

## **Influence of salinity stress on bacterial community composition and $\beta$ -Glucosidase activity in a tropical estuary: Elucidation through microcosm experiments**

**Ranjith Eswaran, Lidita Khandeparker\***

CSIR-National Institute of Oceanography, Dona Paula, Goa-403004, India.

\*Corresponding author. Tel.: +91(0)832-2450432; fax: +91(0)832-2450615.

Email: [klidita@nio.org](mailto:klidita@nio.org)

### **Abstract**

The influence of changing salinity on community composition and functional activity (Bacterial Production (BP) and ectoenzyme activity) of major bacterial taxa was evaluated using microcosm experiments in a tropical monsoon influenced estuary. Natural bacterial inocula at different salinities, representing marine, brackish, and freshwater, were inter-transferred and elucidated their response with an emphasis on community composition and  $\beta$ -Glucosidase (BGase) activity. The results revealed a significant decrease in the total bacterial count (TBC) and BP on the translocation of bacterial inocula to different salinity conditions in the case of freshwater bacteria. However, a significant increase in BGase activity coupled with shifts in the studied bacterial groups was evident in the case of marine as well as freshwater bacteria. Quantitative PCR (qPCR) revealed a shift in major bacterial taxa upon translocation to different waters, which was dependent on salinity and the source of inocula. Redundancy and qPCR analyses showed that members belonging to Gammaproteobacteria and Betaproteobacteria were higher, and possibly influenced BGase activity in marine and freshwater, respectively. Translocation of marine inocula to brackish and freshwater resulted in an emergence of Bacteroidetes, Actinobacteria, and Betaproteobacteria, respectively. Whereas, when freshwater inocula were translocated to marine or brackish water, Alphaproteobacteria and Gammaproteobacteria taxa emerged, and this was coupled with increased BGase activity. In contrast, brackish water bacteria showed a strong persistence in bacterial community composition when translocated to different salinities within this estuary. Such phylogenetic persistence or changes suggests species level shifts in specific bacterial taxa, and unravelling the same using different functional gene markers would ascertain their role in organic matter processing and is way forward.

**Keywords:** Quantitative PCR, Salinity stress, brackish water, Heterotrophic bacteria, Community shift, Freshwater, Estuaries, Ectoenzyme, Bacterioplankton

## 1. Introduction

Estuaries are highly unique systems, where the mixing between the river and ocean water results in steep gradients of different abiotic components that include salinity, temperature, inorganic, and organic nutrients (Santos et al., 2014). Also, such steep environmental gradients significantly influence the estuarine microbial communities either by altering their community composition (Campbell and Kirchman, 2013), or the metabolic functions (Cunha et al., 2001). Among the different bacterial metabolic functions, degradation of organic matter (OM) is one of the major pathways wherein the nutrients are actively reinstated to the food webs (Hu et al., 2014) and are catalyzed by different extracellular hydrolytic enzymes or ectoenzymes (Hoppe, 1983). However, the production of these ectoenzymes by estuarine microbial communities is regulated by the nature of substrate, concentration, as well as the community composition. Moreover, environmental conditions can also modify the enzymatic activities and community composition in which salinity is the prime factor (Cunha et al., 2001). In estuaries, salinity increase or decrease is known to influence the functional activities of the microorganisms. Furthermore, along the estuarine salinity gradient, the combination of marine and riverine influence results in higher bacterial diversity due to the replenishment of bacterial species (Herlemann et al., 2014). Several previous studies have reported a salinity effect on ectoenzymes in both marine and freshwater environments (Cunha et al., 2001; Santos et al., 2009). Results from field and experimental observations show an influence of salinity on bacterial community composition (BCC) (Langenheder et al., 2003; Crump et al., 2004; Herfort et al., 2017; Tang et al., 2012). For instance, Alphaproteobacteria, Gammaproteobacteria, and SAR11 were dominant. They efficiently assimilated OM in marine systems (Cottrell and Kirchman, 2000), while in the estuaries, Bacteroidetes played a dominant role in the degradation of the OM pool (Crump et al., 2004). Contrastingly, Betaproteobacteria efficiently consumes riverine OM and is predominant in freshwater environments (Herlemann et al., 2011). However, very few observations have simultaneously reported the effects of salinity fluctuations on BCC and the ectoenzymes, which they produce (Bullock et al., 2017; Cunha et al., 2000; Langenheder et al., 2003). Such studies are sparse in tropical estuaries. A recent study by Eswaran and Khandeparker (2017) reported that along the estuarine salinity gradient, bacterial abundance, and ectoenzyme expression were high at the saline mouth and during the dry season. Furthermore, high expression was related to Gammaproteobacteria taxa, specifically Pseudomonadales. Whereas, during monsoon, it was Enterobacteriales, which showed high expression (Eswaran and Khandeparker, 2019). The tropical estuaries are inimitable due to the short episodic monsoon spells, which bring high freshwater runoff

resulting in the estuary to be in the non-steady state when compared to the temperate estuaries (Vijith et al., 2009).

