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Original Contribution

Influence of Catastrophic Climatic Events and Human Waste on *Vibrio* Distribution in the Karnaphuli Estuary, Bangladesh

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Abstract: Vibrios are bacteria of marine and estuarine origin that can cause human diseases, such as cholera, and also affect aquatic organisms. The impact of storm-driven changes in salinity and suspended particulate matter (SPM) on cultivable *Vibrio* counts (CVC) and distribution in Karnaphuli estuary, Bangladesh, was compared before and after a strong cyclone in mid May 2007 and after a monsoon landslide a month later. CVC were higher ($\sim 10^3$ colony forming units—cfu/ml) at estuary's mouth (salinity 20–15 parts per thousand, ppt) and steeply declined landwards. CVC and their proportion of total aerobic bacteria were highest after the cyclone and also increased after the landslide, likely due to higher SPM loads. The cyclone did not significantly change previous fecal coliform abundance, contrasting with the ten times increase after the landslide. Sewage input enhanced CVC near the point sources. CVC and salinity correlated highly significantly at salinities <10 ppt; however, at higher values dispersion increased, probably due to the effect of sediment resuspension on CVC. Cyclone or heavy rainfall-mediated turbidity changes jointly with salinity gradients can significantly influence abundance and distribution of estuarine vibrios. Extended salt intrusion and higher turbidities in tropical estuaries by stronger and more frequent storms and deforestation-derived erosion could favor *Vibrio* growth, with increasing risks for aquatic resources and human health in the coastal zone.

Key words: storms, tropical estuaries, *vibrio*, salinity, turbidity, sediment

Introduction

Endemic morbidity and mortality due to diarrheal disease primarily associated with floods and droughts are expected to increase in East, South, and Southeast Asia due to projected changes in the hydrological cycle (IPCC, 2007; Lipp et al., 2002). Particularly in low-lying coasts in tropical regions there is a need for deeper knowledge about the links between hydrology and the ecology of disease agents (Wolanski et al., 2004). *Vibrios* are aquatic bacteria of marine and estuarine origin. *Vibrio cholerae* is the causative agent of cholera, and some other *Vibrios* (e.g., *V. parahaemolyticus, V. vulnificus, V. mimicus*, etc.) are responsible for diarrhea, gastroenteritis, necrotizing fasciitis, and septicemia incidences afflicting the human population worldwide (Chakraborty et al., 1997). Various *Vibrios* (*V. campbelli, V. harveyi*, *V. alginolyticus*, etc.) also can

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cause diseases in fish, shrimp, corals, and other aquatic organisms (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). Most cholera pandemics started in Bangladesh, in the Ganges–Brahmaputra delta, considered as the homeland for cholera since ancient times (Drasar and Forrest, 1996). Cholera epidemics can be related to plankton blooms, and several physicochemical factors in water have been linked to the survival of the pathogen (Miller et al., 1984), including moderate salinity, high nutrient content, warm temperature, and slightly alkaline pH (Singleton et al., 1982).

Through hydrological disturbances, such as those produced by cyclones, *Vibrio* spp. that proliferate in the estuarine brackish region may reach far inland during flooding. With increasing estuaries eutrophication, it is essential to increase our knowledge of *Vibrio* dynamics and diversity in the coastal zone. Hitherto no studies have focused on the relationship of *Vibrio* spp. with suspended particulate matter (SPM) in the estuarine salinity gradient. In most estuaries a turbidity maximum zone occurs close to the salt wedge at flood tide, where most of the suspended terrigenous sediment is trapped. During flood tides, resuspension tends to be maximal, and the sediment is rapidly transported landward. Thus, the extent of risk of pathogenic estuarine bacteria to public health depends critically on their deposition, survival/regrowth in sediment, and subsequent resuspension and transport (Alm et al., 2006; Lee et al., 2006; Whitman et al., 2006; Mallin et al., 2007).

Floodwaters can deteriorate the quality of surface and groundwater through, e.g., sewage and pollutant wastes, frequently causing an increase in enteric diseases in the flood-impacted area (Siddique et al., 1991; Schwartz et al., 2006; Islam et al., 2007). Floodwater in New Orleans from hurricanes Katrina and Rita contained high levels of fecal indicator bacteria and microbial pathogens, raising questions about long-term impacts of these floodwaters on the sediment and water quality of the region (Sinigalliano et al., 2007). Little is known about the influence of an increasing frequency and intensity of tropical storms on microbial dynamics in the coastal zone and its mid- and long-term effects on human health (Fries et al., 2008).

