Wetlands Ecol Manage (2011) 19:109–119
DOI 10.1007/s11273-010-9204-0

ORIGINAL PAPER

Vibrio cholerae in waters of the Sunderban mangrove: relationship with biogeochemical parameters and chitin in seston size fractions

Rubén J. Lara · Sucharit B. Neogi · Mohammad S. Islam · Zahid H. Mahmud · Shafiqul Islam · Debasish Paul · Biniam B. Demoz · Shinji Yamasaki · Gopinath B. Nair · Gerhard Kattner

Received: 28 May 2010 / Accepted: 9 September 2010 / Published online: 2 October 2010

Abstract Wetland dynamics are probably linked to cholera endemicity in South Asia. We focus on links between *Vibrio cholerae* abundance, chitin content and suspended particle load in size fractions of suspended particulate matter (SPM) along the salinity gradient of Sunderban mangrove waters. SPM decreased downstream, while salinity increased from 0.2 to 4. Particulate organic carbon (90 ± 25 μM) and nitrogen (9.1 ± 3.3 μM) highly correlated with SPM and turbidity, suggesting a significant contribution of fine particles to organic matter. Total chitin ranged 1–2 mg/l and decreased downstream. The distribution among size fractions of SPM, chitin and *V. cholerae* O1 (the bacterial serogroup mainly associated with cholera epidemics) was similar, with ~98% of the total in the fraction <20 μm. In comparison, the number of *V. cholerae* O1 attached to zooplankton and microplankton size classes >20 μm was almost negligible, in contrast to usual assumptions. Thus, microdetritus, nanoplankton and fungal cells in size classes <20 μm represent a chitinaceous substrate on which *V. cholerae* can grow and survive. Total bacteria, cultivable vibrios and *V. cholera* O1 increased 5–10 times downstream, together with salinity and nitrite concentration. Overall, nitrate and silicate concentrations were relatively constant (>22 μM N and 100 μM Si). However, nitrite increased ~9 times in the outer sector, reaching ~1.2 μM N, probably as a result of increased abundance of nitrate-reducing vibrios. A characterization of *Vibrio* habitats that takes account of the presence of nitrate-reducing bacteria could improve the understanding of both mangrove nitrogen cycling and cholera seasonality.

Keywords Chitin · Cholera · Sunderban · Tropical estuaries · Vibrios

R. J. Lara (✉)
Leibniz Centre for Tropical Marine Ecology, Fahrenheitstr. 6, 28359 Bremen, Germany
e-mail: ruben.lara@zmt-bremen.de

S. B. Neogi · M. S. Islam · Z. H. Mahmud · S. Islam · D. Paul
International Centre for Diarrhoeal Disease Research, Bangladesh, 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh

B. B. Demoz
Institute of Environmental and Animal Hygiene and Veterinary Medicine, University of Hohenheim, Stuttgart, Germany

S. B. Neogi · S. Yamasaki
Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Sakai 599-8531, Japan

G. B. Nair
National Institute of Cholera and Enteric Diseases, Kolkata 700010, India

G. Kattner
Ecological Chemistry, Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Introduction

The Sunderban in the Ganges–Brahmaputra delta is one of the largest and most vulnerable tracts of mangrove forest in the world. It contains socio-ecological systems that are acutely threatened by climate change, changes in land use and aquatic-borne diseases. Vulnerability to these threats increased following the massive damage inflicted on the Sunderban by Cyclone Sidr in 2007, which damaged about 30% of the mangrove forest and killed ~10,000 people. The marine front of the mangroves and villages located in the forest were particularly affected (Fig. 1). On the other hand, the presence of mangroves mitigated the impact of the cyclone on the coastal region and helped prevent more widespread damage further inland (Ocha 2007).

The dynamics of this aquatic ecosystem, characterized by high inputs of organic matter and severe, recurrent floods are poorly understood. In particular, further research is required into seasonal variability and linkages between water biogeochemistry and microbiology. Although these wetlands have been associated with endemic cholera since ancient times, no systematic studies have been undertaken to establish causal links between hydrological and biogeochemical cycles and the seasonal dynamics of the causative agent of the disease, *Vibrio cholerae*, which is a naturally occurring species in these waters.

As early as 1884, Robert Koch suggested Sunderban mangroves as a principal source of cholera outbreaks where the combination of brackish, organic matter-rich coastal waters, and high human population density in upstream areas can provide ideal conditions for *V. cholerae* proliferation. Difficult access, cyclones, and floods have precluded systematic spatio-temporal monitoring of these habitats. Most of the few studies undertaken have been short-term research based on one-site samplings and do not provide information on seasonal trends (Lara et al. in press). No systematic investigation has been carried out into the influence of monsoons, cyclones, increasing water salinization or pollution on pathogen dynamics in these wetlands. Another topic requiring research is the contribution of aquaculture, with its high input of organic matter, fish feed etc., to accelerated eutrophication of mangrove areas (Anonymous 2008).

Fig. 1 The Sunderban forest at Kotka, severely damaged after the cyclone "Sidr"

In the southern part of the Ganges delta, where cholera is endemic, hydrological disturbances can have prolonged impact on cholera seasonality. Causes of these disturbances include changes in salinity, particle load and associated estuarine biogeochemistry following the construction of Farakka barrage on the Ganges (Mirza 1998), and more rapid melting of Himalayan glaciers (UNEP 2007). As early as 1982, Miller et al. (1982) postulated that dam construction in India could influence *Vibrio* dynamics by facilitating salt intrusion downstream. Dam construction has indeed reduced riverine discharge in the Bay of Bengal, resulting in more extensive salt intrusion into its estuaries, particularly in the SW region of Bengal (Wolf 2001; Adel 2005). This has resulted in changes in land use, with rice cultivation being replaced by shrimp farming in upstream areas of the Sunderban (Gebauer 2007). Salinization of estuaries and inland water bodies due to dam construction or rising sea-level provides conditions that allow halophilic *Vibrio* organisms to expand their range. On the other hand, global warming may increase glacial melting and associated riverine runoff. The interaction of these two factors, in the context of increasing intensity and frequency of cyclones and flooding events, could give rise to an extremely complex situation in the coastal zone that in turn could influence cholera epidemicity.