Zuari estuary is one tropical estuary located at Goa, West Coast of India, which discharges into the Arabian Sea (Fig. 1A). Salinity characteristics of this estuary differ significantly from the low runoff/dry period during November - May to the high runoff period during the summer monsoon from June to September (Shetye et al., 2007). The run-off also contribute to the introduction of high numbers of allochthonous freshwater or terrestrial bacterial communities (Divya et al., 2009; Eswaran and Khandeparker, 2019). During the transport in estuaries, increasing salinity could be a challenging factor for the proliferation of freshwater bacteria as compared to autochthonous estuarine bacteria. Other than bacterial communities, riverine discharge also deposits a high amount of allochthonous materials rich in carbohydrates. Among the carbohydrates, structural polysaccharides are at higher concentration in estuaries due to their recalcitrant nature, when compared to storage polymers (Khodse et al., 2010), and  $\beta$ -Glucosidase (BGase) activity is a useful proxy in assessing their degradation. Our field observations have indicated a shift in the major bacterial taxa from low saline-monsoon to high saline-non monsoon seasons that influence BGase activity/expression in this estuary (Eswaran and Khandeparker, 2017, 2019). Likewise, earlier Del Giorgio and Bouvier (2002) reported that particular bacterial taxa markedly respond to salinity either by increase or decrease in growth and cell-specific activities. Therefore, controlling factors such as salinity fluctuations might act as a barrier to estuarine bacterial communities, resulting in either 'perish' of some species or 'flourish' of others, and that influence the OM degradation. In order to validate this hypothesis, we conducted a series of experiments wherein natural bacterial inocula from different locations of Zuari estuary were inter-transferred in microcosms and examined the response of marine, brackish, and freshwater bacteria to different salinity conditions with an emphasis on major bacterial taxa and BGase activity.

## **2. Materials and Methods**

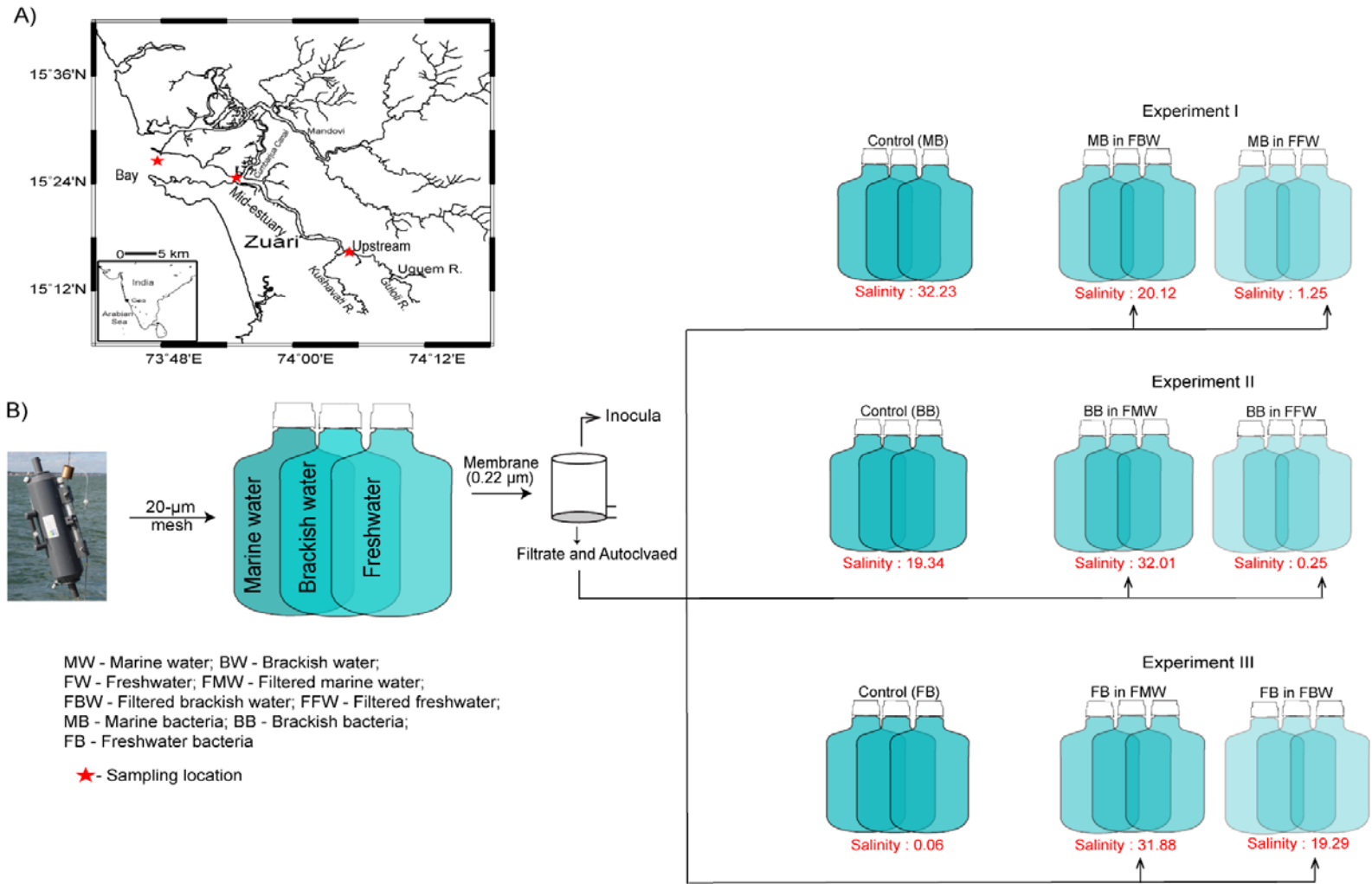
### **2.1. Study area and Field sampling**

Water samples used in the present study were collected from Zuari estuary located in Goa, West coast of India (Fig. 1A). Surface water samples were collected using 5L Niskin water sampler from three different locations based on the salinity representing marine (bay-32.23; 15° 25' 16.9" N 73° 47' 36.9" E), brackish (mid-estuary-19.34; 15° 24' 32.0" N 73° 54' 50.2" E), and freshwater (upstream-0.06; 15° 16' 01.1" N 74° 06' 36.0" E) (Fig. 1A, B). After collection, 200L of water samples from all three stations were passed through 20  $\mu$ m mesh and stored in acid-washed Nalgene bottles (rinsed

with 10% HCl followed by MilliQ water), transported to the laboratory, and used for microcosm experiments.