The present study deals with the Karnaphuli estuary, in southeast Bangladesh. The Karnaphuli River traverses the port city Chittagong and ends in the Bay of Bengal. Like all coastal cities in Bangladesh, Chittagong has insufficient sewage treatment facilities and mostly raw sewage drains directly into its waterways. Chittagong has a population of ~2 million. Population growth in the last decade is ~4.4% per annum, much higher than the national growth of ~1.6%. Approximately 0.7 million are slum dwellers, who do not use septic latrines and defecate directly into waterways. Lack of safe drinking water and sanitation are key challenges for Chittagong's authorities. Waterborne diseases are prevalent, and public health services are insufficient to deal with the problem (Alam et al., 2007).

We focused on the effect of instabilities of the estuarine regime on *Vibrio* distribution in relation to hydrological parameters and microbiological and chemical pollution indicators. Of particular interest were the effects of human-waste inputs and variations in SPM content on *Vibrio* abundance and distribution patterns in the salinity gradient of the turbid waters of this system. The influence of catastrophic climatic events was investigated, including a cyclone and severe, monsoon rainfall-mediated landslides. On May 15, 2007 the first cyclone ("Akash") in the pre-monsoon season produced a tidal surge that swept through the coastal region, including the Karnaphuli estuary. Approximately 80,000 people were evacuated to cyclone shelters. Several fishermen died, crops and shrimp farms were washed away, roads were swamped, and hundreds of homes were damaged (Anonymous, 2007a). On June 11, 2007, Chittagong city was inundated due to exceptional monsoon rainfall (408 mm on that day), the heaviest in 25 years, water logging 1.5 million people. The flash floods caused mud slides and rubble to bury shanties at the foot of the hills at 1–4 km from the river. The worst-affected areas were covered by 3-m high mud and at least 128 died. A major cause of the landslides is hill cutting around Chittagong. During the past decades 30,000 hills have been destroyed to clear land for development, both by private land expropriators and by the City Corporation itself for new residential areas. The 1995 Master Plan forbid the hill cutting but the practice has continued unabated, despite protests and lobbying by concerned citizens. This and the absence of specific policy guidelines on hill management are the main reasons behind the rain-induced landslides in Chittagong. Due to the combination of high tide with concurrent heavy rainfall, and lack of proper drainage system in the hilly areas, most floodwaters along with washed terrestrial sediments and waste were transported into the Karnaphuli estuary, increasing its water level (Alam et al., 2007; OCHA, 2007; Anonymous, 2007b).

In the present work we discuss microbiological and hydrological data from the Karnaphuli estuary immediately before and after these events, focusing on the effect of changes in the environmental setting and human waste input on the abundance and distribution of *Vibrio* spp.

Methods

Study Site and Sampling Strategy

The Karnaphuli estuary (Fig. 1) faces semidiurnal tides with 2- to 4-m range in winter (November–February) and has 8–10 m average channel depth in the external zone. Pre-cyclone sample collection was performed on May 12, 2007, 3 days before "Akash." Post-cyclone sampling took place on May 17. In both cases, the weather still allowed sampling along the axis of the Karnaphuli estuary (Fig. 1). Sample collection began at high tide in the saline estuary's mouth (station 1) sailing during slack tide or starting ebb tide to 30 km inland, well in the freshwater sector. The post-landslide campaign was done on June 15, 2007, 4 days after the event when, due to unfavorable weather, stationary temporal sampling was done at a saline/brackish location at 5 km inland from the river mouth. During this time, water samples were collected every 30 min during a flood tide.

In every sampling, three subsamples of surface water (0.5 m depth, in the center of the river) were collected with sterilized buckets and pooled together. The waters were filtered through a plankton net (20 μm) to remove phyto- and zooplankton, and the filtrates were collected in sterile polycarbonate bottles. All samples were kept under ice and processed for bacteriological analyses within 24 hours of collection following the APHA (2005) protocol.

