrio* spp. in the marine environment (64). It is expected that the predicted rise in global SST may exacerbate the spread of those aquatic *Vibrio* pathogens in the future, with detrimental effects on human and animal health.

Materials and Methods

CPR Samples. The CPR is a plankton-sampling instrument designed to be towed from merchant ships on their normal passage over long distances (65). The CPR is towed on a wire rope at a depth of *ca.* 10 m, and plankton is collected on a band of silk, with a mesh size of 270 μm, that moves across the sampling aperture at a rate that is proportional to the speed of the towing ship (65). The CPR mesh retains larger zooplankton, as well as small planktonic organisms, such as nauplii, microzooplankton, and phytoplankton (66). Within the CPR instrument, the net silk and captured plankton are preserved in 4–10% (vol/vol) buffered formalin until the samples are returned to the laboratory. The silk is removed from the device and divided into individual samples numbered along the route. Each labeled sample represents 10 nautical miles of towing (∼3 m^3 of water filtered). Only odd samples are analyzed, using standard light microscopy procedures, allowing identification of up to 500 different phytoplankton and zooplankton taxa (67, 68). The CPR samples (n = 133) used in this study were collected in nine areas of the temperate North Atlantic (northern North Sea, n = 11; southern North Sea, n = 33; western English Channel, n = 15; Iberian coast, n = 10; Iceland coast, n = 9; Irish Sea, n = 18; Newfoundland, n = 14; Nova Scotia, n = 12; and North Atlantic, n = 11) from 1958 to 2011 (Fig. 1 and Table S1). Because we were interested in detecting multidecadal temporal variations in *Vibrio* relative abundance, all of the analyzed samples were collected in August each year (generally corresponding to the seasonal peak of *Vibrio* counts in seawater) to limit the bias of seasonal variability. For the same reason, samples were collected within restricted areas of 1° longitude × 1° latitude (∼32 × 60 nautical miles) to limit spatial variability bias (Fig. 1 and Table S2). Applying these constraints limited the number of CPR samples for analysis, resulting in a low sample number in some areas (e.g., Iceland coast) and periods of time. It is, however, worth mentioning that a single CPR sample (10 nautical miles) corresponds to multiple point samples, which is the approach traditionally adopted in acquiring environmental microbiological data. The flow of water through the CPR silk filter is reduced by a factor of approximately ×32 compared with other net sampling systems, and, as a result, the silk filter is more likely to entrap small particles, such as mucilage and other material caught by the silk filter. As such,

using CPR samples provides an improvement in spatial sampling resolution by several orders of magnitude. Only unanalyzed samples for phytoplankton and zooplankton (i.e., the even-numbered labeled samples) were used for molecular microbiological analyses (i.e., analysis for *Vibrio*).

Molecular *Vibrio* Studies.

DNA extraction. DNA extraction from CPR samples was carried out as previously described by Vezzulli et al. (21). Briefly, the filtering silk of each CPR sample was cut into five replicate 1 × 1-cm^2 sections, and each section was placed in a sterile tube. Plankton were detached from the silk mesh by adding 25 mL of buffer [10 mM Tris·HCl, 1 mM EDTA (pH 8.0)] and vortexing for 30 s. The resulting plankton pellets were recovered by gentle centrifugation (400 × *g*) and transferred to a sterile tube. Fifty microliters of lysozyme [2 $mg{\cdot}mL^{-1}$ in 10 mM Tris·HCl (pH 8.0)] was added to the tube and vortexed for 1 min. One hundred eighty microliters of 10% SDS and 25 μL of proteinase K (10 $mg{\cdot}mL^{-1}$) were added and vortexed for 30 s. The sample was then incubated at 56 °C for 1 h, heated at 90 °C for 1 h in a dry-block heater, vortexed for 10 s, and centrifuged at 12,000 × *g* for 3 min. After addition of 200 μL of guanidine hydrochloride lysis solution and 200 μL of ethanol, the sample was centrifuged (12,000 × *g* for 10 s). The supernatant was transferred to a QIAamp MinElute column (Qiagen) and processed according to the manufacturer's recommendations. The eluted DNA from each of the five sections was pooled and cleaned to a total variable yield of 100–1,000 ng using an AMICON Ultra 30K membrane (Millipore). The amount of DNA extracted was determined fluorimetrically with a QuantiFluorTM dsDNA System using a QuantiFluorTM-fluorometer (Promega Italia srl). Manipulation of CPR samples and DNA extraction were carried out in a separate laboratory (nonmarine/nonmicrobiological laboratory) using all necessary precautions (e.g., use of a dedicated set of pipettes, reagents, and consumables to avoid cross-contamination of the samples).

