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Spatial Analysis of Bacteria in Brackish Lake Sediment

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- **Highlights:**

- Macro to micro scale analysis of bacterial diversity
- Spatial heterogeneity of physico-chemical variables linked with bacterial distribution
- Salinity plays a major role in bacterial distribution in sediment from a brackish lake
- Streamlines from fish farms are related to decreases in bacterial diversity and increases in pathogens
- Coupling macro-analytical tools (GIS) and micro tools (16S rRNA gene sequencing) to determine the spatial distribution of bacteria in sediment.

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Abstract

The spatial distribution of bacterial communities inhabiting sediment is heterogeneous at different spatial scales, but mostly unexplored. Here, it is postulated that the heterogeneity of the bacterial community composition varies at the same scale of the heterogeneity of sediment chemical properties. The large spatial scale (km) diversity in sediment from a brackish water lagoon (Chilika Lake, India) is studied, considering the large scale physical and chemical characteristics of land cover, climate, pH, and salinity. Seventy-two samples (24 stations, 3 seasons: winter, rainy, and summer) of sediment from Chilika Lake were analyzed by 16S ribosomal ribonucleic acid (rRNA) gene sequencing, and the relations with land cover and other physico-chemical parameters are discussed. More samples were collected after a severe cyclonic storm (Phailin) passed near the lagoon in 2013, to see the impact of the tropical storm on the spatial and temporal distribution of bacteria in the sediment. The results demonstrate clear spatial relations between physico-chemical parameters (e.g., salinity), land surfaces (e.g., drainage area), and the distribution of sediment microbial communities.

1. Introduction

Microorganisms play a major role in global biogeochemical cycles of carbon, nitrogen, and phosphorus. With the growth rate of all microorganisms on earth estimated at over 10^{30} cells/yr, these microorganisms are considered major drivers of geochemical cycling (Withman et al., 1998). Soils and sediment are among the largest microbial reservoirs. In addition to the high number of microorganisms, soils also have relatively high heterogeneity of physical, chemical, and biological characteristics (Beare et al., 1995; Ramette, 2007). This heterogeneity might influence reaction rates through the distances that metabolites must move between different bacteria and archaea in soils, yet, little is known about the spatial distribution of bacteria in soils (and less about sediment) and the drivers of the spatial heterogeneity. Several studies have focused on soil (Dechesne et al., 2003; Grundmann, 2004; Remenant et al., 2009; Vos et al., 2013) and sediment (Córdova-Kreylos et al., 2006; Piza et al., 2005) in order to understand spatial distribution of bacteria at the micro-scale and on soils at the macro-scale (Fierer & Jackson, 2006; Griffiths et al., 2009).

Part of the difficulty is the physical-chemical description of the different samples at the micro-scale. The use of sediment to study spatial-scale effects on microbial community distribution simplifies the micro-heterogeneity of physico-chemical parameters due to the sediment pore water, which increases local nutrient and metabolite diffusion compared to unsaturated soils (Urban et al., 1997). Some studies have evaluated the bacterial composition and spatial distribution in sediment of brackish lakes (Pramanik et al., 2015; Thureborn et al., 2013; Webster et al., 2015; Zaitseva et al., 2014). These studies identified specific functions and microbial communities associated with different types of sediment environments. One marine sediment study identified large-scale shifts in microbial community structure in sediment pore water along a salinity gradient in the Baltic Sea (Herlemann et al., 2011). While salinity clearly has an influence on microbial community composition, several other physical-chemical characteristics also could play important roles. Some of these characteristics could be driven by global land use practices. It is hypothesized that the spatial distribution of the microbial community in large coastal lakes could be modeled in part by sample characteristics and by global land use as determined by satellite data and geographic information systems (GIS). As lake sediment has less physical and chemical heterogeneity than river sediment (Brönmark & Hansson, 2005) and result from the indigenous production and the transport of terrestrial material (organic or inorganic compounds) from the drainage area (Beaulne et al., 2012; Teisserenc et al., 2010), the sub-hypothesis of this paper is that the physico-chemical parameters having a spatial gradient at the scale of the lake (e.g., salinity in this case) will drive bacterial composition and diversity. The combination of marine and fresh water creates a wide range of habitat types that can sustain relatively high microbial diversity in Chilika Lake. This paper examines marine and riverine influences through the use of GIS coupled with standard multivariate analyses.