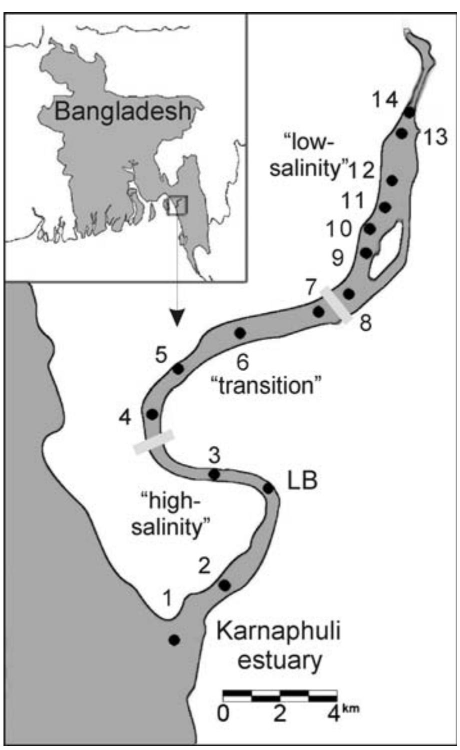

Figure 1. Study site in the Chittagong district, southeast Bangladesh. Numbers 1–14 correspond to stations from pre- and post-cyclone samplings around high-tide. LB designates the position of a land-based, stationary sampling performed after the landslide, approximately every 30 min during flood tide. The grey bars across the estuary divide it in three sectors corresponding to the synoptic pre-cyclone salinity distribution.

Biogeochemical Determinations

Salinity was determined by electric conductivity (WTW 340i with TetraCon325) and expressed in parts per thousand (ppt). Turbidity was measured by nephelometry (Oakton T-100) and expressed in Nephelometric Turbidity Units (NTU). SPM (mg/l) was determined gravimetrically after filtration on Whatman GF/F filters of 300–100 ml of water according to turbidity, and drying at 50°C until constant weight.

Water aliquots for nutrient analysis were preserved following Kattner (1999) and kept frozen until analysis with a Skalar-SANplus autoanalyser after Hansen and Grasshoff (1983). Only nitrate data, as a conspicuous nutrient in polluted urban runoff, are presented.

Bacterial Analyses

Sample duplicates were subjected to each bacteriological analysis. Total aerobic bacteria (TAB) counts were determined on nutrient agar (Difco, Detroit, Mich.) plate by decimal dilutions in normal saline following the spread plate technique. Water samples (1 and 10 ml) were passed through 0.2-μm filters (Millipore) and fecal coliform (FC) pollution was detected using MFC agar (Difco) following methods described earlier (APHA, 2005; Islam et al., 1994a, b, 2001). For cultivable *Vibrio* counts (CVC), water samples were inoculated onto selective thiosulfate citrate bile salts sucrose (TCBS) agar media (Difco) and characteristic colonies were counted after overnight incubation at 37°C. The cultivable *Vibrio* counts were confirmed by verifying the characteristics of bacteria grown on TCBS, e.g., oxidase, gelatinase, and a series of biochemical tests for identification of individual *Vibrio* species according to West and

Colwell (1984). NaCl concentration in each medium was adjusted to sample salinity.

Results

Salinity and Suspended Particle Load

Water temperature range was 27.2–31.4°C (average 29.9°C). Salinity varied from 19.4 ppt at the estuary's mouth to 0.1 ppt at ~30 km inland. Three different zones—"high-salinity," "transition," and "low-salinity"—were distinguishable along the Karnaphuli estuary. In the pre-cyclone expedition (Figure 2a) the "high-salinity" (20–15 ppt) sector reached until ~12 km inland, "transition" (15–3 ppt) extended along a distance of 8 km, ~12–20 km inland and "low-salinity" (3.0–0.1 ppt) started from ~20 km away from river mouth towards inland. After the cyclone, the high-salinity zone extended to 16 km inland (Fig. 2b). During the post-landslide sampling comprising the tidal cycle at 5 km inland, salinity levels at low tide were <0.5 ppt, comparable to the freshwater sector at high tide. During the flood, salinity in this location increased up to only 9 ppt (Fig. 3a).