## **2.2. Microcosm setup**

The culture media were prepared by filtering 20 L of water samples from each station (i.e., marine, brackish, and freshwater ( $n = 3 \times 3$ )) through the manifold filtration system fitted with multiple polycarbonate filter papers, which has 0.22  $\mu\text{m}$  pore size and 47 mm diameter (HTTP04700; Millipore, USA). Subsequently, the filtrates were autoclaved and kept in the dark. Meanwhile, filter papers with particulate materials were stored in sterile Petri dishes (GEN-TCD-60-S, Genaxy, India) at 4 °C until the filtrates were autoclaved. The particulate materials contained the particles which are smaller than 20  $\mu\text{m}$  in size such as, nanophytoplankton (3–20  $\mu\text{m}$ ), picophytoplankton (0.2 to 3  $\mu\text{m}$ ), bacteria (0.2 to 2  $\mu\text{m}$  free-living; > 2  $\mu\text{m}$  particle-associated), and suspended particles (inorganic and organic). In translocation experiment I, the marine bacterial inocula were transferred to filtered and autoclaved fresh, brackish, and marine waters. In experiment II, the brackish water bacterial inocula were transferred to filtered and autoclaved fresh, brackish, and marine waters, respectively. In experiment III, the freshwater inocula were transferred to filtered and autoclaved fresh, brackish, and marine waters, respectively (Fig. 1B). The bacterial inocula, which were retained in the water of the same station, were referred to as controls (Fig. 1B). Hereafter, for simplicity, the filtered and autoclaved fresh, brackish, and marine waters are referred to as fresh, brackish, and marine waters, respectively. The experiments were continued for 21 days under 12 hr light: dark cycle at a controlled temperature ( $26 \pm 2$  °C). Further, incubated bottles were agitated periodically everyday throughout the experiment to avoid the aggregation of suspended particles at the bottom. Before sub-sampling, the incubated bottles were mixed thoroughly and 2.1 L of water from each experiment was transferred to clean beakers for the estimation of dissolved inorganic nutrients, transparent exopolysaccharides (TEP), suspended particulate matter (SPM), dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*), total bacterial count (TBC), bacterial production (BP), major bacterial taxa, and BGase activity. Sub-samples were collected on days 0, 1, 4, 7, 11, 17, and 21, respectively.



**Fig. 1(A)** Sampling locations in Zuari estuary; **(B)** Schematic representation of the experimental setup

### **2.3. Nutrient analyses**

Dissolved nutrient concentrations (silicate, phosphate, nitrite, and nitrate) were determined colorimetrically with an automated nutrient analyzer (SKALAR SAN PLUS 8505 Interface V3.331, Netherland) following the protocol described in Grasshoff et al. (1983).

### **2.4. Suspended particulate matter (SPM), dissolved organic carbon (DOC) and Chlorophyll-a (Chl a) analyses**

SPM was enumerated by filtering 100 mL of water sample through the pre-combusted, weighed GF/F (0.7  $\mu\text{m}$ , Whatman) 25 mm filter papers, and subsequently retained materials were dried in an oven at 60 °C for 24 hr. After drying, the samples were reweighed, and values are expressed as mg L<sup>-1</sup>. Water samples for DOC analysis were filtered through 0.22  $\mu\text{m}$  acetate cellulose filter papers and acidified with 2 % H<sub>2</sub>SO<sub>4</sub> and analysed using a total organic carbon analyser (Shimazu; Model TOC-L), and the values are expressed as mg L<sup>-1</sup>. Chl *a* was measured by filtering 100 mL of water samples through GF/F filter papers, followed by overnight extraction using 90 % acetone at 4 °C in the dark. After extraction, Chl *a* concentration was determined using a fluorometer (Turner, 10-AU) that was standardized with Chl *a* standard (Sigma, USA).

### **2.5. Transparent exopolysaccharides (TEP) estimation**

TEP were quantified using the modified alcian blue method described by Klein et al. (2011). Briefly, 250 mL of water samples from each experiment were filtered through 0.4  $\mu\text{m}$  polycarbonate filter papers (HTTP 4700; Millipore, USA), and materials retained on the papers were scrapped using GF/F filtered seawater. The resuspended materials were centrifuged at 3200  $\times g$  for 10 min, and obtained pellets were mixed with 2 mL of 0.02% alcian blue (prepared using 0.06 % of acetic acid). Subsequently, the mixtures were centrifuged at 3200  $\times g$  for 20 min to remove excess dye. The stained pellets were rinsed with 2 mL of distilled water and centrifuged. The rinsing step was repeated until the supernatant has become clear. Acid digestion of stained pellets was done using 4 mL volume of 80% H<sub>2</sub>SO<sub>4</sub> and kept for 2 hr. After incubation, absorbance was measured at 787 nm by spectrophotometer (UV-VIS-2550; SHIMADZU) against alcian blue as a blank solution. A standard calibration curve was obtained using Xanthan Gum following the above protocol. The measured TEP are expressed as microgram Xanthan Gum equivalent per litre ( $\mu\text{g XG equiv L}^{-1}$ ).

## **2.6. Flow cytometry analyses**

### **2.6.1. Enumeration of the total bacterial count (TBC)**

Subsamples of 4.5 mL were collected in triplicates from each experiment and fixed with filtered paraformaldehyde (1 % final concentration), stored at  $-80\text{ }^{\circ}\text{C}$  until analyses. During analyses, samples were brought back to room temperature, and aliquots of 1 mL were stained with SYBR Green I (1:10000 final concentration; Molecular Probes, USA) and incubated in the dark for 15 min. Subsequently, the stained samples were analysed with BD FACS Aria II flow cytometer configured with a 488 nm nuclear blue laser. The TBC was recorded based on cell granularity (side scatter signal (SSC)) and green fluorescence from SYBR Green I nucleic acid stain (Khandeparker et al., 2017). The SSC and DNA fluorescence data from the cytometer were processed using built-in BD FACS Diva version 6.2 software. Yellow-green fluorescence size beads (1  $\mu\text{m}$ ) were used as an internal standard to calibrate the SSC and FL-1 channel (Polysciences, USA).