2. Material and Methods

2.1. Study area

Chilika Lake, which is the second largest brackish lagoon in the world and the largest in Asia, is situated on the east coast of India (between 19°28' and 19°54' N and 85°05' and 85° 38' E). Since 1981, the site has been designated as an Indian wetland

of international importance (Ramsar Site). The size of the lagoon fluctuates significantly during a year from 906 km² (summer) to 1165 km² (monsoon). The hydrological pattern of Chilika Lake is influenced by three subsystems, namely, Mahanadi River distributaries, streams of the western catchment that bring in freshwater to the lake, and the Bay of Bengal that contributes highly saline sea-water. The lagoon is a collection of very shallow marine, brackish, and freshwater ecosystems. The whole area is categorically organized into four sectors. The northern sector, which receives direct discharge of freshwater from the Mahanadi Delta; the central sector intermixing zone, which is brackish; the southern sector, which has higher salinity levels as compared to central sector; and the outer channel, which exchanges water between the lagoon and the sea. Due to a high salinity gradient, the lake hosts a wide range of macro-organism biodiversity: 314 species of fish, 224 species of water birds, and 729 angiosperms. That high biodiversity sustains an important population of fishermen estimated at about 140,000 individuals (Kumar & Pattanaik, 2012). After a decrease in fishing productivity in the 1990's, the lake water quality has been monitored and the lake mouth to the outer channel was reopened.

2.2. Sampling and physico-chemical analyses

The monitoring of the sediment and water was done at 24 stations spread over the entire lake (Fig. 1 – monitoring and sampling stations) in summer (March to June 2011), monsoon (July to October 2011), and winter seasons (November 2011 to February 2012). Sediment and water samples were collected from stations from all four ecological sectors; northern, central, southern, and outer channel (Fig. 1). Bottom surface sediment samples were collected with a Van Veen type of grab sampler (KC, Denmark,) from each station during summer, monsoon, and winter seasons. Both sediment and water (water column) samples were immediately transferred to sterile plastic 500 ml bottles and transported to the laboratory on ice and stored at 4°C. Sediment samples were partially dewatered to improve lysis efficiency and homogenized manually with a sterile mortar and pestle inside a laminar flow hood.

For each station, pH, conductivity (mS/cm), turbidity (NTU), salinity (parts per thousand; ppt) and temperature of air and water (°C) were measured onboard immediately with a water quality probe analyser (TOA DKK 24, Japan). Depth and transparency/turbidity (cm) of the water column were measured with a measuring tape and Secchi disk, respectively. Biochemical oxygen demand (ppm) and dissolved oxygen (ppm) were measured with an automated portable probe analyzer (VSI 07, VSI Electronics, Mohali, India). Concentration of nitrite, nitrate, ammonia, phosphate, silicate, and total iron were measured on the collected water and total organic carbon on sediment samples (APHA, 1998). In order to determine seasonal effects, each station was sampled 3 times, during summer, winter, and monsoon seasons. They were also sampled after a major storm (as described in section 2.4).

Fig. 1

2.3. GIS analysis (Land surfaces, hydrogeology and spatial analysis)

The watershed boundaries were determined using a Digital Elevation Model (DEM) from Shuttle Radar Topography Mission (SRTM) images (U.S. National Aeronautics and Space Administration, NASA) using Geographical Resources Analysis Support System (GRASS), an open-source GIS software (Beaulne, 2008). Hydrological modeling was also done in order to create stream, slope, and drainage layers (GRASS). The sub-watersheds were merged into four large watersheds to correspond to the scale of the sediment and water sampling.

Land cover classes were assigned using four Landsat Thematic Mapper 7 (TM) satellite images (July 2003) with a 27 m resolution. Land cover classifications were then determined using an unsupervised maximum likelihood analysis method using ArcGIS software. The percentage of forested area, cultivated land, urban area, bare soil, and wetland were calculated for each of the created watersheds (Fig. 2). Areas of aquaculture (mainly shrimp and fish landing centers) also were determined from satellite images (Centre National d'Etudes Spatiales (CNES), 2012, distribution *Astrium* service's spot image). These were considered to be important activities in Chilika Lake, but problematic in terms of water quality in other similar environments (Pushparajan & Soundarapandian, 2010).

Fig. 2

Interpolations were done with GRASS software for the physico-chemical parameters measured for each sampled point and each season to find geographical and temporal concentration gradients.