The SPM and water turbidity range was 35–1890 mg/L and 30–1770 NTU, respectively. Both parameters correlated highly significantly ($r = 0.99$, $n = 28$, $p < 0.001$), therefore, turbidity—easily measurable in the field—was used as SPM proxy. During the pre-cyclone expedition, turbidity was relatively low, ranging from 30 to 70 NTU in the high-salinity and transition zones (Fig. 2a), whereas in the freshwater end it was 50–220 NTU, reaching the maximum at ~25 km from the river mouth. After the cyclone, the turbidity maximum occurred in the high-salinity zone, with 1770 NTU ~10 times higher than before the cyclone (Fig. 2b). During the stationary post-landslide sampling, turbidity increased from ~30 at low tide to ~1100 NTU during the flood tide, similar to the post-cyclone maximum.

Bacterial Populations: Effects of the Cyclone and the Landslide

Before and after the cyclone, TAB ranged from 10^4 to 10^6 cfu/ml, with high values at the estuary's mouth and a count rise at ~13 km from it (Fig. 2c and d), which occurred together with an increase in *Vibrio* counts. Before the cyclone, CVC distribution in the transition zone was similar to FC and nitrate, which respectively increased from ~0 to 140 cfu/ml and from ~30 to ~45 μM, both parameters peaking at salinities ~ 5 ppt. After the cyclone, CVC increased from ~10^2 cfu/ml to ~10^3 cfu/ml in the high-salinity zone (Fig. 2c and d), same as turbidity. FC

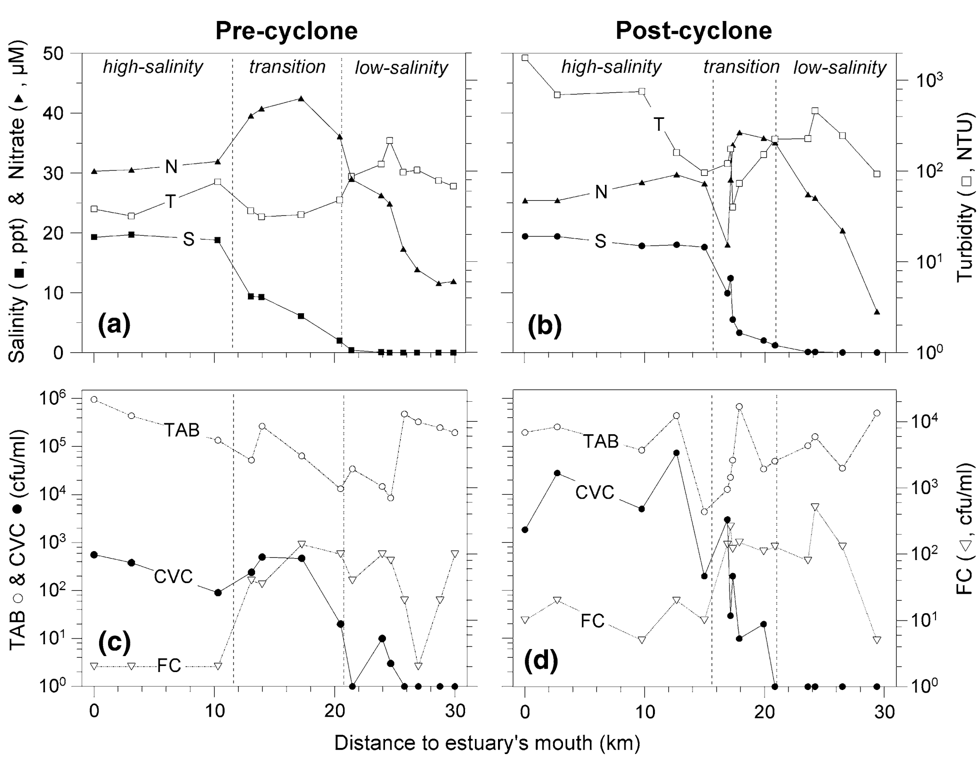

Figure 2. Distribution of hydrological (**a**, **b**) and microbiological (**c**, **d**) parameters along the Karnaphuli estuary few days before and after the cyclone Akash. Bacteria numbers are the average of triplicate counts. Standard deviation bars are omitted for clarity. T = turbidity, S = salinity, N = nitrate, TAB = total aerobic bacteria, CVC = cultivable *Vibrio* counts, FC = fecal coliforms.

