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# Influence of hydrologic and anthropogenic factors on the abundance variability of enteropathogens in the Ganges estuary, a cholera endemic region

Prasenjit Batabyal [a], Marc H. Einsporn [b,*], Subham Mookerjee [a], Anup Palit [a], Sucharit B. Neogi [c,d], Gopinath B. Nair [a,f], Rubén J. Lara [b,e]

[a] National Institute of Cholera Enteric Diseases (ICMR), 700010 Kolkata, India
[b] Leibniz Center for Marine Tropical Ecology (ZMT), 28359 Bremen, Germany
[c] International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Mohakhali, Dhaka 1212, Bangladesh
[d] Graduate School of Life & Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan
[e] Instituto Argentino de Oceanografía, 8000 Bahía Blanca, Argentina
[f] Translational Health Science and Technology Institute, Udyog Vihar, Gurgaon-122016, Haryana, India

## HIGHLIGHTS

- Aquatic enteropathogen dynamics with respect to variations between winter and monsoon
- Synchronous water and sediment sampling from two sites of the River Hooghly
- Tidal and seasonal variation of physico-chemistry correlated to bacterial abundance
- Indication of bentho-pelagic coupling of Vibrio dynamics

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## ABSTRACT

This study deals with the influence of water physico-chemical properties, tides, rainfall and fecal pollution on the abundance of enteropathogens in a main distributary of the Ganges, in the endemic cholera belt of West Bengal. Between January and June 2011, water and sediments were sampled from two sites of the Hooghly River by Kolkata and Diamond Harbour. Counts of cultivable *Vibrio* (CVC, from ~$10^2$ to ~$10^5$ CFU/L) and total bacteria (TBC, from ~$10^5$ to ~$10^9$ CFU/L) increased with water temperature (17 °C to 37 °C). A combination of variations in tidal height, salinity and turbidity had a distinct influence on CVC, TBC and coliform counts. At Diamond Harbour, a salinity increase from 0.6 to 7.9 was accompanied by a 1000-fold amplification of initial CVC ~ $10^2$ CFU/L, whereas higher prevalence of coliforms in Kolkata was related to greater disposal of untreated sewage into the river. Turbidity-dependent variation of CVC was noteworthy, particularly at Diamond Harbour, where CVC in intertidal surface sediments showed an analogous trend as in surface waters, suggesting bentho-pelagic coupling of *Vibrio* dynamics. Besides the influence of salinity variation with tidal cycles, sediment re-suspension from tidal flats can play a role on *Vibrio* abundance in aquatic ecosystems.



## 1. Introduction

Diarrheal diseases associated with floods and droughts are common in East, South and Southeast Asia and are expected to increase due to changes in the hydrological cycle (Lipp et al., 2002; IPCC, 2007). Yet, our knowledge on the influence of environmental factors on the regulation of different pathogenic enteric bacteria in estuarine environments is still limited (Lara et al., 2011). Particularly for low-lying coasts in tropical regions, there is a need for comprehensive knowledge on the links between hydrology and the ecology of disease causing agents (Wolanski et al., 2004).

*Vibrio cholerae* O1 and O139 are the causative agent of cholera, whereas other vibrios (e.g., *V. parahaemolyticus, V. vulnificus, V. mimicus, etc.*) are responsible for diarrhea, gastroenteritis, necrotizing fasciitis, various further skin diseases and septicemia affecting humans worldwide (Thompson et al., 2004). Cholera is an important cause of morbidity and mortality in many developing countries in Asia, Africa and Latin America due to lack of safe water supply and poor hygienic practices (Colwell, 1996), together with the resulting massive use of untreated,

* Corresponding author at: Leibniz Center for Marine Tropical Ecology (ZMT), Fahrenheitstrasse 6, D - 28359 Bremen, Germany. Tel.: +49 421 238 00 46; fax: +49 421 238 00 30.
*E-mail address:* marc.einsporn@zmt-bremen.de (M.H. Einsporn).

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often highly polluted surface waters for human consumption. Even in the present decade, hundred thousands of people suffer from cholera every year, e.g., during 2010, there were approx. 318,000 reported cases of cholera, with 7543 deaths, though the actual death toll could have been as high as 120,000 (WHO, 2012).

Most cholera pandemics started in the Ganges–Brahmaputra delta, considered as the homeland for cholera since ancient times (Drasar and Forrest, 1996). *Vibrio cholerae* is an endemic inhabitant of tropical estuaries, and cholera epidemics can be related to changes in the physico-chemical properties of water, its main transmission vehicle (Singleton et al., 1982). Further, the input of untreated sewage from cities into the riverine environment increases the risk of pathogen transmission between humans using those waters for bathing, cooking or drinking. But *Vibrio cholerae* can be further concentrated in the aquatic environment. For example, zooplankton, fish or shrimps can host the bacteria and represent an additional threat to human health consuming these organisms (Colwell, 1996). Although a few studies were conducted to identify the favorable physico-chemical conditions for *Vibrio* survivability and growth (Chatterjee and Gupta, 1959, Miller et al., 1982, Dunlap, 2009), the microbial dynamics of this large aquatic ecosystem, characterized by high loads of organic matter and suspended sediments, is poorly understood.