### **2.6.2. Bacterial production**

Bacterial production (BP) was measured using 5-Bromo-2-deoxyuridine (BrdU), which is a synthetic nucleoside and an analog of thymidine (Eswaran and Khandeparker, 2017; Khandeparker et al., 2018). Subsamples from each experiment were incubated with 1  $\mu\text{M}$  final concentration of BrdU for 3 hr at in situ temperature in dark condition. After incubation, the bacterial activities were stopped by adding filtered paraformaldehyde (1% final concentration) and stored at  $-80\text{ }^{\circ}\text{C}$  until immunofluorescent detection. The immunofluorescent detection was performed using the anti-BrdU antibody labeled with FITC fluorochrome. In brief, anti-BrdU with DNase I was added to 1 mL of BrdU incorporated water samples and incubated for 30 min in the dark, and subsequently, anti-BrdU labeled cells were counted using BD FACS Aria II flow cytometer with 488 nm nuclear blue laser. Bacterial carbon production was calculated using 11 fg C per bacterium as a cell-to-carbon conversion factor, and obtained values are expressed as  $\mu\text{g C L}^{-1} \text{ h}^{-1}$  (Garrison et al., 2000).

## **2.7. Ectoenzyme analyses**

### **2.7.1. Total $\beta$ - Glucosidase (BGase) activity**

Total BGase activity (includes cell-associated and free enzyme) was estimated by measuring the fluorescence released from 4-methylumbelliferyl (MUF)  $\beta$  - D-glucosidase substrate as described by Hoppe (1983). The working stock was prepared by diluting the MUF tagged substrate in sterile seawater with the addition of methyl cellosolve. A standard curve was prepared using a series of MUF  $\beta$  - D-glucosidase substrate (Sigma, USA) concentrations ranging from 10nM to 500nM, which were incubated in triplicates with 10 mL seawater for an incubation period ranging from 30min

to 24 hr. Based on the substrate concentration and linearity of the observed fluorescence, the standard concentration of 50 nM and an incubation period of 3 hr were selected for this study. For sample analyses, 10  $\mu\text{L}$  of the MUF substrates (50 nM final concentration) were added to 10 mL of experimental samples and incubated for 3 hr. After incubation, 100  $\mu\text{L}$  of Tris – HCl buffer (pH 10.3) was added to each 1 mL of the sample mixture, and fluorescence was measured using a fluorescence spectrophotometer (Shimadzu RF5031) equipped with 365-440nm excitation and emission wavelength. The rate of enzyme activity is expressed as  $\text{nM C L}^{-1} \text{ h}^{-1}$ .

### **2.7.2. Enumeration of the number of cells expressing $\beta$ -Glucosidase**

Fluorescein di- $\beta$ -D-glucopyranoside (FDGlu; Molecular Probes) was used to enumerate the number of cells expressing  $\beta$ -Glucosidase (BGase) from the TBC. Briefly, water samples were incubated in the dark with FDGlu, which is a substrate for BGase. During the incubation, BGase expressing bacteria cleaved the non-fluorescent FDGlu to highly fluorescent fluorescein. Subsequently, the fluorescence from BGase expressing bacterial populations were enumerated using a flow cytometer equipped with a 488 nm nuclear blue laser. 1 $\mu\text{m}$  yellow-green fluorescent beads were used as an internal standard for calibrating the fluorescence detector voltage. Obtained data were processed using BD FACS Diva software version 6.2 (Eswaran and Khandeparker, 2017).

### **2.8. Environmental DNA extraction**

For extraction of environmental DNA, 1.5L volume of subsamples from each experiment were filtered through 0.22 $\mu\text{m}$  pore size filter papers (GSWP04700; Millipore) under constant low vacuum pressure and stored at -20  $^{\circ}\text{C}$  until the extraction. The DNA extraction was carried out in batches soon after the filtration (not more than 24 hr) using a power water DNA isolation kit (MOBio, USA) according to manufacturer's instructions.

### **2.9. Quantitative PCR (Q-PCR) analyses**

#### **2.9.1. Calibration of the standard curve**

Before environmental DNA analyses, the standard curves were obtained for different bacterial taxa using group-specific primers that are listed in Table 1. Q-PCR standards were prepared using genomic DNA (gDNA) that were extracted from different bacterial pure cultures, which are classified under different taxa (For instance, *Exiguobacterium indicum* used for Firmicutes and *Serratia marcescens* for Gammaproteobacteria primer set). Briefly, amplified PCR fragments were purified using pure link PCR purification kit (Invitrogen, USA) and pooled to specific concentrations (Checked using Bio spec, Eppendorf) that were diluted to six orders of magnitude ranging from  $10^9$  to  $10^4$  gene copies  $\mu\text{L}^{-1}$ , and used as standards. The standard curves were generated for each bacterial



taxa by plotting the cyclic threshold ( $C_T$ ) values against the concentration of each diluted standard. The  $C_T$  indicates the number of cycles taken for the fluorescence signal to exceed the threshold level. All the standard curves, which were generated during the study, were linear (Table 1) with efficiency ranging from 0.93 to 1.12.

