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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.

# Spatial analysis of bacteria in brackish lake sediment

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1    **Abstract**

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

12

13

## 14 **1. Introduction**

15 Microorganisms play a major role in global biogeochemical cycles of carbon, nitrogen, and phosphorus. With the growth rate  
16 of all microorganisms on earth estimated at over  $10^{30}$  cells/yr, these microorganisms are considered major drivers of  
17 geochemical cycling (Withman et al., 1998). Soils and sediment are among the largest microbial reservoirs. In addition to the  
18 high number of microorganisms, soils also have relatively high heterogeneity of physical, chemical, and biological  
19 characteristics (Beare et al., 1995; Ramette, 2007). This heterogeneity might influence reaction rates through the distances  
20 that metabolites must move between different bacteria and archaea in soils, yet, little is known about the spatial distribution  
21 of bacteria in soils (and less about sediment) and the drivers of the spatial heterogeneity. Several studies have focused on soil  
22 (Dechesne et al., 2003; Grundmann, 2004; Remenant et al., 2009; Vos et al., 2013) and sediment (Córdova-Kreylos et al.,  
23 2006; Piza et al., 2005) in order to understand spatial distribution of bacteria at the micro-scale and on soils at the macro-  
24 scale (Fierer & Jackson, 2006; Griffiths et al., 2009).

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

## 43 **2. Material and Methods**

### 44 *2.1. Study area*

45 Chilika Lake, which is the second largest brackish lagoon in the world and the largest in Asia, is situated on the east coast of  
46 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

47 of international importance (Ramsar Site). The size of the lagoon fluctuates significantly during a year from 906 km<sup>2</sup>  
48 (summer) to 1165 km<sup>2</sup> (monsoon). The hydrological pattern of Chilika Lake is influenced by three subsystems, namely,  
49 Mahanadi River distributaries, streams of the western catchment that bring in freshwater to the lake, and the Bay of Bengal  
50 that contributes highly saline sea-water. The lagoon is a collection of very shallow marine, brackish, and freshwater  
51 ecosystems. The whole area is categorically organized into four sectors. The northern sector, which receives direct discharge  
52 of freshwater from the Mahanadi Delta; the central sector intermixing zone, which is brackish; the southern sector, which has  
53 higher salinity levels as compared to central sector; and the outer channel, which exchanges water between the lagoon and the  
54 sea. Due to a high salinity gradient, the lake hosts a wide range of macro-organism biodiversity: 314 species of fish, 224  
55 species of water birds, and 729 angiosperms. That high biodiversity sustains an important population of fishermen estimated  
56 at about 140,000 individuals (Kumar & Pattanaik, 2012). After a decrease in fishing productivity in the 1990's, the lake water  
57 quality has been monitored and the lake mouth to the outer channel was reopened.

58

## 59 *2.2. Sampling and physico-chemical analyses*

60 The monitoring of the sediment and water was done at 24 stations spread over the entire lake (Fig. 1 – monitoring and  
61 sampling stations) in summer (March to June 2011), monsoon (July to October 2011), and winter seasons (November 2011 to  
62 February 2012). Sediment and water samples were collected from stations from all four ecological sectors; northern, central,  
63 southern, and outer channel (Fig. 1). Bottom surface sediment samples were collected with a Van Veen type of grab sampler  
64 (KC, Denmark,) from each station during summer, monsoon, and winter seasons. Both sediment and water (water column)  
65 samples were immediately transferred to sterile plastic 500 ml bottles and transported to the laboratory on ice and stored at  
66 4°C. Sediment samples were partially dewatered to improve lysis efficiency and homogenized manually with a sterile mortar  
67 and pestle inside a laminar flow hood.

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

76 Fig. 1

77

## 78 *2.3. GIS analysis (Land surfaces, hydrogeology and spatial analysis)*

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

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

#### 91 **Fig. 2**

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

94

#### 95 *2.4. DNA analysis*

96 Total DNA was extracted from 0.5 g of sediment sample in duplicate using FastDNA® *Spin* Kit for soil DNA extraction (MP  
97 Biomedicals, Santa Ana, CA, U.S.) as per the manufacturer's protocol. DNA was extracted in duplicates for each sampling  
98 station and pooled to one tube per individual station and season. Quality and quantity of metagenomic DNA were verified  
99 using 1.2% of agarose gel (0.5XTAE) and Nanodrop (Epoch, BioTek Instruments, U.S.) analysis ( $A_{260}/A_{280}$ ). DNA from a  
100 total of 72 samples from the 24 station (3 seasons) from 2011 and 2012 and twelve more samples after the "Phailin", a very  
101 severe cyclonic storm (VSCS) which made landfall near  $19.26^{\circ}\text{N} / 84.82^{\circ}\text{E}$  (near Gopalpur, Odisha) at 1600 UTC on 12th  
102 October, 2013 were extracted to determine bacterial diversity and community composition. Total DNA extracted from the  
103 sediment was amplified for 16S rRNA genes by Polymerase Chain Reaction (PCR). The hypervariable (V4-V6) region of the  
104 16S rRNA genes was amplified from using the bacterial primer pair 515F and 1061R (16S-0515F 5'-  
105 TGYCAGCMGCCGCGGTAA-3' 16S-1061R 5'-TCACGRCACGAGCTGACG-3' ~560bp V4-V6 region (M774, Promega,  
106 Madison, WI, U.S.)). PCR was done with the modified primers containing an adapter and a barcode sequence (Schloss et al.,  
107 2009). Paired end 454 pyrosequencing was done on the GS-FLX 454 Titanium platform.