As well as being pathogens, many *Vibrio* species play a role in the biological cycling of refractory organic matter. Vibrios can produce different chitinases to degrade various chitin types (Svitil et al. 1997) in marine, estuarine and freshwater environments. Each year ca. 10^{11} tons of chitin are produced in aquatic environments (Keyhani and Roseman 1999).

Its turnover is essential since otherwise large amounts of carbon and nitrogen would become inaccessible to most organisms. Interestingly, it has been recently discovered that chitin also induces exogenous DNA uptake in *Vibrio* sp. in aquatic environments, thereby facilitating diversification (Meibom et al. 2005). There is experimental evidence of lytic phage mediated transfer of the cholera toxin gene from one *V. cholerae* strain to another aided by chitin-induced competence (Udden et al. 2008). Bhowmick et al. (2007) showed that *V. cholerae* strains were more chitinolytic than non-cholerae vibrios, and the activity of the chitinase genes increased under higher nutrient concentrations and salinities.

Although there has been much speculation about possible ocean/land interactions, based on empirical correlations, the *causal* links between changes in oceanic parameters and the incidence of cholera in riparian inland villages remain obscure. Plankton in both freshwater and marine environments is considered to be a principal means of transmission of *V. cholerae* to humans (Colwell et al. 2003; Islam et al. 1990, 1994b, c; Huq and Colwell 1995). Its abundance can be estimated by remote sensing, and has been related to the incidence of cholera in the Bay of Bengal region by Lobitz et al. (2000). Yet, although tidal transport of *Vibrio*-carrying marine plankton towards inland areas is likely, it is improbable that marine plankton survive long enough to be a source of infection in freshwater, which is what people drink. Microbiological investigations in mangroves have hitherto focused mainly on material transformation and fluxes, and little is known about pathogen dynamics in their different compartments.

The present work is part of a long-term investigation into links between annual cycles of biogeochemical and hydrological parameters in mangroves and estuaries in Bengal, the seasonal dynamics of *V. cholerae,* and the incidence of cholera. In a first phase, an exploratory screening was carried out, focusing on *Vibrio* microhabitats and bacterial distribution along the salinity gradient in one of the main North–South channels of the Bangladeshi Sunderban. Particular emphasis was placed on the characterization of particulate organic nutrients and the relationship between *V. cholerae* abundance, chitin content and particle load in different size fractions of suspended matter.

Materials and methods

Sampling was carried out in 2007 during the winter (dry season) on December 3 and 4. This was shortly after Cyclone Sidr, the strongest named tropical storm ever to hit the Bay of Bengal, made landfall in Bangladesh on November 15 2007. Setting out from Mongla harbor, samples were collected over 2 days at 22 stations between Mirgamari and Kotka in the eastern Sunderban (Fig. 2). At each station, three samples of surface water (0.5 m depth, in the center of the river) were collected with sterilized buckets and the combined samples were analyzed to determine microbiological and biogeochemical characteristics. At 7 of the 22 stations (A–G; Fig. 2), 400 l water were taken, and the suspended particulate matter (SPM) therein was fractionated through nets with mesh sizes of 20, 60, 200 and 500 μm. Aliquots (mostly 100 ml) of the concentrated size fractions (>500, 500–200, 200–60, 60–20, <20 μm) were taken for SPM weight and chitin determinations as well as for microbiological analysis.

Salinity was determined by electric conductivity (WTW 340i with TetraCon325, Weilheim, Germany) and recorded in dimensionless Practical Salinity Units. Turbidity was measured by nephelometry (Oakton T-100, Vernon Hills, IL, USA) and expressed in Nephelometric Turbidity Units (NTU). Water samples were filtered on board through GF/F filters (Whatman, precombusted at 450°C, 3 h). Filtrates were poisoned with $HgCl_2$ and stored at 4°C in 50 ml PE bottles for later nutrient analyses (Kattner 1999). Nitrate, nitrite, ammonium (all in μM N), silicate (μM Si) and phosphate (μM P) were determined according to seawater standard methods (Kattner and Becker 1991). Filters were stored frozen at −20°C until analysis for SPM, particulate organic carbon (POC) and nitrogen (PON), and chitin.

SPM (mg/l) was determined gravimetrically after drying at 50°C to a constant weight. Filters for the determination of POC and PON were dried at 50°C for 12 h and kept at room temperature in a desiccator until analysis. POC was determined after removing inorganic C by acidification with 1N HCl. POC and PON were quantified with an elemental analyzer (Fisons, NA 2100). Standard Reference Material 1515 was used for calibration and as a quality standard.

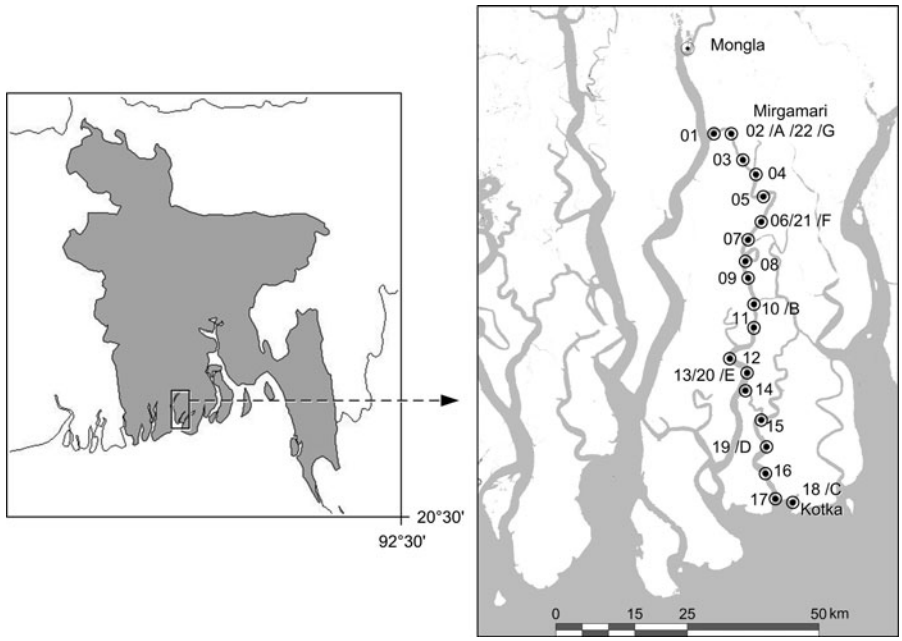

Fig. 2 Study region in the eastern Sunderban mangrove forest, Bangladesh. *Numbers 1* to *22* indicate sampling stations and letters *A* to *G* are stations where larger volumes of water were fractionated using nets of various sizes