2.4. DNA analysis

Total DNA was extracted from 0.5 g of sediment sample in duplicate using FastDNA® *Spin* Kit for soil DNA extraction (MP Biomedicals, Santa Ana, CA, U.S.) as per the manufacturer's protocol. DNA was extracted in duplicates for each sampling station and pooled to one tube per individual station and season. Quality and quantity of metagenomic DNA were verified using 1.2% of agarose gel (0.5XTAE) and Nanodrop (Epoch, BioTek Instruments, U.S.) analysis (A_{260}/A_{280}). DNA from a total of 72 samples from the 24 station (3 seasons) from 2011 and 2012 and twelve more samples after the "Phailin", a very severe cyclonic storm (VSCS) which made landfall near 19.26°N / 84.82°E (near Gopalpur, Odisha) at 1600 UTC on 12th October, 2013 were extracted to determine bacterial diversity and community composition. Total DNA extracted from the sediment was amplified for 16S rRNA genes by Polymerase Chain Reaction (PCR). The hypervariable (V4-V6) region of the 16S rRNA genes was amplified from using the bacterial primer pair 515F and 1061R (16S-0515F 5'-TGTCAGCMGCCGCGGT-3' 16S-1061R 5'-TCACGRCACGAGCTGACG-3' ~560bp V4-V6 region (M774, Promega, Madison, WI, U.S.)). PCR was done with the modified primers containing an adapter and a barcode sequence (Schloss et al., 2009). Paired end 454 pyrosequencing was done on the GS-FLX 454 Titanium platform.

2.5. Analysis of the community composition

The reads were analyzed through the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (<http://qiime.org>) (default parameters) and taxonomic identity of each read was assigned using the Bayesian rRNA gene classifier at the 80% confidence threshold and the Ribosomal Database Project (RDP) database (Caporaso et al., 2010; Cole et al., 2009; Wang et al., 2007). Sequences that could not be classified to at least a kingdom level were excluded from subsequent analysis. For phylotyping of 16S rRNA gene data, sequences were aligned using the QIIME pyrosequencing pipeline. Operational taxonomic units (OTUs) were determined at phylum (80%), class (90%), order (92%), genus (95%), and species (97%) level.

2.6. Statistical and multivariate analysis

Statistical analyses were done using the JMP 11 software (JMP®, Version 11. SAS Institute Inc., Cary, NC, 1989-2019). In order to find the link between physico-chemical parameters and relative abundance of OTU's, a large set of relations was evaluated at different taxonomic levels. To find similarities of community composition between samples, CANOCO 4.5 (CANOCO™ CanoDraw™ and the Cornell Ecology Programs, Microcomputer Power, Ithaca, U.S.) was used to draw two major components of the principal component analysis (PCA). Canonical correspondence analysis (CCA) also was done to find which variables are driving the separation of the samples in terms of community composition in the environmental dataset.

3. Results

3.1. Physico-chemical distribution in the lake and spatial distribution of bacteria

In order to identify correlations between physico-chemical parameters and the bacterial spatial distribution, the spatial distribution of the physico-chemical parameters was first examined. The pH varied between 6.9 and 9.5. No spatial pattern was found in the pH distribution. Only one sampled point (KG) had a pH under 7.8 and only during the monsoon season (the pH was 8.6 during the summer at the same point), probably due to the increase of fresh water (lower pH) coming from the watershed during the monsoon season. Turbidity was in a range between 1.1 to 171 NTUs, but again the nonlinear geographical gradient (patchy distribution) mainly depended on the seasonal effect. The highest turbidity was found in almost all post-Phaillin (cyclonic storm) samples because the cyclonic storm stirred up bottom sediment. While salinity and conductivity were correlated, the salinity was focused on because it can have a strong effect on the microbial spatial distribution and diversity. Salinity was measured between 0.1 and 32.3 ppt (g/L). Both a strong temporal variation and a spatial gradient in salinity values were observed.

The interpolated maps of the geographical variability of the salinity measured for each station during the three seasons showed the highest seasonal variation in the mixing zone near the output channel (Fig. 3a, 3b, 3c). Nitrite, nitrate, ammonia, phosphate, silicate, total iron, and total organic carbon varied considerably between samples at different locations and for different seasons from the same location. The post cyclonic storm samples generally had higher nutrient concentrations due,

in part, to higher flux coming from the watersheds. The concentrations of nitrite (0.04 to 8.6 $\mu\text{mol/L}$), nitrate (0.52 to 25.67 $\mu\text{mol/L}$) and ammonia (7.31 to 206.41 $\mu\text{mol/L}$) generally were low.