108

#### 109 *2.5. Analysis of the community composition*

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

## 116 2.6. *Statistical and multivariate analysis*

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

124

## 125 3. Results

126

### 127 3.1. *Physico-chemical distribution in the lake and spatial distribution of bacteria*

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

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



142 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  
143  $\mu\text{mol/L}$ ) and ammonia (7.31 to 206.41  $\mu\text{mol/L}$ ) generally were low.

144 Fig. 3

### 145 3.2. GIS analysis (land surfaces, hydrogeology and spatial analysis)

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

### 152 3.3- Statistical analysis

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

### 159 Fig. 4

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

### 165 Fig. 5

166 The same twelve sample points were compared before and after the storm (Fig. 6). A small shift in the community structure  
167 occurred after the storm probably due to the mixing of the sediment (sediment was sampled at a water depth between 1 and  
168 3.2 m) and a major influx of organic matter from the watershed. The sample points located near the ocean were not  
169 significantly different after the storm. The major change during the storm is the intrusion of saltwater in to the lake, affecting  
170 principally the zone that used to have low salinity.

### 171 Fig. 6

### 172 3.4. Analysis of the microbial community structure

173 The number of reads per samples were between 5,387 to 38,265. Taxonomy was done using QIIME pipeline and the RDP  
174 database. The most dominant phylum in the current sample was *Proteobacteria* with a relative abundance up to 0.79 and  
175 never under 0.37 (average 0.573). A second more abundant phylum was the *Chloroflexi* (0.01 to 0.24, average 0.086). In  
176 order, the next phyla were *Bacteroidetes* (0.006 to 0.17, average 0.047), *Planctomycetes* (0.001 to 0.08, average 0.038),  
177 *Acidobacteria* (0.002 to 0.07, average 0.031), *Firmicutes* (0.0004 to 0.15, average 0.028 ), *Gemmatimonadetes* (0.002 to  
178 0.08, average 0.027), *Actinobacteria* (0.004 to 0.08, average 0.023), *Verrucomicrobia* (0.0004 to 0.014, average 0.021) and  
179 *Nitrospirae* (0.0005 to 0.06, average 0.017). Looking more specifically at *Proteobacteria* (the largely dominant phylum in all  
180 the samples), a specific distribution was observed at the class level. The most dominant class of *Proteobacteria* was  
181 *Gammaproteobacteria* with an average relative abundance of 0.252, followed by *Deltaproteobacteria* (0.178),  
182 *Betaproteobacteria* (0.066), *Alphaproteobacteria* (0.045), and *Epsilonproteobacteria* (0.026). The high abundance of  
183 *Gammaproteobacteria* was mainly due to four orders, *Chromatiales* (up to 0.19), *Xanthomonadales* (up to 0.12),  
184 *Pseudomonadales* (up to 0.21) and *Thiotrichales* (up to 0.21). The dominance of *Gammaproteobacteria* is similar to what  
185 have been describe in marine sediment (Fang et al. 2017). Some families and genus were also measured at a high relative  
186 abundance in some of the current samples: *Piscirickettsiaceae* (up to 0.21 relative abundance) and *Pseudomonas* (up to 0.09).  
187 *Acidobacteria* was observed at a relatively high abundance even if the pH was above 7 in almost every part of the lake. No  
188 correlation between the *Acidobacteria* relative abundance and pH was observed.

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

194 Fig. 7

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

#### 200 4. Discussion of the drivers of the community structure

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

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

210 The distributions of other variables measured in the current study had either a much higher or a much lower spatial scale of  
211 variation. Water temperature and depth that could, respectively, correspond to temporal and spatial distribution were not  
212 significantly correlated to community structure, probably due to the relative homogeneity of those parameters in Chilika  
213 Lake. pH has been described to have strong effect on regulation of life processes and nutrient availability in aquatic systems  
214 (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  
215 correlated to the spatial distribution of bacteria and diversity index. Although the relative abundance of *Acidobacteria* has  
216 been described to correlate with soil pH (Fierer & Jackson, 2006; Griffith et al., 2011), no correlation between the pH and the  
217 relative abundance of the phylum *Acidobacteria* nor with the genera within this phylum was found.