The waters of the Ganges are used by millions of people for an enormous variety of personal, societal and industrial purposes; especially near the megacity of Kolkata, a cholera endemic region. The present study deals with links between variations in hydrologic conditions, water biogeochemistry and *Vibrio* abundance in the Hooghly River, a main distributary of the Ganges. Furthermore, under consideration of microbiological indicators of inputs from Kolkata, this approach aims to discriminate between hydrologic and anthropogenic drivers of change of enteric pathogen abundance, as a contribution to the understanding of cholera dynamics in West Bengal.

## 2. Methods

### 2.1. Study sites

The Hooghly River originates at the junction of the rivers Bhargirathi, Jalangi and Mathabhanga. Since simultaneous samplings at several locations along the estuarine gradient would have involved an unaffordable logistic effort, two stations with contrasting environmental settings, Howrah Bridge and Diamond Harbour, were chosen and sampled during winter and summer, from January to June 2011 (Fig. 1).

The Howrah Bridge site (HB) is within an urban setting connecting the City of Kolkata and the township of Howrah (approx. 15 million inhabitants). Although 130 km away from the sea mouth of the Hooghly River, this site has an estuarine condition due to a significant tidal oscillation of ~3 m. Upstream of HB, the river flows through the densely populated region in south-east West Bengal, as well as through many areas of agricultural and aquaculture use. The river water is accessed for drinking, washing, bathing, cleaning utensils and for many religious rituals. Mostly untreated sewage from both sides of the river is disposed off into its waters.

Diamond Harbour (DH) hosts an important commercial port. It is surrounded by a rural hinterland and located about 50 km downstream of HB. Being closer to the estuary mouth, the river is ca. five times wider than in Kolkata and has a higher turbulence. Also the tidal influence is larger, with higher seawater intrusion during the flood, while large mud flats get exposed at low tide. The human impact on the estuarine environment is less at this site due to lower population density, strong water currents and exchange with the sea.

### 2.2. Sampling of surface waters and sediments

Sampling was carried out every three weeks, in order to embrace the effect of hydrological changes due to the different tidal range in alternating spring and neap tides, as well as during increasing and decreasing moon phases. Synchronized sampling was undertaken at both sites. At each sampling day, 5 to 7 samples were collected at regular intervals (every 2 h) covering a 12 – 14 h period of a tidal cycle including low and high tides. During each sample collection, three to four subsamples of surface water from the midstream were taken with a metal bucket and pooled together in a sterile 10 L container. Subsequently, the samples were shaken and divided into 1 L aliquots. Additionally, surface sediment samples were taken from a tidal flat at Diamond Harbour. At low tide, 10 sub-samples of 3 $cm^3$ of mud were taken with syringes from a surface of 1 $m^2$ and pooled together in a sterile glass flask. After collection, water and mud samples were put into ice-chilled, lightproof boxes and transported to the laboratory for further processing and analysis within 12 h of collection.

### 2.3. Physico-chemical and hydrological parameters

Immediately after sample collection, temperature, pH, conductivity and salinity were measured with a Multi-meter (pH/Cond 340i WTW, Weilheim, Germany). Turbidity was measured by a portable turbidimeter TD-100 (Eurotech, Singapore) and expressed as Nephelometric

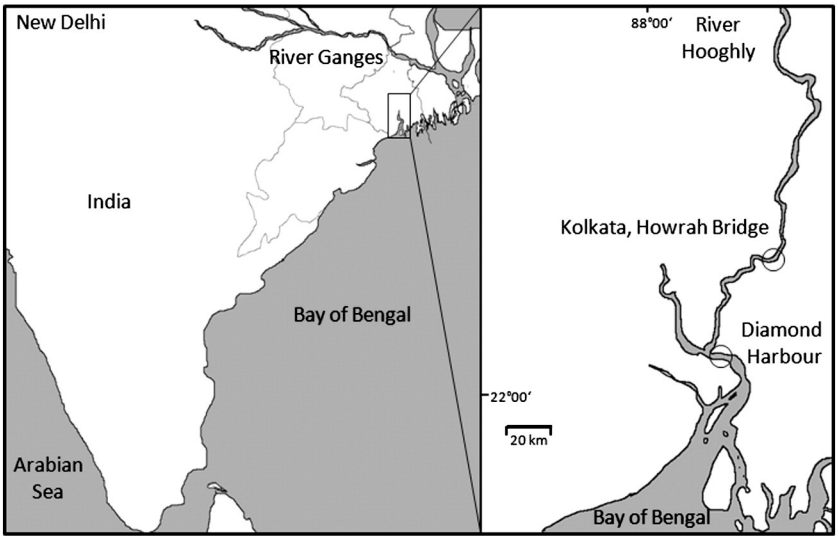


**Fig. 1.** Map of sampling sites in the estuary of the Hooghly River. Both sites, Howrah Bridge (HB, 22° 35′ 6″ N, 88° 20′ 49″ E) and Diamond Harbour (DH, 22° 11′ 37″ N, 88° 10′ 48″ E).

Turbidity Units (NTU). Salinity is expressed as Practical Salinity Units (PSU), which for practical purposes is almost identical to g/L or parts per thousand. Tidal heights were taken from official websites (www.mobilegeographics.com). Rainfall data were extracted from the web-based meteorological database of the Utah State University, USA (http://climate.usurf.usu.edu/mapGUI/mapGUI.php) and precipitation heights were accumulated from prior 21 days, including the sampling day.