Chitin content in unfractionated particulate matter and its size fractions was determined by the WGA-FITC method after Montgomery et al. (1990). WGA (wheat germ agglutinin) has a high affinity for *N*-acetyl glucosamine residues in chitin and binds specifically to it even when samples contain high concentrations of cellulose, clay and bacteria. Gallagher et al. (1985) reported that WGA can also bind to polylactosamine-type oligosaccharides and has a lower affinity to *N*-acetylneuraminic acid. Thus, the possibility can not be ruled out that WGA might also bind to other glycopeptides in environmental samples. The method was calibrated with purified crab chitin (Sigma-Aldrich, St Louis, MO, USA) as standard, and quantified by fluorimetric measurement of excess WGA after reaction with fluorescein isocyathionate (FITC).

Total aerobic bacteria counts (TBC) were determined on nutrient agar (Difco, Detroit, MI, USA) plates by decimal dilutions in normal saline (0.85% NaCl, w/v) solution following spread plate technique. Water samples (1 and 10 ml) were passed through 0.2 µm filters (Millipore), and total coliforms (TC) were quantified using mFC agar (Difco) following methods described earlier (APHA 2005; Islam et al. 1994b, 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 was carried out to identify individual Vibrio species according to West and Colwell (1984). The NaCl concentration in each medium was adjusted to sample salinity. Pathogenic *V. cholerae* O1 were enumerated by means of the Direct Fluorescence Antibody (DFA) technique using the Cholera DFA kit (New Horizon Diagnostics Corp., Maryland, USA) according to its instruction manual, and cells were finally observed under an epifluorescence microscope (Olympus, model AH-2). All analyses were done in triplicate.

Statistical analyses of the obtained data sets were made using 'Xact' (version 7.21d, SciLab GmbH, Hamburg, Germany) and Statistica (ver. 10.0, StatSoft Inc., Tulsa, OK, USA) programs. Linear and curvilinear regression fits were applied to explore correlations between variables. A P value < 0.05 was considered as significant.

Results and discussion

Synoptic biogeochemical trends and relationships

The amount of SPM and the optical turbidity ranged from 45 to 170 mg/l (average 105 ± 40 mg/l) and 45–385 NTU (165 ± 97 NTU), respectively. Salinity

was low, ranging from 0.2 to 4. Both SPM and turbidity decreased towards the outer part of the estuary and displayed a non-linear, hyperbolic relationship with salinity (Fig. 3a, b). SPM and turbidity were similar to or slightly higher than values measured in the Karnaphuli estuary (located approx. 300 km east) before Cyclone Akash (May 15, 2007) (Lara et al. 2009). Although the Sunderban, located in the south western part of Bangladesh, had been hit by the much stronger cyclone "Sidr" shortly before our sampling, the particle load was much lower than in the Karnaphuli estuary, located in the south eastern part of Bangladesh, immediately (2 days) after Cyclone Akash which caused ten times higher values in turbidity than those recorded before the cyclone. One reason for the lower turbidity after cyclone in the Sunderban estuary was probably the fact that the high vegetation density and root network of the mangrove trapped particles more efficiently than in the deforested Karnaphuli estuary, where one month after "Akash" a severe landslide occurred (Lara et al. 2009). Further, during the 2 week time after 'Sidr', exchange of mangrove water with the coastal region might have further reduced the suspended particle load. However, samples taken previously in the Sunderban in May, 2007 (Lara, unpublished data) showed similar turbidities (222 ± 187 NTU) as those registered in December 2007, after Cyclone Sidr.

The percentage content of organic matter in SPM was relatively constant along the estuary: The proportion (w/w) of organic C was 1.09 ± 0.2% and of organic N 0.12 ± 0.04%. POC concentrations ranged from 48–128 μM (average 90 ± 25 μM) and PON varied between 3.6 and 15.7 μM N (average 9.1 ± 3.3 μM). There was a highly significant linear positive correlation between SPM and POC and PON ($r = 0.93$ and 0.80, respectively, $n = 22$, $P < 0.001$). A highly significant correlation between POC and optical turbidity ($r = 0.80$, $n = 22$, $P < 0.001$) suggests that a significant fraction of this suspended organic carbon consists of very fine particles. The POC and PON values in Sunderban were lower but approximately