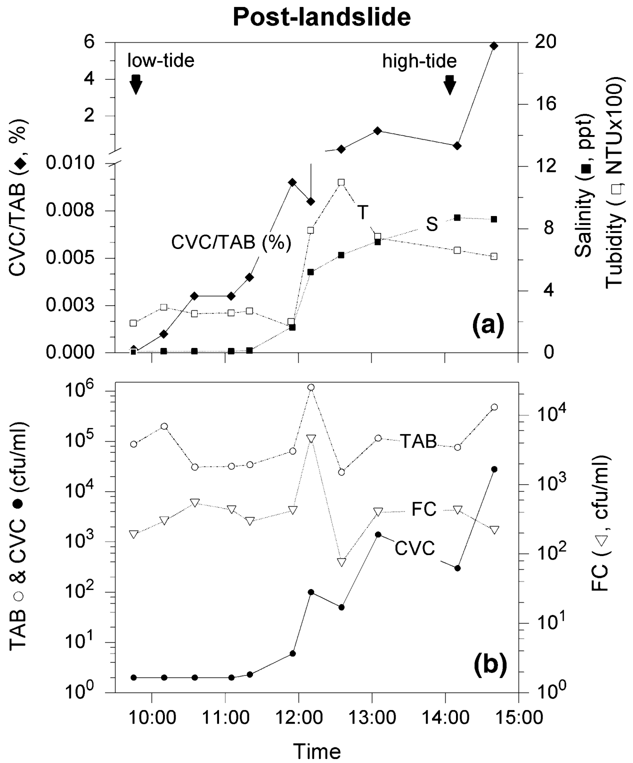

Figure 3. Temporal trends during a flood-tide stationary sampling near the mouth of the Karnaphuli estuary (station LB, Fig. 1) after the landslides. Bacteria numbers are the average of triplicate counts. Standard deviation bars are omitted for clarity. T = turbidity, S = salinity, TAB = total aerobic bacteria, CVC = cultivable *Vibrio* counts, FC = fecal coliforms, CVC/TAB (%) = percentage *Vibrio* proportion of total aerobic bacteria (TAB) counts.

increased from ~ 1 cfu/ml to ~ 10^1 cfu/ml in the high-salinity zone but changed little in the transition and low-salinity sectors, apart from point variations. Nitrate slightly decreased along estuary (Fig. 2a and b) and the other nutrients did not suffer major changes either (not shown). In the narrower transition zone, CVC sharply decreased with salinity despite high FC and increasing TAB and nitrate. However, before and after the cyclone, the counts of *Vibrio* spp. decreased sharply at salinities <5 ppt.

After the landslide, TAB ranged from 10^4 to 10^6 cfu/ml as in the previous two expeditions and presented a strong peak during a steep salinity rise near the middle of the flood tide (Fig. 3b). Interestingly, FC counts in this part of the estuary were ~10 times higher than in the pre- and post-cyclone expeditions and a peak of ~10^4 cfu/ml occurred around the middle of the flood tide. During low tide and beginning of flood tide, CVC in this location (~5 km inland from river mouth) were similar to those in low-salinity zone of the two previous expeditions; however, it started a rapid increase around noon at the middle of the flood tide when salinity and turbidity also gradually increased (Fig. 3a and b).

In random samples from all three expeditions, we identified 150 *Vibrio* strains at species level. The major part of the cultivable *Vibrio* population comprised *V. parahaemolyticus* (15%), *V. vulnificus* (15%), *V. splendidus* (13%), *V. cholerae* (8%), and *V. mimicus* (4%), whereas *V. campbelli, V. harveyi, V. alginolyticus, V. pelagious, V. fluvialis* etc. were present in much lower numbers.

Discussion

Salinity and Suspended Particle Load

The cyclone produced shifts in the salinity and turbidity distribution. After the cyclone the transition zone became narrower (~4 km), with a steep salinity gradient. This deeper intrusion of seawater into the estuary suggests a situation of higher energy with strong sediment resuspension from tidal flats, because turbidity maximum was directly at the estuary's mouth and not in the low-salinity zone, as before the cyclone (Fig. 2a and b), where it was associated to sediment input from shallow sectors and a mud bank (Fig. 1). Due to tidal mixing and increased runoff, turbidity in the low-salinity zone also was higher after the cyclone but less than in the high-salinity sector. Most likely the energy of the wind-driven tidal surge and the resulting flooding more than the rainfall were responsible for the observed setting, because the salinity range did not substantially change.