### 2.4. Bacteriological analysis

The samples were analyzed for Total Bacterial Count (TBC), Total Coliform Count (TCC), Total *Escherichia coli* Count (TEC), Total Fecal Coliform Count (TFCC) and Cultivable *Vibrio* Count (CVC) following standard procedures (APHA/AWWA/WEF, 2005; Lara et al., 2009, 2011). Briefly, 0.1 mL of each sample (diluted or concentrated) was spread plated on Nutrient Agar (Becton, Dickinson, USA) for TBC; Chromocult Coliform Agar (Merck, Darmstadt, Germany) for TCC and TEC; MFC agar (Hi-Media, Mumbai, India) for TFCC, and Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS; Becton, Dickinson, USA) for CVC determination, respectively. This was followed by an overnight incubation at 37 °C for all determinations, except at 44 °C for fecal coliform count. All microbiological enumerations were done in triplicate. Suspected *Vibrio*, coliform and *E. coli* isolates grown on selective media were verified by biochemical tests following standard procedures, e.g. oxidase, gelatinase, motility, reactivity to Kligler's Iron Agar slant, carbohydrate fermentation assay (arabinose, glucose, mannose, sucrose, mannitol, inositol, citrate, etc.), decarboxylase reaction in amino acids (arginine, lysine, ornithine), salt tolerance tests (0%, 6.5%, 8%) etc. (APHA, 2005; West and Colwell, 1984). Some of the biochemically identified strains belonging to pathogenic species, e.g., *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, etc. were verified by PCR based molecular methods following previously established protocols (Hoshino et al., 1998; Neogi et al., 2010).

### 2.5. Data processing and statistical analysis

Statistical analyses and graphics were carried out using 'Xact' (version 7.21d, SciLab GmbH, Hamburg, Germany) and Sigma Plot (version 12.0, SYSTAT Software Inc, San Jose, USA). Single or median values were used to describe the temporal sequences. In box plots, the bottom and top of the box represent the 25th and 75th percentile, respectively. Horizontal lines in the box indicate the median values, while vertical bars show the standard deviations.

## 3. Results

In the periods from January to June 2011, 6 sampling campaigns were carried out. A total of 67 surface water samples were collected from the two stations (DH and HB) in Hooghly River, and six composite surface sediment samples at DH.

### 3.1. Hydrological and physico-chemical parameters

The sampling period started off with a dry phase in winter, was followed by pre-monsoon rain in March and ended up in early south-west monsoon with 120 mm of accumulated precipitation (Fig. 2A). Surface water temperature gradually increased from 17 °C to 37 °C (Fig. 2B and C). The rise in temperature was similar at both sites.

At DH, the average tidal height varied between 4.55 ± 0.75 m at high tide and 0.55 ± 0.65 m at low tide; salinity varied from 0.3 to 7.5 PSU, was mostly >3 PSU, peaking during April (Fig. 2D); and pH ranged from 7.60 to 8.20. At this site water turbidity ranged from 40 to 920 NTU (Fig. 2F). At HB, average tidal range was 4.16 ± 0.9 m at high tide to 1.27 ± 0.65 m at low tide. Although salinity values were too low to be expressed in PSU, water electrical conductivity varied between 320 and 610 μS/cm (Fig. 2E), with consistently higher values at high tide and lower ones at low tide. The pH values ranged from 7.70 to 8.60 and turbidity varied between 50 and 250 NTU (Fig. 2G). At both sites, the variation patterns of turbidity were similar, with maxima during April (218 NTU at HB and 661 NTU at DH, Fig. 2F and G).

### 3.2. Bacterial counts

From January to May, TBC varied from ~$10^5$ to ~$10^7$ CFU/L at DH and ~$10^6$ to ~$10^7$ CFU/L at HB; yet, a drastic increase to ~$10^9$ CFU/L was observed in June (Fig. 3A and B). Despite similar TBC ranges, generally higher CVC values were observed at DH (~$10^3$ – $10^5$ CFU/L) in comparison to HB (~$10^2$ – $10^4$ CFU/L; Fig. 3C and D). In contrast, HB was characterized by higher prevalence of total coliforms including *E. coli* (~$10^5$ – $10^6$ CFU/L of both TCC and TEC) in comparison to DH (~$10^3$ – $10^5$ CFU/L of TCC and ~1–$10^4$ CFU/L of TEC; Fig. 3E, F, I and J). Similarly, there was a higher prevalence of fecal coliforms at HB, with TFCC ranging from 65,000 to 750,000 CFU/L, substantially more than the values observed at DH (1 – 78,000 CFU/L, Fig. 3G and H). Another difference between these sites is that at DH, the TCC, TEC and TFCC values increased with water temperature up to April, while at HB there was a relatively constant abundance of coliforms and fecal coliforms throughout the sampling period irrespective of temperature variations (Fig. 3E to J).

Analysis of benthic samples from DH (Fig. 4) revealed a trend of gradually increasing TBC [$4 \times 10^5$ to $2.9 \times 10^6$ CFU/g (dry weight)] and CVC [1400 to 4500 CFU/g (dry weight)] along with water temperature increase except for the month of May, when a fall of both TBC and CVC occurred. Remarkably, there was also an abrupt drop of bacterial abundance in water at DH in May, with simultaneously a much lower yet perceptible decrease also at HB (Fig. 3C and D).