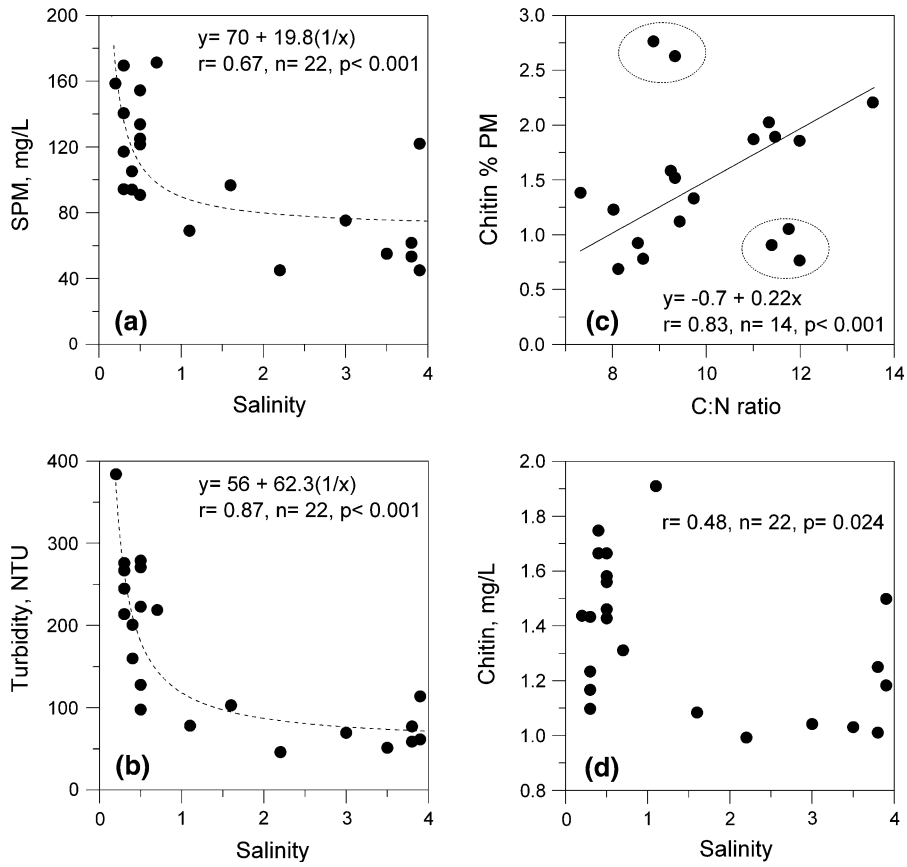

Fig. 3 Relationships between biogeochemical parameters in unfractionated water from stations along the estuarine gradient in the Sunderban mangrove. Turbidity and SPM showed a hyperbolic dependence on salinity (*dotted curves*). This trend was also observed in the chitin data set, though it was statistically not significant. The *encircled* data points in (**c**) were not included in the regression

within ranges measured in mangroves in other regions. For example, in studies of Amazonian mangroves, creek water had average annual values of 240 and 29 μM of POC and PON, respectively (Dittmar and Lara 2001).

The slope of the linear regression between POC and PON is a good estimate of the C:N ratio of natural organic matter in aquatic ecosystems. The sample data produced a slope of 6.95 ($r = 0.88$, $n = 22$). Data from Biswas et al. (2010) indicate that the highest phytoplankton biomass in the Indian Sunderban occurs during the post-monsoon season (October–December). This is consistent with the C:N ratio derived from the POC versus PON regression in our study since this value is close to the Redfield ratio for active phytoplankton (Laws et al. 2001), indicating that a significant proportion of the variability in suspended organic matter was due to phytoplankton. The higher C:N ratio obtained from simple data average includes the contribution of detritus and zooplankton. The average C/N ratio in Sunderban (10.05 ± 1.7) was also similar to the average annual value of ~8 in Amazonia (Dittmar and Lara 2001).

There was no significant correlation between chitin and POC ($r = 0.23$, $n = 22$, $P = 0.31$), nor with PON ($r = 0.13$, $n = 19$, $P = 0.61$), C:N ($r = -0.11$, $n = 19$, $P = 0.65$) or SPM ($r = 0.23$, $n = 22$, $P = 0.29$). However, the proportion of SPM accounted for by chitin showed a positive, highly significant correlation with the C:N ratio of SPM ($r = 0.83$, $n = 14$, $P < 0.001$), except for few cases (Fig. 3c), suggesting that detrital material makes a contribution to the chitin pool. The proportion of chitin to SPM (w/w), representing contribution of bulk seston composition, ranged from ~0.5 to 3%. Total chitin in SPM ranged between roughly 1 and 2 mg/l. The relationship between chitin and salinity (Fig. 3d) showed a similar pattern to that found between SPM and turbidity, i.e., higher values in low salinity waters (Fig. 3a, b), which basically reflects their analogous distribution along the estuary. However, the correlation of chitin versus salinity was not statistically significant ($r = 0.48$, $n = 22$, $P = 0.024$). During the sampling campaign in the upstream, low-salinity part of the estuary we observed hundreds of boat capturing shrimp seed for aquaculture. We assume that shrimp exoskeleton was partly responsible for the abundance of chitinaceous particles and wide data dispersion in this sector of the estuary. Chitin data was also highly dispersed at salinities around four comparable to SPM (Fig. 3d) which might be due to characteristic of fine particles that are more abundant and have a greater influence on water transparency (see discussion on size classes below).

Distribution of bacteria and inorganic nutrients in estuary sectors

The main features in the distribution of bacterial counts, turbidity and salinity along the estuarine transect allow a rough grouping of the stations in three sectors: upstream, middle and downstream (Fig. 4).

The upstream sector had salinities of ~0.5, turbidity on average of 203 ± 74 NTU and the highest total coliform counts of the three sectors (1433 ± 674 CFU/l), most probably due to anthropogenic inputs from Mongla city. TBC averaged $1.2 \pm 0.8 \times 10^6$ CFU/l, *V. cholera* O1 had $1.5 \pm 1.2 \times 10^4$ cells/l and CVC of 50 ± 22 CFU/l.

In the middle sector, salinities of 0.4 ± 0.3 were similar to upstream, while turbidity was on average higher (228 ± 90). Coliform counts were lower and on average about 50% of the upstream values (650 ± 346 CFU/l). Interestingly coliform values showed a similar distribution to turbidity and there was a significant correlation between these two variables ($r = 0.72$, $n = 8$, $P < 0.05$). These observations suggest an input from the mudflats and the mangroves fringing the estuary, but no anthropogenic influence. Average TBC, *V. cholera* O1 and CVC values were also slightly lower or similar to those in the upstream sector.