In contrast, after the landslide the maximum salinity at the stationary sampling site (Fig. 1) was strongly reduced (~50%), being similar to the pre-monsoon salinity at the beginning of the transition zone (Fig. 2a). Thus, the previous limit of the high-salinity zones was shifted toward the river mouth due to the enormous freshwater runoff, which also transported high loads of particulates and waste from the nearby hilly areas to the coastal area during ebb tides. Subsequently, during flood tides the highly turbid water would partly return back into the estuary with increased salinity (Fig. 3a).

Bacterial Populations: Effects of the Cyclone and the Landslide

In general, in the high-salinity sector the higher TAB counts coincided with higher CVC. Before the cyclone this pattern

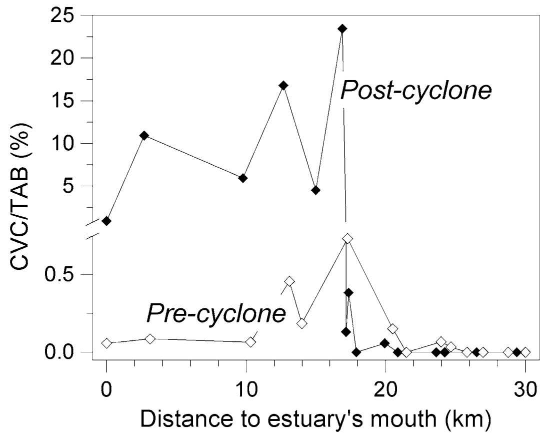

Figure 4. Cultivable *Vibrio* (CVC) percent proportion of total aerobic bacteria (TAB) counts (CVC/TAB) along the Karnaphuli estuary before (white squares) and after (black squares) the cyclone Akash.

also occurred in the transition zone, where in addition CVC, FC, and nitrate presented a similar, bell-shaped distribution (Fig. 2a and c), FC and nitrate peaking close to Chittagong metropolitan area. Thus, a significant fraction of *Vibrio* increase in this sector likely originated from human waste inputs. In the posterior transition and low-salinity zones, despite high FC and increasing TAB and nitrate loads indicating significant human input, CVC further decreased, probably due to suboptimal salinities. A salinity range of 5–25 ppt has been reported as favorable for *Vibrio* spp growth and persistence in the environment (Singleton et al., 1982). The relatively higher turbidity in the low-salinity zone also did not show any discernible impact on CVC. After the cyclone, the generally slightly elevated FC values were likely due to flushing out of human waste from upstream densely populated urban sectors (Fig. 2d).

Because *Vibrio* population may be significantly altered by the combined variation of salinity and SPM, whereas other aerobic bacteria are less influenced, variations in the CVC/TAB ratio can serve as sensitive indicator of such impacts. In the pre-cyclone high-salinity and transition zones, CVC/TAB ranged from 0.02 to 0.75%, increasing after the cyclone to 0.75 to 25% (Fig. 4). This was linked to a strong SPM increase at a similar range of higher salinities as before the cyclone (Fig. 2a and b), involving better conditions for *Vibrio* growth. In contrast, FC have better survival chances at lower salinities (Chigbu et al., 2004). In this study FC was generally higher that accepted by water quality standards for most human use forms (Hespanhol, 1997). This and differential salinity tolerance could lead to an ecological zonation of different causative agents for diarrheal diseases along the estuary, with sectors where vibrios or FC could respectively represent a higher health risk, depending on types of water use.

After the landslide, the strong FC increase in comparison to the previous expeditions indicates the outwelling of human waste from the affected villages. The CVC/TAB ratio increased steadily with salinity and turbidity (Fig. 3a), reaching a maximum of 6% at a higher salinity ($\sim$9 ppt). Still, this CVC/TAB value was much lower than the maximum registered after the cyclone. Besides the lower salinities, the terrestrial material introduced into the estuary by the strong rainfall was probably not significantly colonized by *Vibrio*.

Effect of Particulate Matter and Salinity on Vibrio Populations

Although during the pre-cyclone expedition a direct relationship between CVC and turbidity variations in the estuary was not obvious, after the cyclone and landslide events, high *Vibrio* counts occurred in patches of turbid water having higher salinity (Fig. 2b, d and 3a, b). The CVC values in the high-salinity sector increased more than proportionally to the increased FC and seemed to be more influenced by the much higher SPM compared to the pre-cyclone sampling. Besides, the cyclone probably introduced resuspended *Vibrio*-rich estuarine sediment into the water column of the high-salinity zone.