A tidal oscillation was also observed at both study sites. At DH, located near the river mouth, influence of tide on intraday variation of CVC was evident, whereas at HB, ca. 130 km away from the river mouth, this effect was damped (Fig. 5). The influence of tide on vibrios is discernible throughout the whole sampling period, even superimposed on the effect of increasing temperature.

The tidal influence was particularly noticeable in the DH data from March at new moon (Fig. 6). During flood tide, salinity increased from 0.70 to 2.0 PSU at high tide (Fig. 6A), which was accompanied by rising TBC ($1.2 \times 10^6$ to $7.5 \times 10^6$ CFU/L) and CVC ($5 \times 10^2$ to $6 \times 10^3$ CFU/L) (Fig. 6B). However, a positive influence of high tide was not evident on TCC, which rather increased during ebb tide from a minimum of $1 \times 10^4$ CFU/L at high tide to $2 \times 10^5$ CFU/L at low tide (Fig. 6C).

## 4. Discussion

### 4.1. Variations in physico-chemical parameters

At DH salinities were higher (0.3 – 7.5) than at HB (ca. 0.0) due to the proximity to the sea mouth. Yet, tide-driven conductivity variations could be detected at both stations. Higher turbidities at DH (40 to 920 NTU) than at HB (50 – 250 NTU) are likely due to sediment resuspension from the wide mud flats along the riverbanks due to stronger tidal influence. Despite different ranges, turbidity at both sites, DH and HB, peaked in April, with a strong decrease in May with the beginning of the monsoon (Fig. 1F and G). Salinities also reflect the increase in rainfall along with the melting of ice in the Himalayan glaciers due to rising temperature from the end of March. Taking into account the observed trends in salinity, a simple multiple regression model was built, which supported the influence of tidal range, temperature, precipitation and evapotranspiration on conductivity with highly significant correlations for both stations, despite very low values in HB. Since salinity is determined via conductivity measurement, for consistency we used conductivity in both stations.

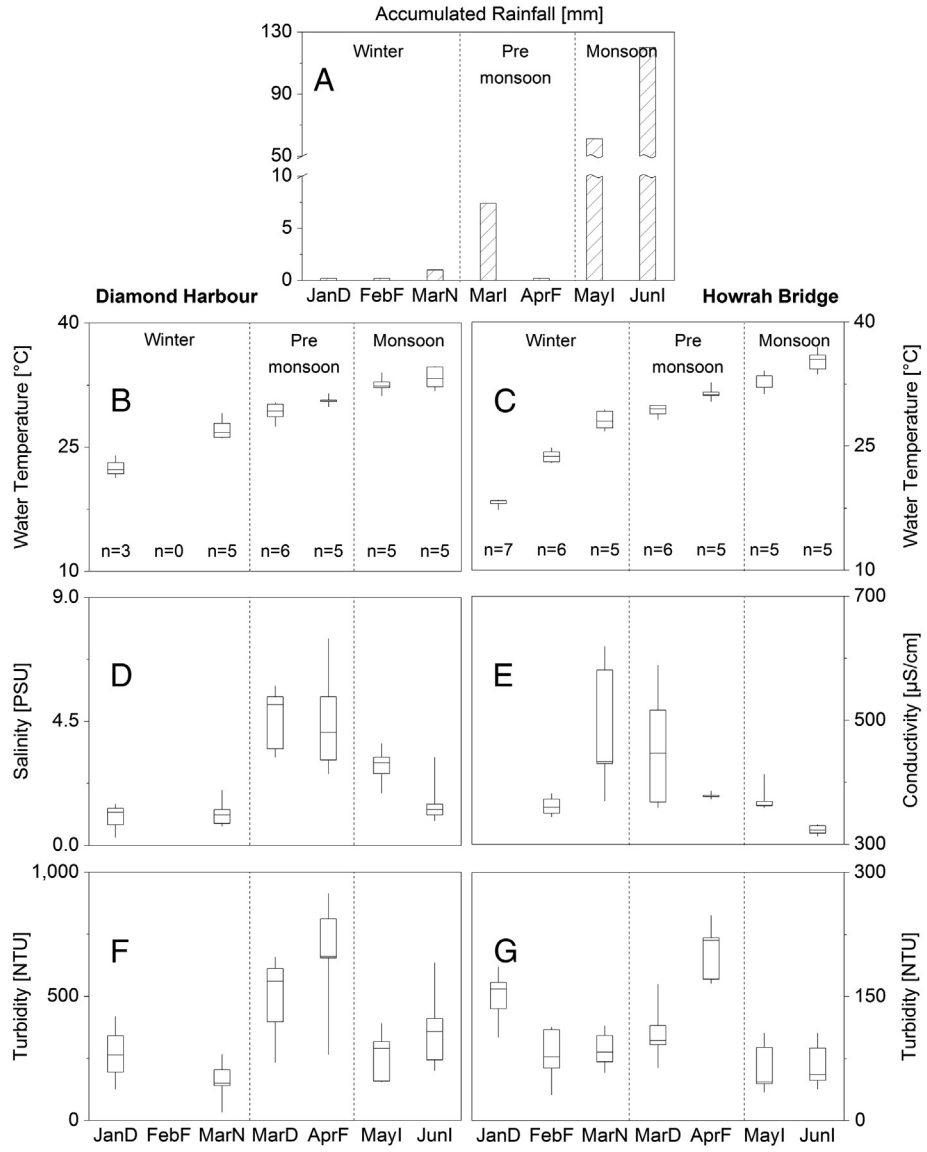


**Fig. 2.** Temporal variation of precipitation and water physico-chemical parameters at Diamond Harbour and Howrah Bridge. The month abbreviation includes a code for the moon phase. I: increasing, F: full, D: decreasing, N: new.