At the downstream stations, salinities were higher at 3.2 ± 0.9, and average turbidity of 73 ± 24 NTU was lower than in the other two sectors. The higher salinities, lower particle densities and the absence of significant human impacts were reflected in coliform values that were lowest in this sector. The correlation between turbidity and coliform counts of the pooled data from the middle and downstream stations was also significant ($r = 0.86$, $n = 16$, $P < 0.001$). TBC in the downstream sector showed an average value about five times higher than at the upstream and middle stations, though highly variable ($5.2 \pm 9.3 \times 10^6$ CFU/l). Halophilic bacteria were more abundant in this sector and average values obtained for *V. cholerae* O1 ($2 \pm 4 \times 10^5$ cells/l) and CVC (517 ± 1093 CFU/l) were about an order of

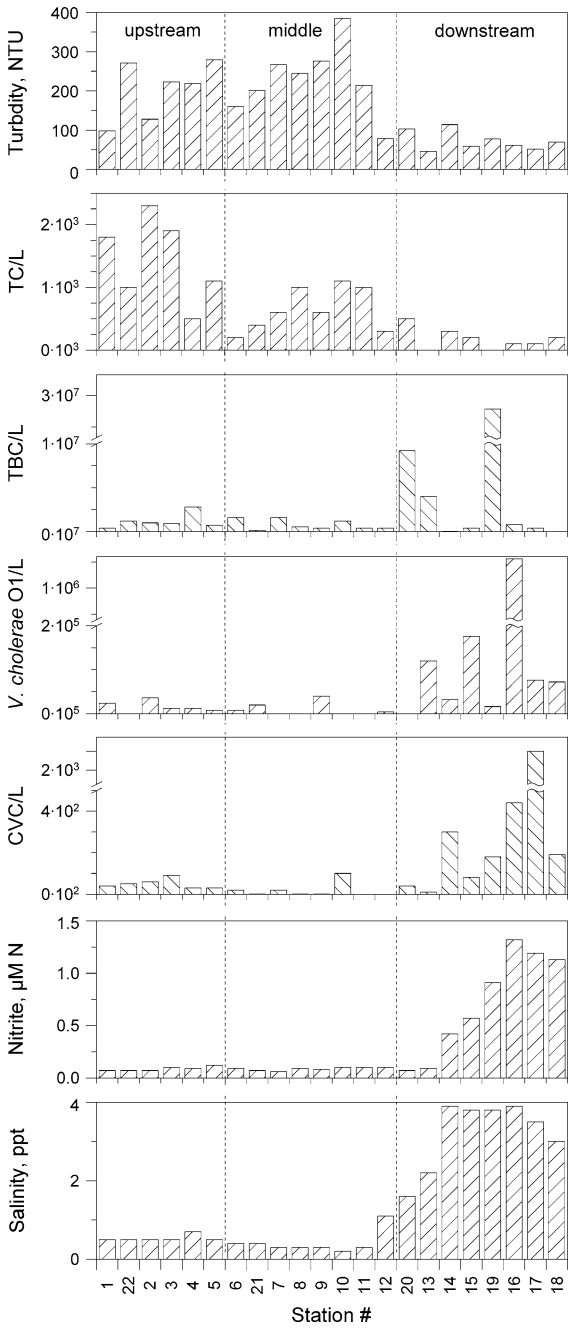

Fig. 4 Distribution of biogeochemical and microbiological parameters along the estuarine gradient in waters of the Sunderban mangrove. *CVC* cultivable *Vibrio* counts (CFU/l), *Vibrio cholerae O1* DFA-counts/l, *TBC* total bacteria counts (CFU/l), *TC* total coliforms (CFU/l)

magnitude higher than in the other two sectors. There was, however, no correlation between chitin concentration and *V. cholerae* O1 and CVC abundance along the entire estuary; the most likely reason is that any correlation present in the lower sectors was masked by data from the upstream sector, where high chitin concentrations were recorded in areas where salinities were too low for *Vibrio* development.

The distribution of inorganic nutrients along the estuary was relatively constant and did not present any relevant features, with the exception of nitrite. Mean nitrate values in each sector of the estuary were higher than 22 µM, with an overall average of 23.3 ± 1.2 µM. Phosphate levels increased from 0.65 ± 0.2 µM in the upstream sector to 0.88 ± 0.1 µM in the middle one, and to 1.07 ± 0.1 µM in the downstream sector. Silicate was on average 117 ± 6 µM and at all stations >100 µM. This was the only nutrient to present a significant inverse, hyperbolic correlation with salinity ($r = 0.7$, $n = 22$, $P < 0.001$), similar to those found for turbidity, SPM and chitin. Yet, this is unlikely to be ecologically relevant, since all silicate concentrations were consistently higher than 100 µM Si.

Nitrite showed a remarkable pattern compared to the other nutrients although concentrations were low and nearly constant in the upstream and middle sectors with 0.08 ± 0.01 µM N, average concentrations in the downstream portion increased ~9 times to 0.71 ± 0.5 µM N, reaching ~1.2 µM N in the outermost part at the highest salinities. These higher nitrite values do not seem to be relevant from the point of view of plankton nitrogen requirements, since nitrate levels were consistently high, but rather point to locally enhanced heterotrophic activity. Nitrite distribution showed a similar pattern to salinity, which excludes a riverine source. However, neither is a marine source plausible, since the nitrite maxima occurred at salinities of ~4, i.e., ~9 times lower than average seawater, where nitrite is usually markedly lower than 1 µM N. Exceptions are regions where nitrate-reducing bacteria are abundant, or where nitrite is produced from ammonia by the action of nitrifying bacteria and accumulated (Brandhorst 1959). In an undisturbed mangrove forest along the central west coast of India, ammonium and nitrite regeneration rates were generally highest in nearshore waters, and nitrite production and use were closely coupled to those of ammonium. Nitrogen balance analyses showed that proximity to mangrove vegetation enhanced the flux rates (Dham et al. 2002). In the downstream sector, the Sunderban mangroves have a

denser creek network and thus the sampled channel may be more influenced than the upstream sectors by exchange processes between surface sediments and vegetation and/or by regular flooding. Under such conditions one would expect higher water turbidities, which however were lowest in this sector. Thus, nitrite production is probably mainly the result of activity of halophilic, nitrate-reducing bacteria. Many *Vibrio* species have the capability to reduce nitrate, including those commonly found in nearshore water, sediment and mangrove habitat (e.g., *V. mangrovi*, *V. cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, *V. campbellii*, *V. harvey*, etc.) (Rameshkumar et al. 2010, Ishimaru et al. 1996, MacFarlane and Herbert 1982). Thus, it is likely that the higher nitrite values in the nearshore sites can be at least partially explained by the presence of high numbers of vibrios.