Fries et al. (2008) found that salinity was the strongest predictor of *Vibrio* counts ($r = 0.62$) in the Neuse River Estuary, USA, during two periods dominated by different suspension types: runoff and resuspension. In our study, the relationship between log CVC and salinity values from the three expeditions showed two different patterns. In the salinity range 0–10 ppt, log CVC and salinity strongly correlated ($r = 0.93$, $n = 19$, $p < 0.001$; Fig. 5) but did not show any obvious correlation at higher salinities, possibly due to a SPM effect on *Vibrio* abundance.

Koh et al. (1994) found higher *Vibrio* counts in water close to the water–sediment interface compared with surface water, attributing this to resuspension of benthic vibrios. Epidemiological data in Florida revealed a predominance of non-O1 *V. cholerae* infections at the time the organisms flourished in the sediment (Williams and LaRock, 1985). This calls attention on the hitherto probably underestimated quantitative role of suspended sediments as microhabitat for *Vibrio* organisms.

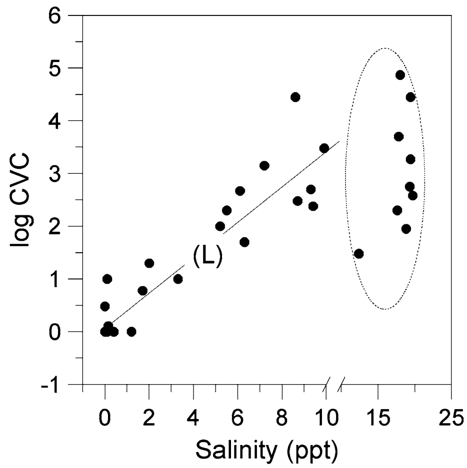

Figure 5. Relationship between the logarithm of CVC (cultivable *Vibrio* counts) and water salinity (ppt) in the Karnaphuli estuary. Pooled data from all three samplings. (L): regression line for both variables in the salinity range 0–10 ppt. Encircled data: log CVC for salinities >10 ppt.

Based on a comprehensive analysis (Alam et al., 2007) of the local sanitation and environmental situation and the comparison with other densely populated coastal regions (Anonymous, 2007c), several measures can be proposed to reduce the impact of floods on the population health. These include from community-based actions, such as latrine construction in villages and slums, to government-based programs, such as improvement of solid waste and sewage management, as well as reforestation projects for erosion and runoff control. Two critical issues are the improvement of rain drainage systems, to reduce city flooding during heavy rainfall, and the modification of sewer systems to avoid intrusion of estuarine/river water during storms through, e.g., the construction of sewer ports that can be closed during storm surges. The latter includes the creation of reservoirs with enough retention capacity and able to act as waste stabilization ponds to allow settling of particulate matter and a significant die-off of pathogenic bacteria before the effluent is released into the estuary (Polprasert et al., 2002 and references therein).

Conclusions

Cultivable *Vibrio* distribution patterns in the Karnaphuli estuary are mainly influenced by salinity and suspended particle load. Human waste in point pollution sources also can enrich *Vibrio* population, but this input is significant only in a limited area and does not greatly influence the general trend governed by the changes in salinity, turbidity, and climatic events. Cyclones cannot only cause damages by inundating vast coastal areas but also increase estuarine *Vibrio* population by sediment resuspension, likely triggering outbreaks of *Vibrio*-related diseases, including devastating cholera. Vibrios and their proportion of total aerobic bacteria were highest after the cyclone and also increased after the landslide. The cyclone did not significantly change pre-cyclone fecal coliform abundance, whereas this increased approximately ten times after the landslide. Sediment resuspension (e.g., by landslide or cyclone events) could represent an additional source of organic-rich substrate for increased bacterial growth or involve a direct input of particle-attached *Vibrio*. Hence, the characterization and quantification of the benthic and suspended particle-attached microbial community deserves increased attention. The combination of both extended salt intrusion and higher turbidities caused by stronger and more frequent storms and deforestation-derived erosion could increase the amount of vibrios in the coastal zone of the Bay of Bengal, further endangering aquatic resources and human health.

Acknowledgements

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