218 The higher scale of variation would affect all the samples, making it difficult to measure their effect on the spatial  
219 distribution of the bacteria in the sediment (climatic conditions, watershed land cover). On the other hand, the distribution  
220 and variability of nitrite, nitrate, ammonia, phosphate, silicate, total iron, and total organic carbon occurred at a much smaller  
221 scale than the scale of the current samples. Hotspots for those variables are visible at the micro-scale (Parkin, 1987; Schramm  
222 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  
223 used to do chemical analysis and 16S rRNA gene analysis hindered finding a correlation between those micro-scale variables  
224 and potential drivers of the microbial community structure at the scale of the current study (Fig. 8).

225 Fig. 8

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

237 Fig. 9

238 **5. Conclusion**

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

251

252

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258  
259

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261

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411 **List of captions**

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

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

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

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

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

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

426 **Fig. 7.** a) Effect of salinity on the diversity (Shannon Index); b) Effect of salinity on *Proteobacteria* relative abundance  
427 (coefficient of determination ( $R^2$ ) of 0.1896,  $p < 0.0002$ ); c) Effect of the relative abundance of *Proteobacteria* and diversity  
428 (Shannon Index); and d) Effect of salinity on *Gammaproteobactria* relative abundance,  $R^2$  of 0.2829,  $p < 0,0001$

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

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

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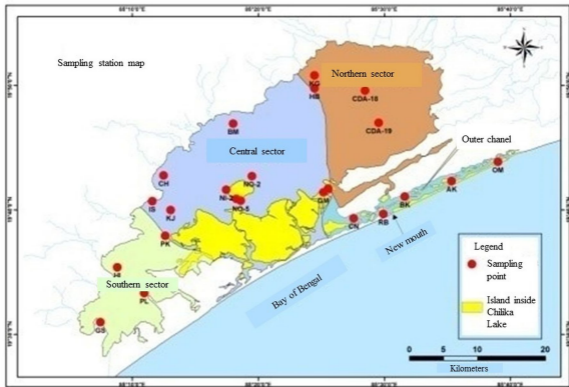
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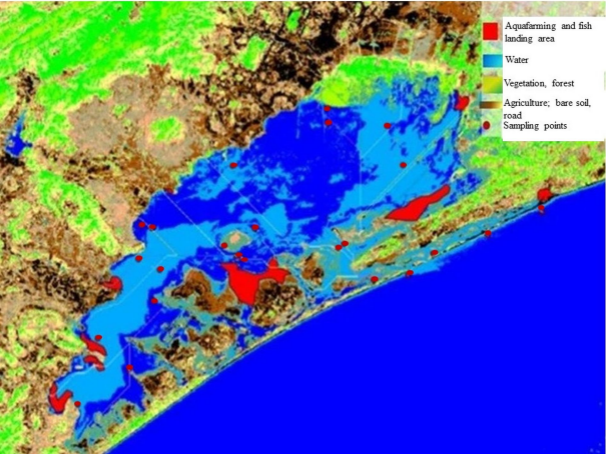
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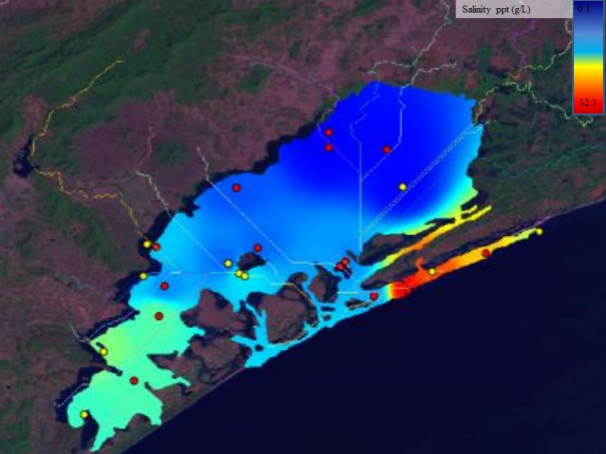
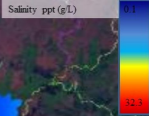
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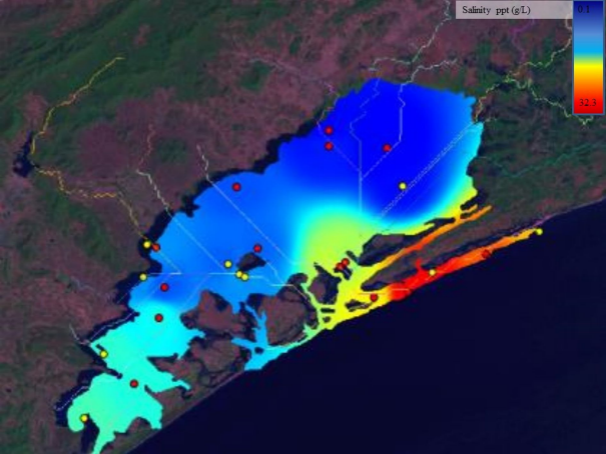
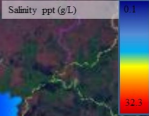
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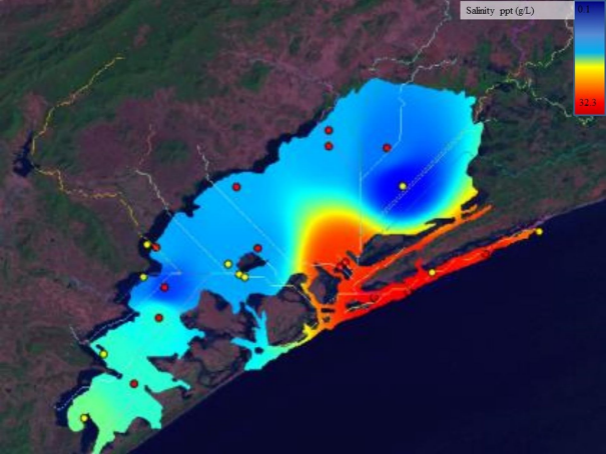