Distribution of chitin, SPM and V. cholerae O1 in size classes

More than 97% of chitin and SPM occurred in the size fraction <20 μm (Fig. 5a). Chitin accounted for about 1.6% (w/w) of SPM in this fraction. The remaining chitin was distributed in the other size fractions and composed of microphytoplankton, mesozooplankton, etc. Slightly higher values were recorded in the fraction 60–200 μm, but these still amounted to less than 1% of the total. SPM decreased from ~98% of the total in the fraction <20 μm to 1% in the size class 20–60 μm to 0.02% in the fraction >500 μm.

As mentioned above, there was a positive correlation between the proportion of chitin in SPM and C:N ratio in the unfractionated samples. Considering

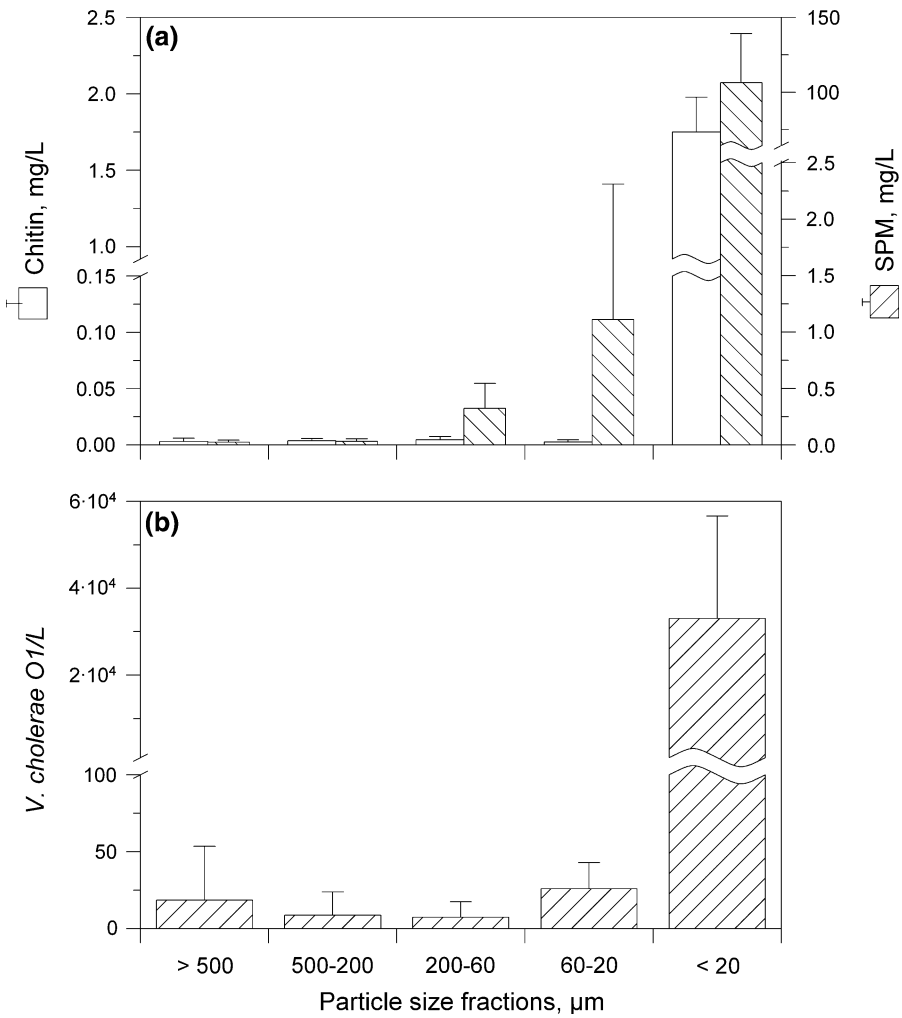

Fig. 5 Distribution of **a** chitin, SPM and **b** *V. cholerae* O1 in size classes of suspended particulate matter

that (i) most of the chitin was in the fraction <20 μm, (ii) pure chitin has a C:N ratio of 8.1, (iii) phytoplankton and living zooplankton have C:N ratios generally in the range from 5 to 10 (e.g., Moloney and Field 1991; Gismervik 1997) and (iv) most zooplankton is >20 μm, the observed trend suggests that a large portion of chitin was probably present in form of degraded micro-detritus such as old exuviae or adsorbed on sediment particles. In addition, fungi may contribute to the chitin pool in the fraction <20 μm because they have cells walls that contain chitin with higher C:N ratios (7–25; Wichern 2004) than bacteria and phytoplankton. There are, however, no data in the literature that allow a comparison with those in the present work.

The DFA counts of *V. cholerae* O1 in the different size fractions revealed that the highest abundance occurred in the fraction <20 μm (Fig. 5b) and showed essentially the same pattern as SPM and chitin. This implies that microdetritus, sediment particles, nanoplankton and fungal cells (with some restrictions, see Lio-Po et al. 2005) represent a chitinaceous substrate on which *V. cholerae* can attach and grow. *V. cholerae* has been reported (Islam et al. 1994b, c) to be associated with the mucilaginous sheath of cyanobacteria such as *Anabaena* sp., which builds long strains. Although these filaments are much larger than the smallest mesh size used in this work (20 μm), it has still to be investigated whether mucin in phytoplankton cells in the nanoplankton fraction represent a significant reservoir for *Vibrio* in the estuarine environment as they do in larger algae.

The fact that the highest proportion of chitinaceous particles was in the smallest size fraction has profound implications for the understanding of the ecology of *V. cholerae*, including its seasonal variation. Up to now investigation has focused on zooplankton in the size class >60 μm and on phytoplankton in the size class 20–60 μm, as reservoirs of *V. cholerae* (Huq and Colwell, 1995; Colwell et al. 2003; Islam et al. 1994a, 2007and references therein). Attention has often focused on living zooplankton, due to its chitinaceous exoskeleton, given that vibrios are chitinolytic bacteria. However, there are reports that chitinaceous detritus could promote growth of *V. parahaemolyticus* more than other particulate or dissolved nutrients. Watkins and Cabelli (1985) found that growth and survival of *V. parahaemolyticus* was stimulated more by addition of pulverized chitin than by living zooplankton, which in turn caused more stimulation than sewage or other nutrients. In vitro experiments with seston >64 μm showed that clinical *V. cholerae* O1 isolates attached preferentially to moulted zooplankton exoskeletons (exuviae) rather than to whole specimens (Tamplin et al. 1990).