*4.1.1. Diamond Harbour (DH)*

$$\begin{aligned}\text{Conductivity} = {} & 6530.785 - (0.226 \times \text{Precipitation}) \\ & + (213.263 \times \text{Accumulated evapotranspiration}) \\ & - (118.226 \times \text{Tidal range}) - (380.413 \times \text{Temperature}) \\ R = 0.81, P = {} & <0.001, N = 29\end{aligned}$$

*4.1.2. Howrah Bridge (HB)*

$$\begin{aligned}\text{Conductivity} = {} & 211.789 - (2.009 \times \text{Precipitation}) \\ & - (3.339 \times \text{Accumulated evapotranspiration}) \\ & - (51.838 \times \text{Tidal range}) - (18.565 \times \textit{Temperature}) \\ R = 0.65, P = {} & 0.005, N = 32\end{aligned}$$

*4.2. Site specific variations in bacteriological parameters*

The application of *t*-test shows statistically significant differences between CVC ($t = 2.34$, $P < 0.05$, DF = 66) and TFCC ($t = 9.050$, $P < 0.001$, DF = 66) at both sites. There was a higher predominance of fecal coliforms at HB in Kolkata than at DH; oppositely, a higher abundance of CVC was generally recorded near the river mouth. This was likely due to the impact of both higher anthropogenic input and lower salinity at HB and *vice versa* at DH. Even though there is an extreme disparity in population density between Kolkata (24,252 people per km$^2$) and Diamond Harbour (South 24 Parganas, 819 people per km$^2$) a significant Pearson correlation of their CVC ($R = 0.694$, $P = 0.008$, $N = 29$) indicates a data co-variation at both sites. The influence of different population densities and land-use forms on CVC at each site cannot be tested with the present data. Yet, regarding *Vibrio* distribution trends in the estuarine environment, it can be hypothesized that salinity acts as site-specific driver with different weighting compared to anthropogenic input at each location.

The impact of salinity gradients on the *Vibrio* distribution in anthropogenically polluted estuaries has been also recorded in other coastal regions of Bay of Bengal (Lara et al., 2009, 2011; Neogi et al., 2012). Additionally, the overall trend of CVC at DH is visibly different from that at HB (Fig. 3C and D). *Vibrio cholerae* was frequently isolated from both study sites. However, while this pathogenic species dominated the HB samples, other halophilic vibrios such as *V. parahaemolyticus,*