### **2.9.2. Q-PCR assay of experimental samples**

The experimental samples were analysed in triplicates with Rotor-Gene Q 5X HRM machine (Qiagen) using 2X Quantitech SYBR Green master mix in 20- $\mu$ L reaction volume (Qiagen). The reaction mixture consisted of 10  $\mu$ L of the qPCR master mix, 0.5  $\mu$ L of each primer, 1  $\mu$ L of environmental DNA template, and 8  $\mu$ L of RNase free water. The raw fluorescence signals from amplification were captured through the green channel at 510 nm with gain settings of 5. Subsequently, data were analysed using built-in Rotor-gene Q software wherein  $C_T$  values of amplified samples were compared with the standard calibration curve. Further, high-resolution melt (HRM) curve analyses were performed to test the specificity of the primers with target samples by increasing the temperature from 70 to 90 °C with 1°C interval. A single bell shape peak at 84 – 86 °C indicated the presence of site-specific qPCR amplicon (Eswaran and Khandeparker, 2017).

**Table 1:** Quantitative PCR primers and conditions used in this study

S.no	Target group/Gene	Primer sequence (5'----3')	Reference	Modified annealing temperature used in this study ( °C)	Q-PCR standard curve (slope and Efficiency)
1.	Actinobacteria	<b>ACT920F3:</b> ACGGCCGCAAGGCTA <b>ACT1200R:</b> TCRTCCCCACCTTCCTCCG	De Gregoris et al., 2011	53	$Y = -5.013x + 49.596$ ; $R^2 = 0.995$ ; $E = 1.12$
2.	Alphaproteobacteria	<b>ALF682F:</b> CIAGTG TAGAGGTGAAATT <b>ALF908R:</b> CCCC GTCAATTCCTTTGAGTT	De Gregoris et al., 2011	53	$Y = -3.100x + 32.011$ ; $R^2 = 0.995$ ; $E = 1.1$
3.	Betaproteobacteria	<b>BETA359F:</b> GGGGAATTTTGGACAATGGG <b>BETA682R:</b> ACGCATTCACTGCTACACG	Ashelford et al., 2002	53	$Y = -3.232x + 30.936$ ; $R^2 = 0.997$ ; $E = 1.04$
4.	Gammaproteobacteria	<b>GAM1080F:</b> TCGTCAGCTCGTGTYGTGA <b>GAM1202R:</b> CGTAAGGGCCATGATG	De Gregoris et al., 2011	53	$Y = -3.468 + 33.271$ ; $R^2 = 0.996$ ; $E = 0.99$
5.	Bacteroidetes	<b>CFB798F:</b> CRAACAGGATTAGATACCCT <b>CFB967R:</b> GGTAAGGTTCCCTCGCGTAT	De Gregoris et al., 2011	53	$Y = -3.065x + 34.067$ ; $R^2 = 0.997$ ; $E = 1.1$
6.	Firmicutes	<b>FIRM928F:</b> TGAAACTYAAAGGAATTGACG <b>FIRM1040R:</b> ACCATGCACCACCTGTC	De Gregoris et al., 2011	53	$Y = -3.065x + 34.067$ ; $R^2 = 0.996$ ; $E = 1.12$

## 2.10. Statistical analyses

TBC, BP, total BGase activity, number of cells expressing BGase, and major bacterial taxa data were  $\log(X + 1)$  transformed to normalize the data. The transformed data sets, which met the normality assumption, were subjected to parametric statistical analysis. A parametric two-way ANOVA followed by post hoc Tukey's HSD test was performed to determine the significant variation in bacterial parameters between treatments ( $n = 3$ ) and incubation days ( $n = 7$ ) (IBM statistics, SPSS version 22.0). Furthermore, the relationship between major bacterial taxa, total BGase activity, number of cells expressing BGase, and environmental variables was evaluated using the correlation matrix (IBM statistics, SPSS v 22.0) and redundancy analysis (RDA). Before RDA, the biotic datasets were evaluated for linear characteristics by performing detrended correspondence analysis (DCA) (ter Braak and Smilauer 2002). In DCA, the length of the first gradient axes was  $< 2$  standard deviation (SD) units, which confirmed the linear characteristics, and thus RDA was applied. During RDA, firstly, scaling was focused on the inter-species correlations, and obtained species scores were divided by SD. Besides, species data sets were standardized by the error variance. Secondly, the significance of the RDA model was tested using a Monte Carlo permutation test with 999 permutations and combination. The species response and relationship with environmental variables was predicted using generalized linear models (GLM) in a stepwise manner followed by  $F$ -statistics at the significance level of  $< 0.05$ . The above statistics were executed using CANOCO software version 4.5 for Windows.