The results of this work show that the number of *V. cholerae* O1 attached to the zooplankton and microplankton size classes above 20 μm is almost negligible compared to that in the fraction <20 μm (Fig. 4b). On average, and in each of the seven samples >99% of DFA counts were in that fraction. The distribution of *V. cholerae* O1 among the different size fractions shows essentially the same pattern as for SPM and chitin. The fractionation study supports the assumptions of a positive influence of suspended, fine-grained material on *Vibrio* abundance, since optical measurements showed that most turbidity in unfractionated water was contributed by the particles in the fraction <20 μm.

Conclusions

In the present study, the main finding is that maximum values for *V. cholerae* O1 and chitin were both found in the same small size fraction <20 μm and not, as usually assumed and studied, in the larger size fractions which mostly harbor zooplankton, larger exuviae or a wide variety of phytoplankton. Since covariation does not imply causality, and SPM also peaks in this fraction, it is not possible to assert which of the particulate parameters had a major influence on *Vibrio* abundance: the amount of chitin or the simply the amount of suspended particles. This consideration is relevant for two main reasons: mangroves are environments naturally rich in chitinaceous organisms (e.g., plankton, detritus, shrimps, and crabs), and chitin abundance can favor *Vibrio* mutations (Meibom et al. 2005). Further, the high density of shrimps and vibrios in aquaculture ponds and the nutrient input from megacities that induces coastal plankton blooms could favor the emergence of new and highly pathogenic *Vibrio* types in and near South Asian mangroves (Faruque et al. 2003). Regarding the role of particle load on *Vibrio* dynamics, higher turbidities in tropical estuaries caused by stronger and more frequent cyclones and

erosion associated with changing patterns of land use, together with more extensive salt intrusion could favor growth and habitat expansion of vibrios, with increasing risks for aquatic resources and human health in the coastal zone (Lara et al. 2009).

To date there are no field studies linking *V. cholerae* diversity to chitin abundance along with other dissolved and particulate components such as mucin in wetland ecosystems, especially in the nanoplankton fraction. Knowledge of the seasonal variations of these parameters could help further clarify current cholera dynamics and future trends. Future *Vibrio* research in mangroves should include the determination of nitrate-reducing halophilic species in order to better characterize the structure of *V. cholerae* habitats and uncover hitherto unknown links to the nitrogen cycle in wetlands.

Acknowledgments This work was funded by the grant LA 868/5-1 from DFG/BMZ, Germany. We thank the Environmental Microbiology Laboratory of ICDDR, B, specially Md. A. Matin, Md. H. Uddin and P. Gope for their support during sample collection and processing. Thanks are due to D. Peterke and D. Dasbach (ZMT) for their technical assistance and to K.U. Ludwichowski (AWI) for the determination of inorganic nutrients.

References

Adel MM (2005) Background state leading to arsenic accumulation in the Bengal basin groundwater. J Water Health 3:435–452

Anonymous (2008) Sunderbans. http://westbengalforest.gov.in/urls_all/forest_wild_life_terrain.htm

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF), Washington, DC

Bhowmick R, Ghosal A, Chatterjee NS (2007) Effect of environmental factors on expression and activity of chitinase genes of vibrios with special reference to *Vibrio cholerae*. J Appl Microbiol 103:97–108

Biswas H, Dey M, Ganguly D, De TK, Ghosh S, Jana TK (2010) Comparative analysis of phytoplankton composition and abundance over a two-decade period at the land–ocean boundary of a tropical mangrove ecosystem. Estuaries Coasts 33:384–394

Brandhorst W (1959) Nitrification and denitrification in the eastern tropical North Pacific. J Cons Int Explor Mer 25:3–20

Colwell RR, Huq A, Islam MS, Aziz KM, Yunus M, Khan NH, Mahmud A, Sack RB, Nair GB, Chakraborty J, Sack DA, Russek-Cohen E (2003) Reduction of cholera in Bangladeshi villages by simple filtration. Proc Natl Acad Sci USA 100:1051–1055

Dham VV, Menezes Heredia A, Wafar S, Wafar M (2002) Seasonal variations in uptake and in situ regeneration of nitrogen in mangrove waters. Limnol Oceanogr 47: 241–254

Dittmar T, Lara RJ (2001) Driving forces behind nutrient and organic matter dynamics in a mangrove tidal creek in North Brazil. Est Coast Shelf Sci 52:249–259

Faruque SM, Sack DA, Sack RB, Colwell RR, Takeda Y, Nair GB (2003) Emergence and evolution of *Vibrio cholerae* O139. Proc Natl Acad Sci USA 100:1304–1309

Gallagher JT, Morris A, Dexter TM (1985) Identification of two binding sites for wheat-germ agglutinin on polylactosamine-type oligosaccharides. Biochem J 231:115–122

Gebauer M (2007) Bangladesh: Wo der Klimawandel nach Salz schmeckt. Spiegel on-line 26. April 2007

Gismervik I (1997) Implications of zooplankton stoichiometry on distribution of N and P among planktonic size fractions. J Plankton Res 19:343–356

Huq A, Colwell RR (1995) Vibrios in marine and estuarine environment. J Mar Biotechnol 3:60–63

Ishimaru K, Akagawa-Matsushita M, Muroga K (1996) *Vibrio ichthyoenteri* sp. nov., a pathogen of Japanese flounder (*Paralichthys olivaceus*) larvae. Int J Syst Bacteriol 46:155–159

Islam MS, Drasar BS, Bradley DJ (1990) Long-term persistence of toxigenic *Vibrio cholerae* O1 in the mucilaginous sheath of a blue-green alga, *Anabaena variabilis*. J Trop Med Hyg 93:133–139