VAI. The VAI, also referred to as the "*Vibrio* index," was measured as previously described (21). This index measures the proportion of *Vibrio* bacteria in relation to total bacteria in CPR samples using quantitative PCR (qPCR), producing small amplicons of similar size (113 vs. 98 bp) to avoid age- and formalin-induced bias [technical aspects and limitations of the molecular analysis applied to the CPR samples can be found in a report by Vezzulli et al. (21)].

The qPCR amplification protocol was set up on a Light Cycler 1.5 instrument (Roche Diagnostics) using Light Cycler SYBR Green I Master Mix chemistry. The oligonucleotide primers were Vib1 f-50-GG CGTAAAGCGCATGCAGGT-30 and Vib2 r-50-GAAATT CTACCCCCCTCTACAG-30 (69), specific for the genus *Vibrio*, and 967f-50-CAACGCG AAGAACCTTACC-30 and 1046r-50-CGACAGCCATGC ANCACCT-30 (70), specific for the domain "bacteria," amplifying positions 567–680 and 965–1,063 (V6 hypervariable region) of the *Escherichia coli* numbering of the 16S rRNA, respectively. Each reaction mixture contained 5.0 mM of $MgCl_2$ and 0.25 μM of each primer in a final volume of 20 μL. The PCR program was as follows: initial denaturation at 95 °C for 10 min, subsequent 40 cycles of denaturation at 95 °C for 5 s, annealing at 58 °C (*Vibrio* spp.) or 57 °C (total bacteria) for 5 s, and elongation at 72 °C for 4 s, followed by a final elongation at 72 °C for 10 min. For each single real-time PCR assay, each DNA template was analyzed in triplicate (coefficient of variation < 5%). An accurately quantified copy number genomic DNA of the *V. cholerae* (39315; American Type Culture Collection) was used as a standard (genetic PCR solution).

Vibrio spp. and total bacterial concentrations were expressed as number of cells per square centimeter of CPR sample by dividing total 16S rDNA copy number by the average 16S rDNA copy number in vibrios ($n = 9$) (71) and Proteobacteria ($n = 3.5$) (72), respectively.

Detection of human pathogenic vibrios. Direct detection of the human pathogenic vibrios in CPR samples was performed using capillary PCR on a Light Cycler 1.5 instrument following the protocol described by Vezzulli et al. (73), Campbell and Wright (74), and Nordstrom et al. (75) for *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*, respectively. Such protocols are based on amplification of short DNA fragments (<210 bp) and were optimized for the analysis of formalin-fixed CPR samples (73). Primers and probes used in the analysis are reported in Table S3. Amplification reaction mixtures (20 μL) contained the following: 1× TaqMan Master Mix, 200 nM primers, 25 nM probe, and DNA sample (0.2–2 ng/μL). Five microliters of DNA template was added to the reaction mixture. The PCR program used was as follows: initial denaturation at 95 °C for 10 min; subsequent 45 cycles of denaturation at 95 °C for 10 s; annealing at 59 °C [*V. cholerae* (Vc) gbpA primers], 60 °C [*V. vulnificus* (vvha) primers], or 59 °C [*V. parahaemolyticus* (vptlh) primers] for 20 s; and elongation at 72 °C for 1 s, followed by a cooling step at 40 °C for 30 s.

For each single real-time PCR assay, each DNA template was analyzed in triplicate (coefficient of variation < 5%). Amplicons were visualized by agarose gel electrophoresis using ethidium bromide solution and sequenced using an automated ABI Prism 3730 DNA sequencer (Applied Biosystems).

Climate Time Series. Climate time series data were collected from different sources. All data used covered the period 1958–2011.

SST. The SST monthly data for each study location were taken from the Hadley Centre dataset (www.metoffice.gov.uk/hadobs). The annual means were calculated by averaging all months. Obtained time series were centered around the mean and scaled to an SD of 1. The standardized time series were calculated as follows:

$$z_i = \frac{(x_i - \mu)}{\sigma},$$

where μ is the mean of the time series and σ is the SD.

Northern Hemisphere mean land–ocean temperature index. Data on the annual surface temperature change for the Northern Hemisphere mean land–ocean temperature index (NHT index) were obtained from the National Aeronautics and Space Administration Goddard Institute for Space Studies (www.giss.nasa.gov).

AMO index. The AMO has been identified as a coherent mode of natural variability occurring in the North Atlantic Ocean, based upon the average anomalies of SSTs (34). The AMO index time series data were obtained from the National Oceanic and Atmospheric Administration (NOAA) Earth System Research Laboratory (www.esrl.noaa.gov/psd/data/timeseries/AMO/). The time series are calculated from the Kaplan SST dataset, which is updated monthly. Yearly means of the AMO index were used in this study.