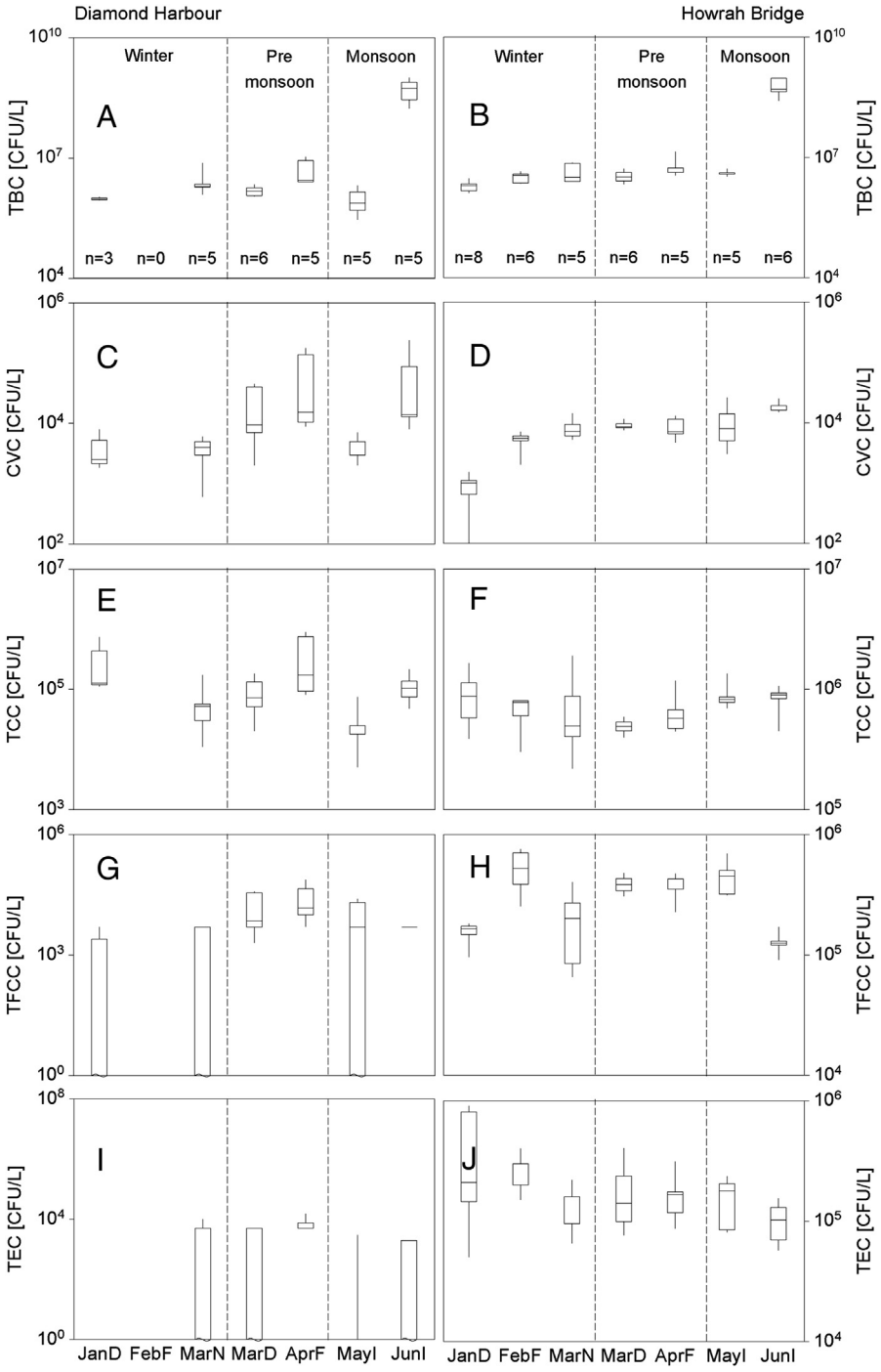


**Fig. 3.** Temporal variation of cultivable bacterial abundance in colony-forming units per litre (CFU/L) at both study sites. TBC, total bacterial count; CVC, cultivable *Vibrio* count, TCC, total coliform count, TEC, total *E. coli* count, TFCC, total fecal coliform count. The month abbreviation includes a code for the moon phase. I: increasing, F: full. D: decreasing, N: new.

*V. vulnificus*, etc. were more prevalent in DH samples (data not shown). Thus, the CVC load in HB samples most likely comprised a mixture of indigenous *Vibrio* populations, and a significant component from local anthropogenic input and sewage outlets opening into the Hooghly River from nearby metropolitan settings. Irrespective of the study month, the relatively constant higher abundance of total fecal coliforms at HB (Fig. 3G and H) is attributable to its proximity to Kolkata metropolitan area and high anthropogenic inputs. The scenario at DH is different, showing only a relatively higher TFCC abundance in the pre-monsoon time (Fig. 3G and H).

### 4.3. Effect of physico-chemical variations on bacterial abundance

With a gradual increase in water temperature (17 to 37 °C) there was an overall increasing trend of TBC and CVC in the summer months (Figs. 2B and C, 3A to D). However, there was only a weak correlation between temperature and TBC ($R = 0.43$, $P = 0.019$, $N = 29$ for DH and $R = 0.48$, $P = 0.002$, $N = 32$ for HB). Contrasting with this, CVC and temperature correlated highly significant at HB ($R = 0.733$, $P = <0.001$, $N = 32$) which is supported by earlier findings reporting an increase of CVC with temperature in natural waters (e.g., Nair et al.,

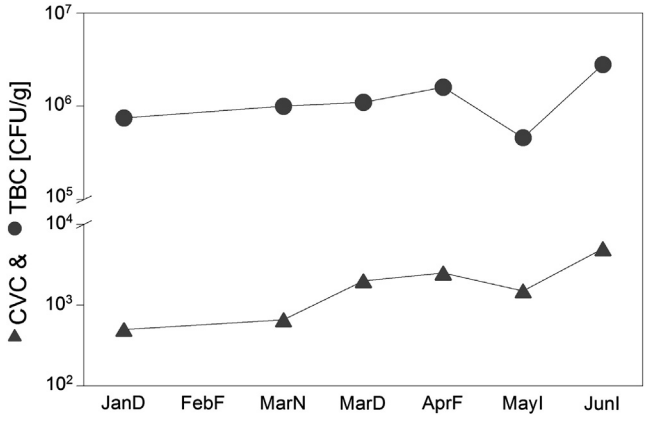


**Fig. 4.** Temporal variation of total bacterial counts (TBC) and cultivable *Vibrio* counts (CVC) in benthic samples at Diamond Harbour. The month abbreviation includes a code for the moon phase. I: increasing, F: full, D: decreasing, N: new.

1988; Das et al., 2009; Neogi et al., 2011). However, in our case within the same estuary, but at higher salinities, CVC and temperature show a much lower correlation ($R = 0.332$, $P = 0.078$, $N = 29$) at DH, which stresses the importance of further environmental parameters. At DH, salinity changes from 0.3 to 7.5 PSU likely favored the increasing trend of CVC (Fig. 3C). Even at the extremely low salinities found at HB, a conductivity gradient of 350–620 μS/cm seems to favor higher CVC ($1 \times 10^2 - 2 \times 10^4$ CFU/L), although this only happened from January to March (Fig. 3D) and could be a concomitant variation with rapidly increasing water temperatures, thus not necessarily indicating causality.

Yet, this observation could support the hypothesis of Shikuma and Yildiz (2009) on the capacity of *V. cholerae* to adapt to a low saline environment through osmoregulation.