Fig. 3

3.2. GIS analysis (land surfaces, hydrogeology and spatial analysis)

Interpolation of physico-chemical parameters was done for each season. Only salinity showed a clear spatial gradient, although the gradient varied from season to season. Interpolation of the bacterial diversity (Shannon index at the genus level) (Morris et al., 2014) was also done using the GRASS software. Figure 3d shows the interpolation of bacterial diversity after merging the samples from the three seasons. A significant “hot spot” for bacterial diversity can be seen. The geographical area where the highest microbial diversity was measured (Figure 3d) also was where the salinity had the highest variability during the year (seasonal effect, Fig 3a, 3b, 3c).

3.3- Statistical analysis

Multivariate analysis (PCA, CCA) was done with CANOCO v4.5 to test in part whether one of the main drivers of the microbial community variability was salinity. A separation of the samples was observed following principally the variation of the salinity and conductivity (Fig. 4). The spatial effect also can be responsible for the differences in community structure as all the samples with the higher salinity were located in the same geographical zone, i.e. the outer channel. A PCA also was done to determine the seasonal effect on the bacterial community structure. No clear evidence of a major shift in the community structure as a function of season was observed.

Fig. 4

In order to test the hypothesis that the rare severe storms smooth out spatial heterogeneity, twelve samples were collected just after a cyclonic storm in October 2013. The samples were collected one week after the storm, 3 samples were taken in each of the four zone of the lake. The samples with high salinity before (dark green points) and after the storm (dark red points) had similar community structures (Fig. 5). The low salinity samples taken after the storm (light red points) had different community structures than those sampled before the storm (light green points).

Fig. 5

The same twelve sample points were compared before and after the storm (Fig. 6). A small shift in the community structure occurred after the storm probably due to the mixing of the sediment (sediment was sampled at a water depth between 1 and 3.2 m) and a major influx of organic matter from the watershed. The sample points located near the ocean were not significantly different after the storm. The major change during the storm is the intrusion of saltwater in to the lake, affecting principally the zone that used to have low salinity.

Fig. 6

3.4. Analysis of the microbial community structure

The number of reads per samples were between 5,387 to 38,265. Taxonomy was done using QIIME pipeline and the RDP database. The most dominant phylum in the current sample was *Proteobacteria* with a relative abundance up to 0.79 and never under 0.37 (average 0.573). A second more abundant phylum was the *Chloroflexi* (0.01 to 0.24, average 0.086). In order, the next phyla were *Bacteroidetes* (0.006 to 0.17, average 0.047), *Planctomycetes* (0.001 to 0.08, average 0.038), *Acidobacteria* (0.002 to 0.07, average 0.031), *Firmicutes* (0.0004 to 0.15, average 0.028), *Gemmatimonadetes* (0.002 to 0.08, average 0.027), *Actinobacteria* (0.004 to 0.08, average 0.023), *Verrucomicrobia* (0.0004 to 0.014, average 0.021) and *Nitrospirae* (0.0005 to 0.06, average 0.017). Looking more specifically at *Proteobacteria* (the largely dominant phylum in all the samples), a specific distribution was observed at the class level. The most dominant class of *Proteobacteria* was *Gammaproteobacteria* with an average relative abundance of 0.252, followed by *Deltaproteobacteria* (0.178), *Betaproteobacteria* (0.066), *Alphaproteobacteria* (0.045), and *Epsilonproteobacteria* (0.026). The high abundance of *Gammaproteobacteria* was mainly due to four orders, *Chromatiales* (up to 0.19), *Xanthomonadales* (up to 0.12), *Pseudomonadales* (up to 0.21) and *Thiotrichales* (up to 0.21). The dominance of *Gammaproteobacteria* is similar to what have been describe in marine sediment (Fang et al. 2017). Some families and genus were also measured at a high relative abundance in some of the current samples: *Piscirickettsiaceae* (up to 0.21 relative abundance) and *Pseudomonas* (up to 0.09). *Acidobacteria* was observed at a relatively high abundance even if the pH was above 7 in almost every part of the lake. No correlation between the *Acidobacteria* relative abundance and pH was observed.

A negative effect of the salinity on the diversity (except for the site located in the mixing zone) (Fig. 7a) also can be seen. The decrease of diversity related to salinity is similar of what have been observed in other study comparing sediment from fresh to salt water (Wang et al., 2012). A positive relation between the salinity and the relative abundance of *Proteobacteria* was found (Fig. 7b). Since the relative abundance of *Proteobacteria* was generally high but variable, a strong negative relation also was found between the abundance of *Proteobacteria* and the Shannon diversity (Fig. 7c)

Fig. 7

Links between physico-chemical variables and bacterial community structure were observed with the distribution of the most relatively abundant bacterial phylum and class found in the Chilika Lake sediment (i.e. between *Proteobacteria* and *Gammaproteobacteria* and salinity). With a R^2 of 0.2829 ($p < 0.0001$) (Fig. 7d), there is a trend for increasing salinity to relate to the distribution of the *Gammaproteobacteria* in the sediment of Chilika Lake, but the data noise is quite high. At the phylum level, the trend ($R^2 = 0.1896$, $p < 0.0002$) (Fig. 7b) was less obvious.