NAO index. The NAO is a large-scale alternation of atmospheric mass with centers near the IL and AH (35). An annual principal component-based index of the NAO for the period 1958–2011 was obtained from the Climate Analysis Section of the US National Center for Atmospheric Research (https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-pc-based).

EAP. The EAP is structurally similar to the NAO and consists of a north/south dipole of anomaly centers (31). Monthly data for the East Atlantic teleconnection index were obtained from the NOAA at www.cpc.ncep.noaa.gov/data/teledoc/ea.shtml. Yearly means of the EAP index were used in this study.

GSNW index. The GSNW index data, which are a measure of the latitudinal position of the north wall of the Gulf Stream where it breaks away from the east coast of North America (38), were obtained from Plymouth Marine laboratory (www.pml.ac.uk) for each month and year from 1966 to the present. Yearly GSNW index values were used in this study.

Plankton Studies. Plankton data from 1958 to 2011 were obtained from the CPR database (65) of the Sir Alister Hardy Foundation for Ocean Science. Estimates of plankton abundance from CPR samples are considered semi-quantitative, although reflecting real changes in abundance [a detailed description of how CPR data are generated is provided by Richardson et al. (76)]. Yearly means for the PCI (an index of total phytoplankton biomass and a proxy of water trophic status) and the total number of copepods per CPR sample (TotCop) were averaged for 1958–1969, 1970–1979, 1980–1989, 1990–1999, and 2000–2011 across each of the nine geographic areas to provide background environmental data over the entire time interval (Table 1). However, in consideration of their restricted geographic extension, only two locations (northern and southern North Sea) had a sufficient number of CPR samples collected over the study period to allow plankton time series analysis to be performed. In these two areas, multiscale temporal eigenfunction analysis, namely, distance-based Moran's eigenvector maps, were applied to PCI and TotCop data (77) (*SI Materials and Methods* and Fig. S2). This method, based on the computation of the principal coordinates of a matrix of temporal neighbors among the samples, was used to identify temporal patterns across the entire range of scales present in the dataset (78, 79). By identifying and quantifying temporal patterns at which both PCI and TotCop abundance were structured, the analysis allowed us to recreate time series of abundance that corresponded to large temporal scales (i.e., superior to a year), which are believed to be mostly driven by multidecadal climate changes over the study period (1958–2011).

To investigate the long-term change in plankton community structure in the same locations, geostatistical and temporal interpolation techniques (i.e., inverse square distance method and ratio estimator method) were also used to interpolate CPR plankton abundance data on a regular grid in the North Sea between 51°N and 62°N and between 4°W and 8°E using MATLAB software (Release 2008a) (68) (*SI Materials and Methods*). Month-by-year contour plots of plankton abundance for the period 1958–2011 were produced for the northern and southern North Sea for the 10 most abundant

zooplankton and phytoplankton taxa collected by the CPR survey according to Johns and Reid (80). The data were log-transformed before they were analyzed.

Epidemiological Data. Epidemiological data on *Vibrio* cases in Northern Europe were collected from peer-reviewed publications obtained from multidisciplinary search engines (e.g., Web of Science, Scopus, National Center for Biotechnology Information/PubMed, Google Scholar) (Table S4). "Gray literature" (e.g., surveillance data from national communicable disease agencies, medical profession newsletters, bulletins from European Union laboratories, European surveillance websites) were also examined. Information regarding the responsible microbiological agent, timing, and geographical location were collated (Table S4). *Vibrio* infections imported by travelers from known sources abroad, if known, were omitted from the dataset.

Vibrio case data (excluding toxigenic *V. cholerae*) for the US Atlantic coast were collected from the CDC Cholera and Other *Vibrio* Illness Surveillance system (www.cdc.gov/nationalsurveillance/cholera-vibrio-surveillance.html). According to the CDC "Atlantic region," geographical locations reporting cases included in the analysis were South Carolina; North Carolina; Virginia; Washington, DC; Maryland; Delaware; New Jersey; New York; Connecticut, Rhode island, Massachusetts, New Hampshire; and Maine.

Statistical Analysis. A GAM (81, 82) with Gaussian error distribution and identity link function was used to study multidecadal relationships between *Vibrio* prokaryote abundance and the main climatic variables in the North Atlantic region. GAMs were preferred over generalized linear models because they can estimate nonlinear relationships between the target variable and the explanatory variables [a detailed explanation of the GAM applied to the *Vibrio* data is provided by Martinez-Urtaza et al. (83)]. The thin-plate regression spline was used as a smooth function (82), with optimized degree of freedom selected by restricted (or residual) maximum likelihood (84). Assumptions of normal distribution and constant variance of residuals were checked for overall models using the gam.check function. Autocorrelation in the data was also determined by analysis of the regression residuals and was found not to be significant. Computation was carried out using the mgcv package of programming language R (R Development Core Team, 2012).

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