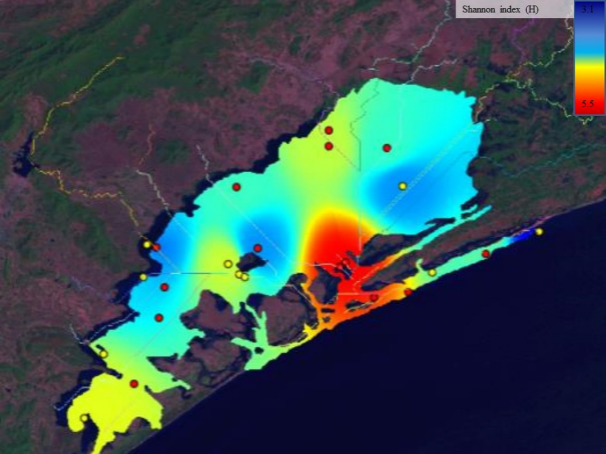


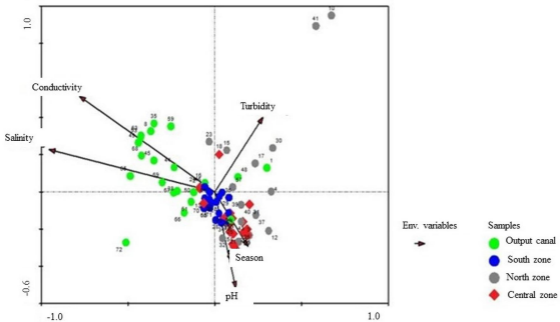


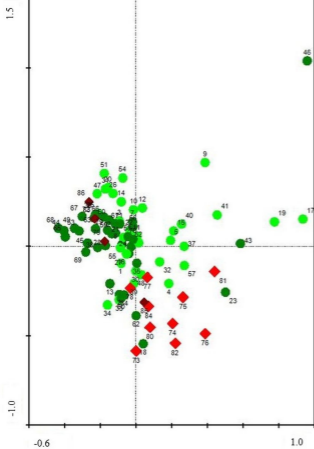
Salinity ppt (g/L)



Shannon index (H)



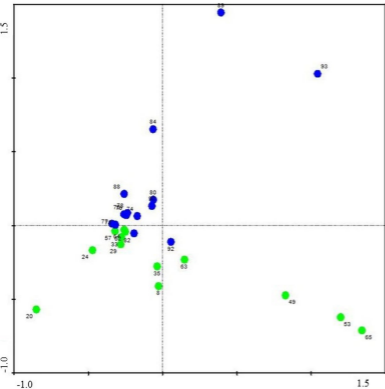




Samples

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





Samples

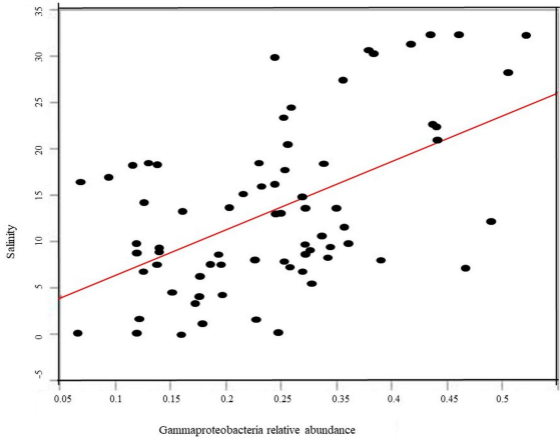
● Before storm (summer)

● After storm









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