4. Discussion of the drivers of the community structure

Recent research has shown that macrobiotic ecological changes in the Chilika Lake system and its fisheries were due to the effect of water exchange between the lake and the sea that is responsible for control of the salinity, siltation, macrophyte infestation, and increase of marine forms (Ghosh et al., 2006; Sahu et al., 2014; Satyanarayana et al., 1999). It is hypothesized that the microbial community would be similarly affected. The community structure and distribution in Chlika Lake observed in the current study was similar to that observed in 16S rRNA gene studies on brackish water, in the Baltic

Sea (Herlemann et al., 2011), in sediment of Sundarbans Mangrove Wetland (Basak et al., 2014), in two samples in Chilika Lake (Pramanik et al., 2015), and in a Brazilian mangrove (Andreote et al., 2012). Salinity was the only variable that was identified as having a spatial gradient of distribution at the scale of the current sampling in the lagoon. Salinity has been described as the major factor shaping microbial communities (Lozupone & Knight, 2007).

The distributions of other variables measured in the current study had either a much higher or a much lower spatial scale of variation. Water temperature and depth that could, respectively, correspond to temporal and spatial distribution were not significantly correlated to community structure, probably due to the relative homogeneity of those parameters in Chilika Lake. pH has been described to have strong effect on regulation of life processes and nutrient availability in aquatic systems (Tank et al., 2009). In the case of Chilika Lake (and at the scale of the current study), pH did not appear to be strongly correlated to the spatial distribution of bacteria and diversity index. Although the relative abundance of *Acidobacteria* has been described to correlate with soil pH (Fierer & Jackson, 2006; Griffith et al., 2011), no correlation between the pH and the relative abundance of the phylum *Acidobacteria* nor with the genera within this phylum was found.

The higher scale of variation would affect all the samples, making it difficult to measure their effect on the spatial distribution of the bacteria in the sediment (climatic conditions, watershed land cover). On the other hand, the distribution and variability of nitrite, nitrate, ammonia, phosphate, silicate, total iron, and total organic carbon occurred at a much smaller scale than the scale of the current samples. Hotspots for those variables are visible at the micro-scale (Parkin, 1987; Schramm et al., 1999). The size of samples (0.5 g), the nesting of the sampling effort and the fact that the exact same sample was not used to do chemical analysis and 16S rRNA gene analysis hindered finding a correlation between those micro-scale variables and potential drivers of the microbial community structure at the scale of the current study (Fig. 8).

Fig. 8

Another limitation in the current analysis of the spatial distribution of bacteria and the identification of the physico-chemical drivers is the importance of the transport of material in the lake (watershed - lake, stream). All the samples with high relative abundances (more than 0.08 relative abundance) of a specific family (*Chromatiales*, *Xanthomonadales*, *Pseudomonadales*, and *Thiotrichales*) or specific genus (*Piscirickettsiaceae* and *Pseudomonas*, more than 0.05 relative abundance) were identified. Coupling a map of the fishfarming and a stream map (produced with Hydrological Modeling, GRASS), it was observed that almost all the points with high abundance of a specific *Gammaproteobacteria* family or genus were located on a streamline from the fishfarming areas (Fig. 9). Those four taxonomic families and two genera are known to contain some pathogen species; some of them have been associated with fish pathogens in salmon fishfarming (Austin & Austin, 2007; Birkbeck et al., 2011). The spatial analysis also highlighted hotspots of bacterial diversity where the seasonal variation of the salinity was high. The more drastic change in salinity was mainly due to the freshwater coming into the lagoon during the monsoon season. Some changes of salinity could be due to the tidal effect on a daily basis.