In the investigated period, TBC and CVC derived from mud samples at DH showed a significant correlation ($R = 0.92$, $P = 0.01$, $N = 6$). Furthermore, CVC in water (Fig. 3C) and sediment (Fig. 4) significantly correlated ($R = 0.86$, $P < 0.05$ $N = 6$), and was reflected in a similar variation pattern, which was in turn analogous to those of salinity and turbidity. At this site, the correlation between CVC, turbidity and salinity is statistically highly significant ($R = 0.57$, $P = 0.006$, $N = 29$).

This setting was especially noteworthy in April/May, when a sharp decrease in turbidity occurred, in a setting of declining salinities (Fig. 2D and F). Then, the sudden fall of all investigated bacterial populations in water at DH, was accompanied by decreasing counts of CVC and TBC in sediment and occurred simultaneously with a low tidal range (2.78 m) and an increase in rainfall. It is likely that lower turbidity and bacterial counts are associated with low tidal range and a concomitant decreased material transport from intertidal mud flat habitats. Onset of heavier rainfall likely produced a decrease in salinity, however, a higher runoff and associated increase in turbidity and bacterial load would be expected under such conditions. Oppositely, lower turbidity and bacterial counts were registered.

The involved mechanisms and the simultaneous decrease of CVC in both water and sediment samples deserve further investigation to understand changes in abundance and composition of *Vibrio* and other bacterial populations by the hydrologically-driven variations in the amount of suspended particulate matter. Additionally, comprehensive information on the barrage management upstream of the Hooghly River would likely improve the understanding of the influence of changes in the hydraulic regime on *Vibrio* dynamics in this estuarine environment. There are also uncertainties on the links between precipitation regime and *Vibrio* dynamics: the weekly number of cholera cases in the period 1996 – 2002 in Dhaka, Bangladesh was only partly triggered by changes in rainfall, suggesting that river level and associated regular mud plain inundation – without reaching catastrophic flooding – may also play a role on *Vibrio* ecology (Hashizume et al., 2008).

Even though Constantin de Magny et al. (2012) found statistically significant correlations between sea surface temperature, rainfall, respective floods and the increase of cholera cases in Senegal in August 2005, these parameters do not stringently explain the disease outbreaks. Lobitz et al. (2000) found similar patterns in changes of sea surface temperature, sea surface height and cholera incidence. By the application of an indirect approach using remote sensing, they stated that cholera cannot be explained either by SST or SSH data but needs to be supplemented by, at least, salinity and phytoplankton data. Constantin de Magny et al. (2008) describe SST and rainfall as trigger for the growth of zooplankton and its intrusion to inland waters. They hypothesize that high plankton abundance leads to more contact between humans and hosts of V. cholerae and therewith to an increased infection rate. However, Bouma and Pascual (2001) described clear differences between the winter and summer peak of cholera, and determined the correlation to the sea surface temperature of the Bay of Bengal less consistent in winter and with increasing distance from the river mouth. Furthermore, they support the theory of different reservoirs for Vibrio in either season, making the prediction, even by consideration of each known influencing variable, complex.

### 4.4. Effect of lunar phase and tidal cycles on bacterial abundance

Daily tidal oscillation can produce strong variations of bacterial abundance (Fig. 5). CVC often reached maxima close to or at high tide, this being likely related to higher salinities and/or turbidities. The pattern of variation is not always the same, but this may be a hysteresis-like effect, depending on how a given tidal height is reached, from the flood or the ebb tide, i.e. the dependence of a system not only on its current status but also on its past environment. For logistics reason it was not possible to perform sampling always during the same tidal sequence, yet a clear example of a tide-dependent variation in bacterial counts accompanied by maxima in CVC at high tide and TCC at low tide was recorded at DH (Fig. 6).

Tidal height variations due to monthly lunar phases were found to influence physico-chemical and bacterial dynamics in the Hooghly estuary. A higher abundance of fecal coliforms in water (up to 78,000 CFU/L),

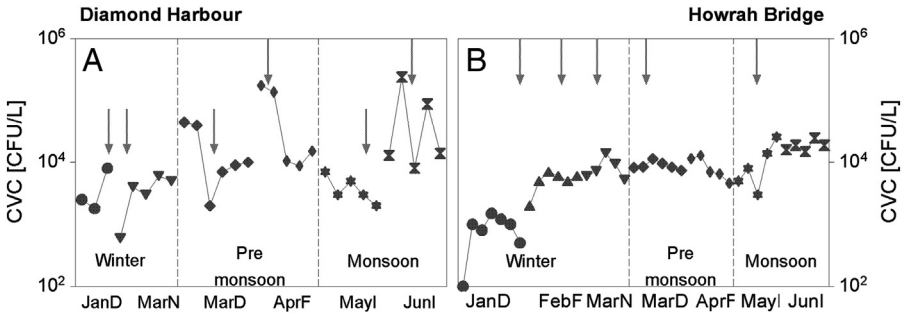


**Fig. 5.** Influence of tides on the abundance of cultivable *Vibrio* counts (CVC) in water in different months of the year at both study sites. During each sampling event, water samples were collected at every 2 h intervals. The arrows indicate the high tides. The month abbreviation includes a code for the moon phase. I: increasing, F: full. D: decreasing, N: new.