Islam MS, Alam MJ, Khan SI, Huq A (1994a) Fecal pollution of freshwater environments in Bangladesh. Int J Environ Stud 46:161–165

Islam MS, Drasar BS, Sack RB (1994b) The aquatic flora and fauna as reservoirs of *Vibrio cholerae*: a review. J Diarrhoeal Dis Res 12:87–96

Islam MS, Drasar BS, Sack RB (1994c) Probable role of blue-green algae in maintaining endemicity and seasonality of cholera in Bangladesh: a hypothesis. J Diarrhoeal Dis Res 12:245–256

Islam MS, Siddika A, Khan MNH, Goldar MM, Sadique MA, Kabir A (2001) Microbiological analysis of tube-well water in a rural area of Bangladesh. Appl Environ Microbiol 67:3328–3330

Islam MS, Brooks A, Kabir MS, Jahid IK, Shafiqul Islam M, Goswami D, Nair GB, Larson C, Yukiko W, Luby S (2007) Faecal contamination of drinking water sources of Dhaka city during the 2004 flood in Bangladesh and use of disinfectants for water treatment. J Appl Microbiol 103:80–87

Kattner G (1999) Storage of dissolved inorganic nutrients in seawater: poisoning with mercuric chloride. Mar Chem 67:61–66

Kattner G, Becker H (1991) Nutrients and organic nitrogenous compounds in the marginal ice zone of the Fram Strait. J Mar Syst 2:385–394

Keyhani NO, Roseman S (1999) Physiological aspects of chitin catabolism in marine bacteria. Biochim Biophys Acta 1473:108–122

Koch R (1884) An address on cholera and its bacillus. Br Med J 6:453–459

Lara RJ, Neogi SB, Islam S, Mahmud ZH, Yamasaki S, Nair GB (2009) Influence of estuarine dynamics and catastrophic

climatic events on *Vibrio* distribution in the Karnaphuli estuary, Bangladesh. EcoHealth 6:279–286

Lara RJ, Islam MS, Yamasaki S, Neogi SB, Nair GB (in press) Aquatic ecosystems, human health and ecohydrology. In: E. Wolanski, D. McLusky (eds) Treatise on estuarine and coastal science."Ecohydrology and restoration",Vol. 10 (L. Chícharo, M. Zalewski (eds.)). Elsevier, Amsterdam

Laws EA, Popp B.N, Bidigare RR, Riebesell U, Burkhardt S, Wakeham SG (2001) Controls on the molecular distribution and carbon isotopic composition of alkenones in certain haptophyte algae. Geochem Geophys Geosyst 2. doi:10.1029/2000GC000057

Lio-Po GD, Leaño EM, Peñaranda MMD, Villa-Franco AU, Sombito CD, Guanzon NG Jr (2005) Anti-luminous *Vibrio* factors associated with the 'green water' grow-out culture of the tiger shrimp *Penaeus monodon*. Aquaculture 250:1–7

Lobitz B, Beck L, Huq A, Wood B, Fuchs G, Faruque ASG, Colwell RR (2000) Climate and infectious disease: use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. Proc Natl Acad Sci USA 97:1438–1443

MacFarlane GT, Herbert RA (1982) Nitrate dissimilation by *Vibrio* spp. isolated from estuarine sediments. J Gen Microbiol 128:2463–2468

Meibom KL, Blokesch M, Dolganov NA, Wu CY, Schoolnik GK (2005) Chitin induces natural competence in *Vibrio cholerae*. Science 310:1824–1827

Miller CJ, Drasar BS, Feachem RG (1982) Cholera and estuarine salinity in Calcutta and London. Lancet 29: 1216–1218

Mirza MMQ (1998) Diversion of the Ganges water at Farakka and its effects on salinity in Bangladesh. Environ Manag 22:711–722

Moloney CL, Field JG (1991) The size-based dynamics of plankton food webs. I. a simulation model of carbon and nitrogen flows. J Plankton Res 13:1003–1038

Montgomery MT, Welschmeyer NA, Kirchman DL (1990) A simple assay for chitin: application to sediment trap samples from the subarctic Pacific. Mar Ecol Prog Ser 64:301–308

OCHA (2007) Bangladesh Cyclone Sidr. United Nations Office for the Coordination of Humanitarian Affairs. http://ochaonline.un.org/News/Emergencies/Bangladesh/tabid/2707/Default.aspx. Accessed on December 3, 2007

Rameshkumar N, Sproer C, Lang E, Nair S (2010) *Vibrio mangrovi* sp.nov., a diazotrophic bacterium isolated from mangrove-associated wild rice (*Poteresia coarctata* Tateoka). FEMS Microbiol Lett 307:35–40

Svitil AL, Ni Chadhain SM, Moore JA, Kirchman DL (1997) Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin. Appl Environ Microbiol 63:408–413

Tamplin ML, Gauzens AL, Huq A, Sack DA, Colwell RR (1990) Attachment of *Vibrio cholerae* serogroup O1 to zooplankton and phytoplankton of Bangladesh waters. Appl Environ Microbiol 56:1977–1980

Udden SM, Zahid MS, Biswas K, Ahmad QS, Cravioto A, Nair GB, Mekalanos JJ, Faruque SM (2008) Acquisition of classical CTX prophage from *Vibrio cholerae* O141 by El Tor strains aided by lytic phages and chitin-induced competence. Proc Natl Acad Sci USA 105:11951–11956

UNEP (2007) Fast melting glaciers from rising temperatures expose millions in himalaya to devastating floods and water shortages. UNEP-ROAP News Release 07/10. http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=512&ArticleID=5600&l=en

Watkins WD, Cabelli VJ (1985) Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. Appl Environ Microbiol 49:1307-1313

West PA, Colwell RR (1984) Identification and classification of Vibrionaceae: an overview. In: RR Colwell (ed) Vibrios in the environment, New York, John Wiley, pp 285–363

Wichern F (2004) Fungi vs bacteria: their different roles in decomposition of organic matter. soils are alive newsletter, Vol. 3 Nr 3. http://www.soilhealth.see.uwa.edu.au/fungi_vs_bacteriacomponents/

Wolf AT (2001) Water and human security. J Contemp Water Res Educ 118:29