Fig. 9

5. Conclusion

This first description of the spatial distribution of bacteria in the Chilika Lake sediment has highlighted the predominance of *Gammaproteobacteria*. Trends in the spatial and temporal distribution of that subphylum could be identified in the brackish water lagoon. Hotspots of some families and genus from *Gammaproteobacteria* were linked to the potential transport of material through the lake from fish farming areas. This analysis was made possible by applying GIS-based spatial analysis. The only variable, salinity, that had a spatial pattern of distribution at the same geographic scale as the sampling effort was the only one that could be correlated with the community structure. The relation between salinity and relative abundance of *Gamaproteobacteria* was an indication that the spatial distribution of bacteria in sediment was possibly driven by this physico-chemical parameter. Using only statistical approaches to identify physicochemical drivers of the community structure can be meaningless if both the spatial and the temporal scale are not considered. Environmental parameters that are generally insignificant can have hotspots of higher importance that explain specific niches for bacteria at the micro-scale. Comparing samples of 0.5 g will not address the importance of parameters that vary at a smaller scale. At a scale larger than the sample set, the variability will be integrated in all the samples, and, therefore, will not differentiate between samples.

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List of captions

Fig. 1. Map of Chilika Lake showing the locations of 24 sampling stations spanning four different sectors, northern, central, southern, and outer channel.

Fig. 2. Interpolation of satellite images. Red polygons represent shrimp farming zones and fish landing areas.

Fig. 3. Interpolation of the salinity measured in monsoon (a), winter (b) and summer (c) seasons, color gradient from red (highest concentration) to blue (lowest concentration). Interpolation of the Shannon Index (d). Color gradient from red (highest index) to blue (lowest index).

Fig. 4. Results of PCA of the microbial community at the genus level (72 samples) with overlays of the environmental variables and the different zones denoted in different colors. (note Env. = Environmental).

Fig. 5. Results of PCA of the microbial community at the genus level (84 samples) with sample color referring to their salinity levels before and after the storm.

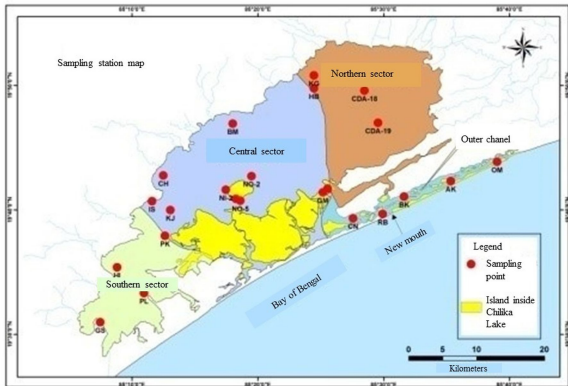
Fig. 6. Results of PCA of the microbial community at the genus level (24 samples) with samples taken before (green) and after (blue) the storm.

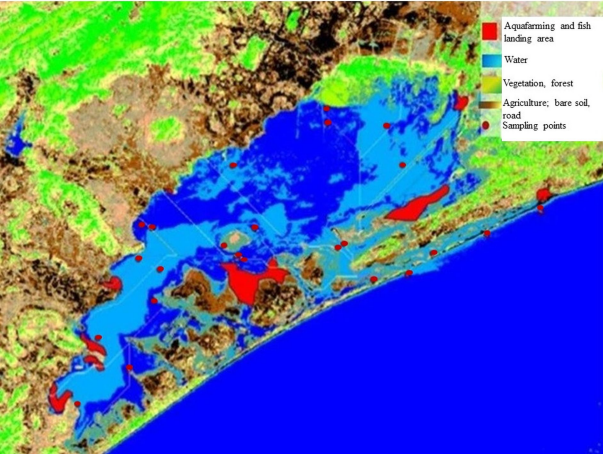
Fig. 7. a) Effect of salinity on the diversity (Shannon Index); b) Effect of salinity on *Proteobacteria* relative abundance (coefficient of determination (R^2) of 0.1896, $p < 0.0002$); c) Effect of the relative abundance of *Proteobacteria* and diversity (Shannon Index); and d) Effect of salinity on *Gammaproteobacteria* relative abundance, R^2 of 0.2829, $p < 0.0001$

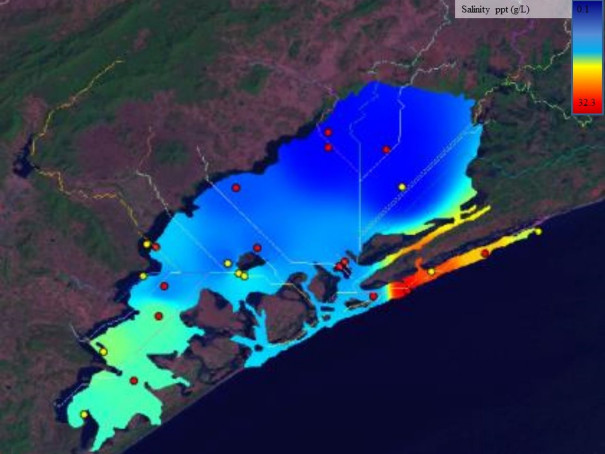
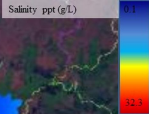
Fig. 8. Multiple scale heterogeneity of the spatial distribution of variables driving the composition of bacterial communities.