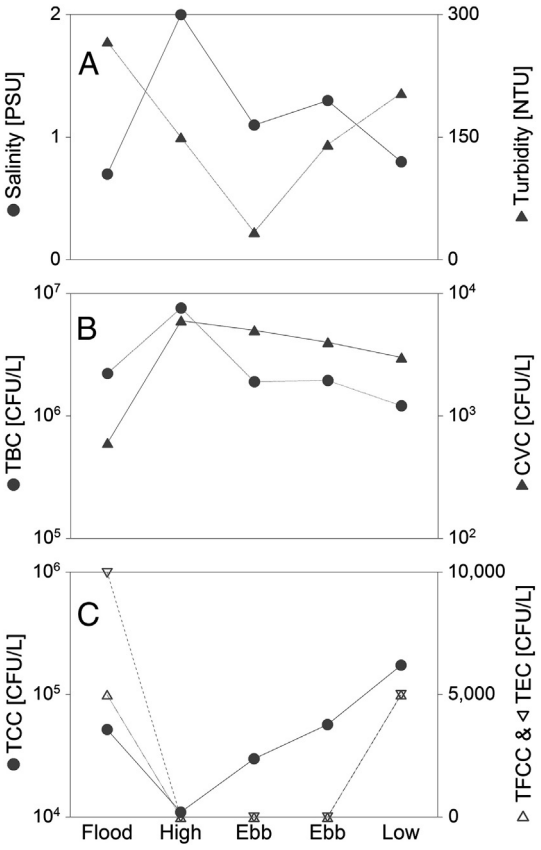


**Fig. 6.** Temporal variation of physico-chemical parameters and comparative abundance of bacterial communities during different tidal phases at Diamond Harbour site in March new moon sampling event. TBC, total bacterial count; CVC, cultivable *Vibrio* count, TCC, total coliform count, TEC, total *E. coli* count, TFCC, total fecal coliform count.

occurred at DH during a spring tide in April, although this less anthropogenically influenced river mouth site had relatively low fecal counts in other occasions. This rise is likely due to transport of more fecal coliforms from the river banks into the mainstream water course due to higher inundation during spring tide. In the May sampling, tidal height was 1.3 m lower than in April and concomitantly, coliforms and all other bacterial counts also decreased strongly. Higher intrusion of marine water at full or new moon (spring tides) induces increases in salinity, sediment resuspension and turbidity in water column and likely favours a preponderance of halophilic bacterial load and *Vibrio* counts, including pathogenic *V. cholerae* species. However, the same variation of tidal height may have a different impact on the pelagic than on the benthic bacterial community, depending on the climatic setting, degree of exposition and desiccation of tidal flats. Yet, despite uncertainties about the involved mechanisms, the local tidal regime through its interaction with bottom sediments seems to be a driving force of bacterial variability to be taken into account for the understanding of *Vibrio* and cholera dynamics.

In a previous work (Lara et al., 2009), attention was drawn on the possibility that sediment resuspension (e.g. by landslide or cyclone events) could either represent an additional source of organic-rich substrate for increased bacterial growth or involve a direct input of particle-attached *Vibrio* into the water column. In concordance, the present study also revealed a similarity of variation in CVC in water column and in surface sediments. This can have an epidemiological relevance, as supported by Williams and LaRock (1985), who found that there was a predominance of non-O1 *Vibrio cholerae* infections in Florida at the time the organisms flourished in the sediment.

This and related evidence (Lara et al., 2011; Neogi et al., 2012) point to an essential question: are *Vibrios* in sediment, part of a benthic community with own characteristics or do they basically consist of a fraction of a pelagic population reaching the sediment after sedimentation of the particles to which they are attached, or *vice versa*? This does not disregard the influence of plankton on *Vibrio* dynamics (Constantin de Magny et al., 2011), yet, the bentho-pelagic coupling effects do not seem to be significantly considered in the mainstream of ecological *Vibrio* research.

## 5. Conclusion

In both study sites there was a trend of increasing cultivable bacterial populations with temperature. There was a higher predominance of coliforms at Howrah Bridge and of cultivable *Vibrio* at Diamond Harbour, respectively, as a result of higher anthropogenic input in Kolkata and increased salinity near the river mouth. This emphasizes the relative importance of salinity as compared to anthropogenic input for *Vibrio* distribution in the estuarine environment. The abundance of *Vibrio* and total bacteria counts also showed a positive dependence on turbidity, which in turn is influenced by tidal regime, turbulence and runoff.

Besides the effect of the changing salinity during a tidal cycle, it is likely that resuspension of sediments from the tidal flats influences *Vibrio* concentration in the water column. In different scales of time, in addition to the influence of climatic variables and tidal amplitude (spring tide, neap tide, etc.), tidal cycles (flood and ebb) have an important effect on intraday variability of bacterial abundance. The large variations in bacterial counts within a tidal cycle and the dependence on tidal amplitude make it advisable to include sampling strategies as the one used in this work for the evaluation of temporal trends of *Vibrio* organisms and cholera incidence in an estuarine environment.

Similarity in the variation patterns of *Vibrio* counts in intertidal sediments and in surface waters points to a possible bentho-pelagic coupling of *Vibrio* dynamics in the Ganges estuarine zone. The confirmation of this coupling as well as the knowledge of its nature requires further investigation. Besides anthropogenic inputs, runoff and sediment-water interactions may play a larger role in the understanding of *Vibrio* dynamics in estuaries, as hitherto assumed by conceptions in which interactions with plankton were dominant. Future studies and efforts to understand and predict cholera outbreak patterns through models relating disease incidence and environmental variables should incorporate these components.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2013.10.093. These data include Google maps of the most important areas described in this article.

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