**Table 2** Background / Initial condition after passing through 20- $\mu$ m mesh during the sampling in Zuari estuary

### 3. Results

<b>Background/Initial conditions</b>				
<b>S.No</b>	<b>Parameters</b>	<b>Marine/ Bay</b>	<b>Brackish/ Mid-estuary</b>	<b>Freshwater/ Upstream</b>
	<i>Abiotic components</i>			
<b>1.</b>	Salinity	32.23	19.34	0.06
<b>2.</b>	Chl <i>a</i> (mg L <sup>-1</sup> )	0.57	0.88	0.22
<b>3.</b>	SPM (mg L <sup>-1</sup> )	49.45	76.9	52.4
<b>4.</b>	DOC (mg L <sup>-1</sup> )	1.42	1.05	1.49
<b>5.</b>	TEP ( $\mu$ g XG eq. L <sup>-1</sup> )	397.20	444.44	518.70
<b>6.</b>	Nitrite ( $\mu$ Mol L <sup>-1</sup> )	2.08	0.80	0.40
<b>7.</b>	Nitrate ( $\mu$ Mol L <sup>-1</sup> )	0.56	1.23	5.95
<b>8.</b>	Phosphate ( $\mu$ Mol L <sup>-1</sup> )	3.52	4.47	3.26
<b>9.</b>	Silicate ( $\mu$ Mol L <sup>-1</sup> )	12.33	28.94	73.58
	<i>Biotic components</i>			
<b>10.</b>	TBC (cells mL <sup>-1</sup> )	$1.95 \times 10^6$	$3.11 \times 10^6$	$2.09 \times 10^6$
<b>11.</b>	BP ( $\mu$ g C L <sup>-1</sup> h <sup>-1</sup> )	25.91	14.20	11.97
<b>12.</b>	Total BGase activity (nM C L <sup>-1</sup> h <sup>-1</sup> )	240.00	185.80	215.25
<b>13.</b>	No of cells exp BGase (cells mL <sup>-1</sup> )	$4.21 \times 10^5$	$2.34 \times 10^5$	$7.56 \times 10^4$
<b>14.</b>	Alphaproteobacteria (copies $\mu$ L <sup>-1</sup> )	$2.40 \times 10^5$	$4.13 \times 10^4$	$1.37 \times 10^4$
<b>15.</b>	Betaproteobacteria (copies $\mu$ L <sup>-1</sup> )	$1.93 \times 10^3$	$1.95 \times 10^2$	$1.26 \times 10^5$
<b>16.</b>	Gammaproteobacteria (copies $\mu$ L <sup>-1</sup> )	$1.53 \times 10^5$	$3.75 \times 10^4$	$4.09 \times 10^3$
<b>17.</b>	Bacteroidetes (copies $\mu$ L <sup>-1</sup> )	$6.10 \times 10^5$	$1.16 \times 10^5$	$1.19 \times 10^5$
<b>18.</b>	Firmicutes (copies $\mu$ L <sup>-1</sup> )	$7.63 \times 10^4$	$1.42 \times 10^4$	$1.94 \times 10^3$
<b>19.</b>	Actinobacteria (copies $\mu$ L <sup>-1</sup> )	$6.41 \times 10^4$	$6.80 \times 10^4$	$4.26 \times 10^4$

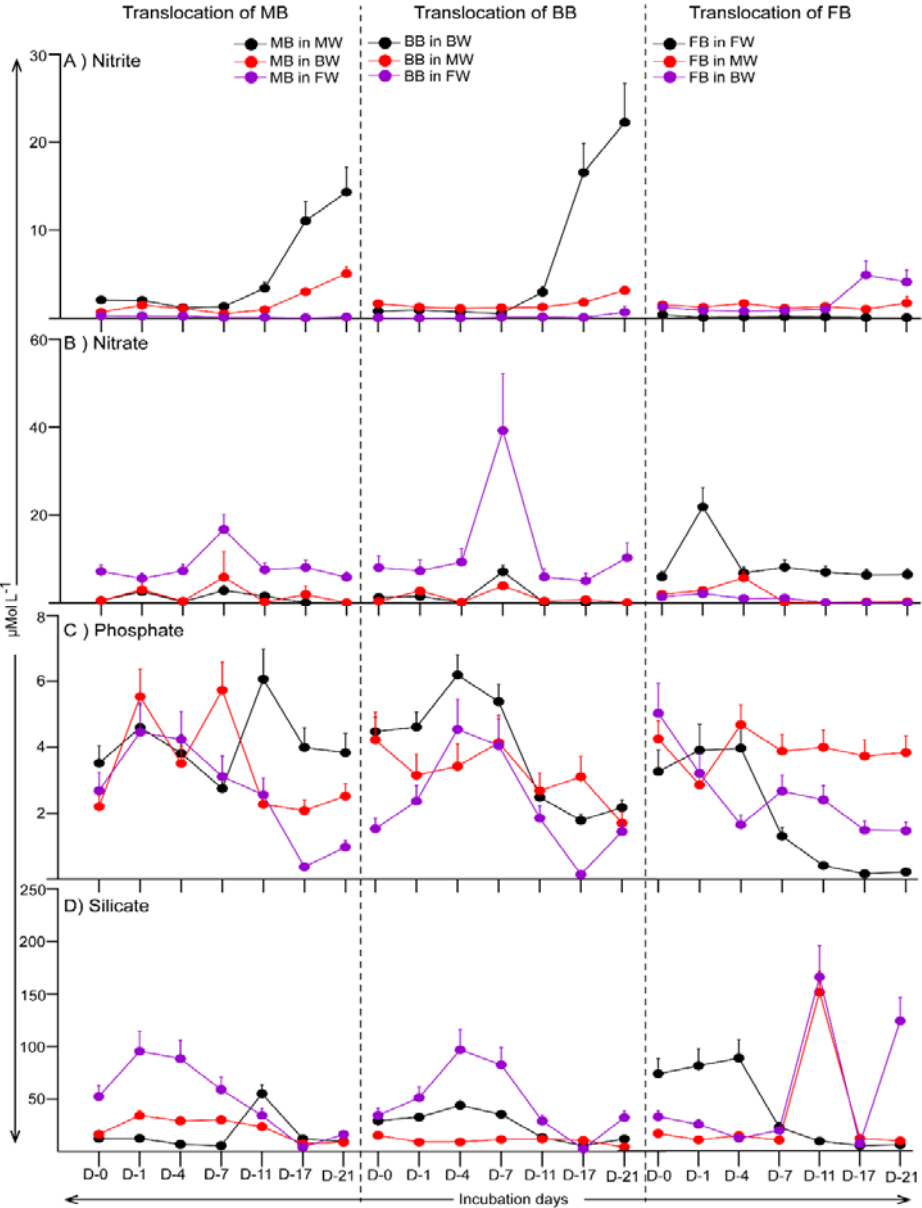
### 3.1. Initial c

During marine, brackish, and freshwater bacteria were higher (2.08  $\mu\text{Mol L}^{-1}$ ) at marine (2.08  $\mu\text{Mol L}^{-1}$ ) (Table. 2).