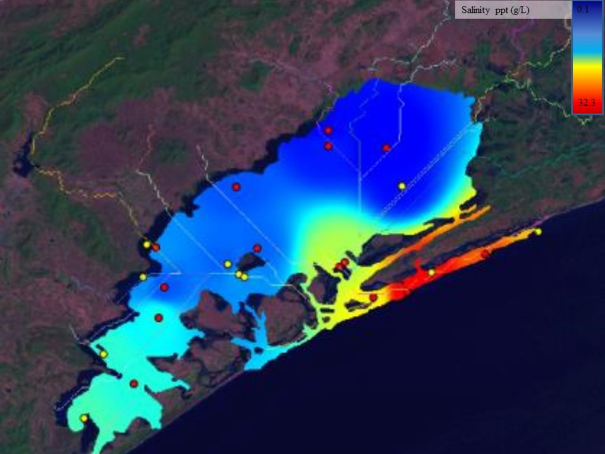
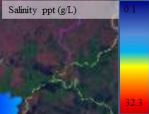
Fig. 9. Satellite view incorporating GIS stream analysis, fish farms (red polygons) and sampling points with relatively high abundance of single genus or family (yellow dot) and corresponding reduction in diversity.

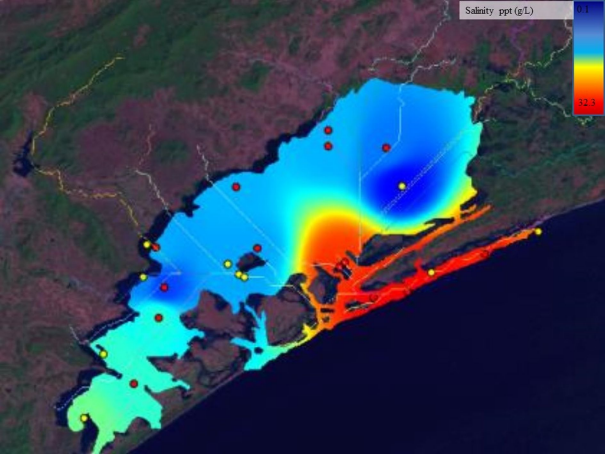
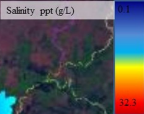
Sampling station map



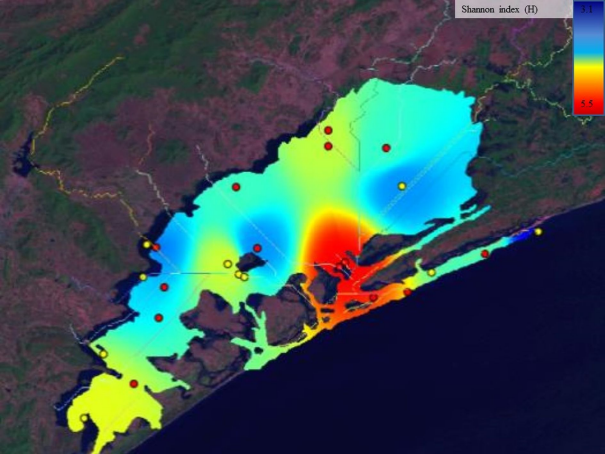


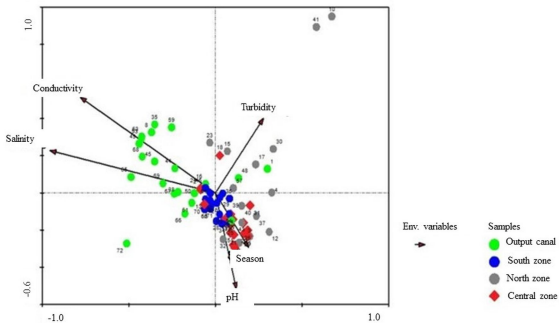


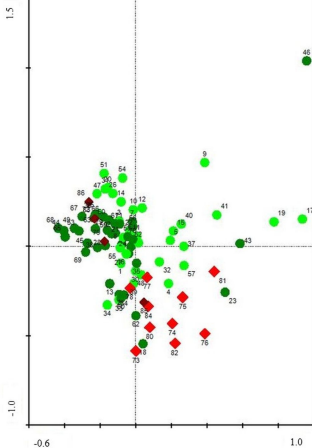




Shannon index (H)

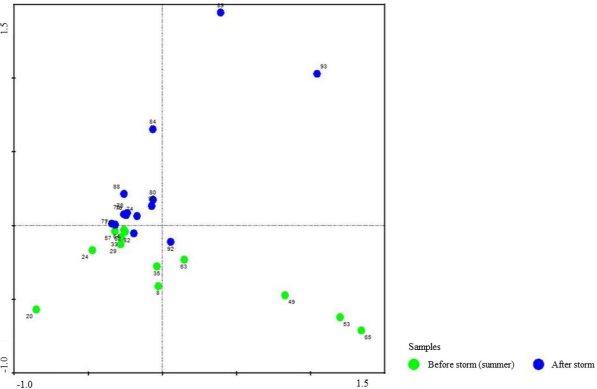


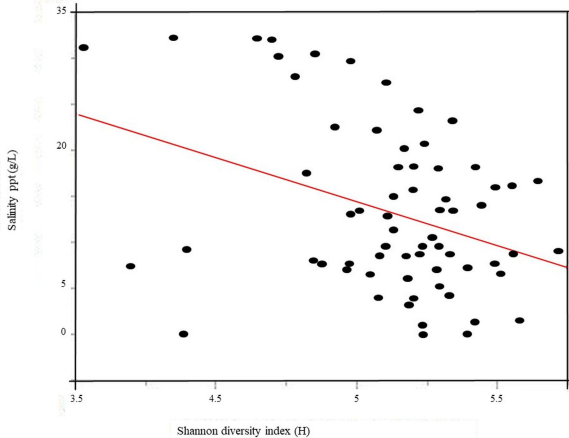


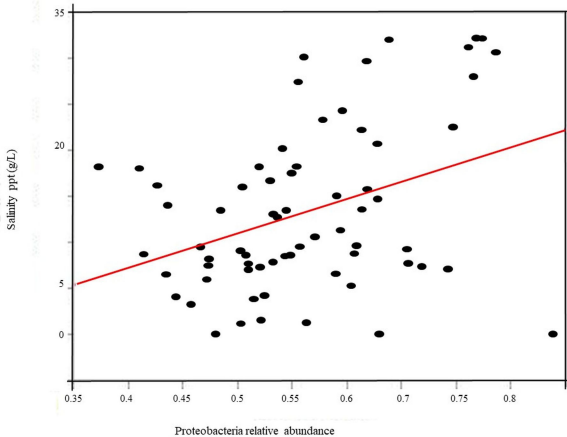


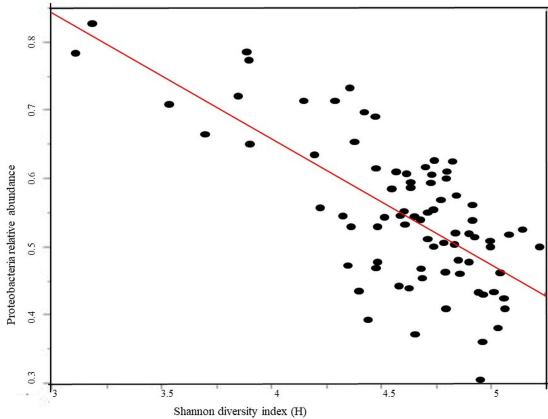
Samples

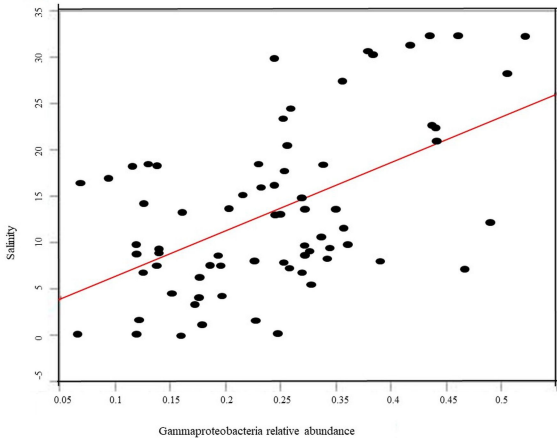
- High salinity
- Low salinity
- High salinity - after storm
- Low salinity - after storm











Field (lake) scale distribution
spatial pattern of physico-
chemical parameter : Salinity