### 3.2. Microc

#### 3.2.1. Envir

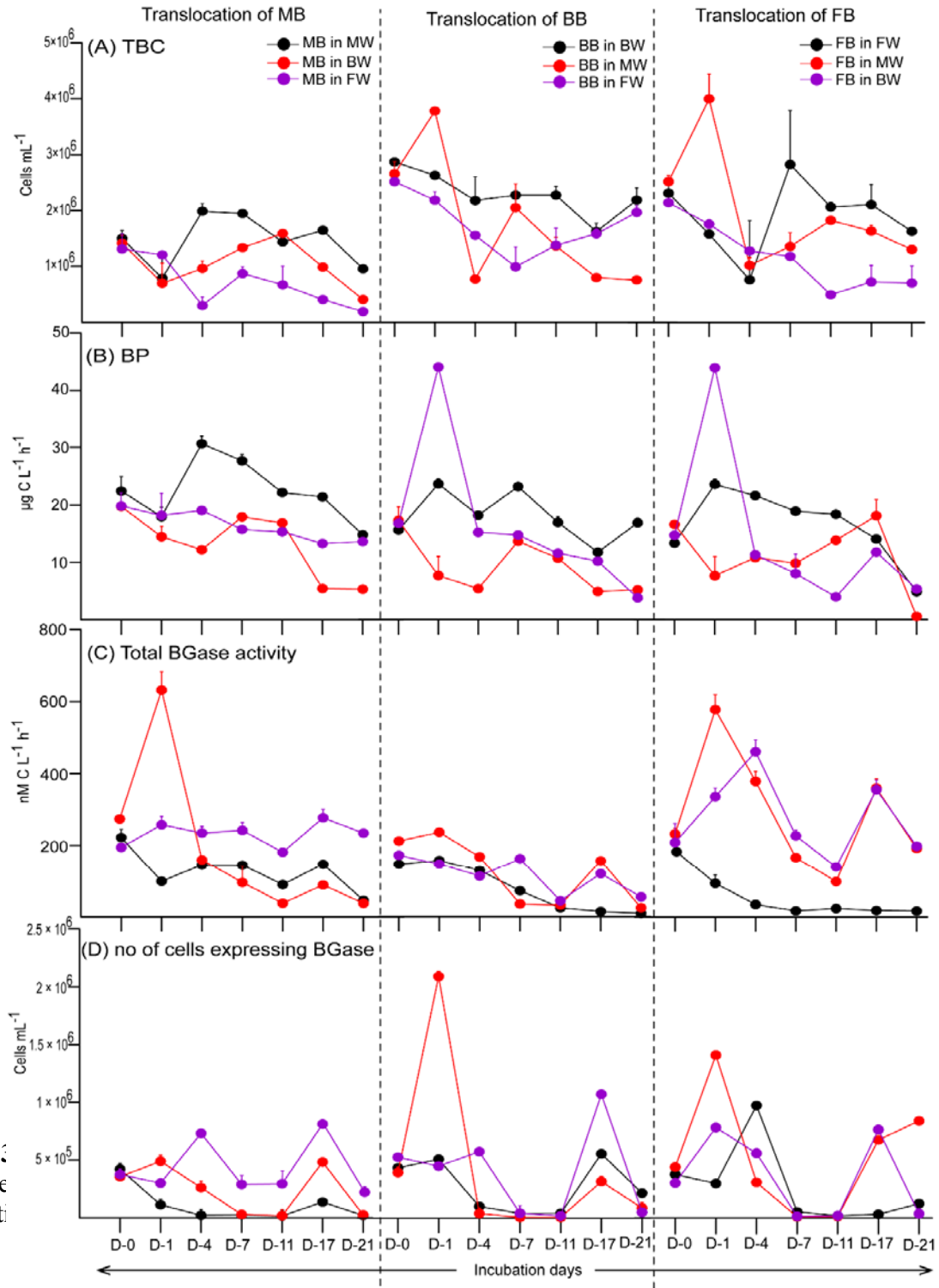
The nitrate concentration between 397  $\mu\text{Mol L}^{-1}$  and 600  $\mu\text{Mol L}^{-1}$  (F = 4.085; 27.16;  $p < 0.05$  in C, and D). The nitrate concentration of the treatment (Fig. 2B). However, meanwhile, translocated marine, brackish, and freshwater bacteria were higher (2.08  $\mu\text{Mol L}^{-1}$ ) at marine (2.08  $\mu\text{Mol L}^{-1}$ ) (Table. 2).



Betaproteobacteria taxa on day 11. Correlation analyses showed a positive relationship between these variables, but it was not significant (Suppl. Table S1). A gradual increase in the DOC concentration was evident until day 4, and vice versa was observed from day 7 to 21 when marine, brackish, and freshwater bacteria were translocated in different waters (F = 9.944;  $P < 0.001$ ; Fig. 3B).



**Fig. 3**  
XG e  
additi



**Fig. 4** Response of (A) TBC (cells mL<sup>-1</sup>), (B) BP (μg C L<sup>-1</sup> h<sup>-1</sup>), (C) Total BGase activity (nM C L<sup>-1</sup> h<sup>-1</sup>), and (D) no of cells expressing BGase (cells mL<sup>-1</sup>) during the translocation of MB, BB and FB to different waters. See Fig. 2 for additional details.

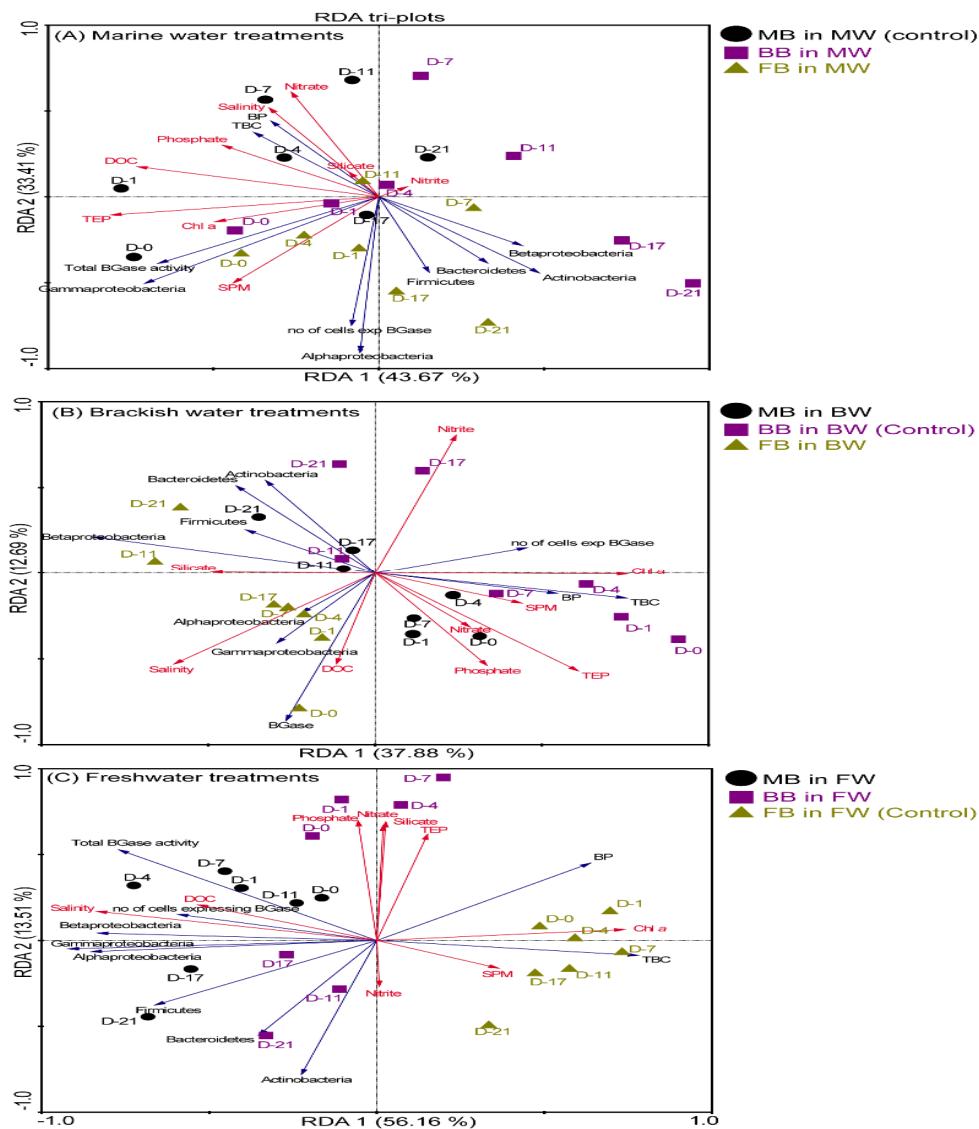
### **3.2.2. Marine bacteria**

When marine bacteria with high TBC were translocated to brackish and fresh water conditions, a significant decrease was evident from day0 ( $1.28 \times 10^6$  and  $1.12 \times 10^6$  cells mL<sup>-1</sup>) today21 ( $4.09 \times 10^5$  and 1.



$93 \times 10^5 \text{ cells mL}^{-1}$ ; ANOVA -  $F=9.17$ ;  $p<0.01$ ; Fig. 4A,B) when compared to control (marine bacteria in marine water). Similar to TBC, BP also decreased except when marine bacteria were translocated in freshwater (Fig. 4B). Whereas, the total BGase activity showed an increasing trend until day 1 in brackish, and was high throughout in fresh water translocation (Fig. 4C). The number of cells expressing BGase also showed a similar trend (Fig. 4D).

In the case of BCC, a clear shift was observed between the control and the treatments (ANOVA-  $F=58.19$ ;  $p<0.0001$ ; Fig. 5A-C). Q-PCR analyses of field samples showed that Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Firmicutes taxa were relatively high in marine inocula (Table. 2). In marine water (control), the relative abundance of Gammaproteobacteria was high and coupled with BGase and BP (Figs. 5A and 6C; Table 3A; Suppl. Table S1). Their abundance was positively related to salinity, DOC, TEP and Chl *a* (Fig. 5A; Suppl. Table S1). However, the translocation of marine bacteria to brackish conditions resulted in a significant decrease in Gammaproteobacteria taxa, and the emergence of Bacteroidetes and Actinobacteria (Figs. 5B, 6C, and 7A, C). Their proliferation in brackish conditions was negatively related to salinity (Fig. 5B; Table. 3B; Suppl. Table S2). A similar trend was also evident when marine inocula were translocated to freshwater, wherein Betaproteobacteria and Firmicutes taxa were predominant and coupled with the number of cells expressing BGase (Fig. 5C; Table. 3C; Suppl. Table S3), which were negatively influenced by Chl *a* and TEP, and positively related to DOC (Figs. 5C, 6B, and 7B; Table 3C; Suppl. Table S3).



**Fig. 5** Redundancy analyses showing the changes in major bacterial taxa and their relationship with environmental variables in (A) marine water (B) Brackish water, and (C) freshwater treatments; blue and red lines in RDA tri-plots indicate biotic (response variable) and abiotic variables (explanatory variables), respectively; Circles, boxes and up-triangle in RDA tri-plots represent MB, BB, and FB in different waters, respectively; D = Days.

### 3.2.3. Brackish bacteria

When brackish water bacteria were translocated to marine water, TBC (ANOVA:  $F = 29.89$ ,  $p < 0.0001$ ) and BP ( $F = 13.26$ ,  $p < 0.001$ ) decreased compared to control (Fig. 4A, B). In the case of freshwater translocation, the decrease was evident in BP (Fig. 4B). While total BGase activity and the number of cells expressing BGase showed an increasing trend until day 4 and vice versa observed from day 7 to 21 (Fig. 4C, D). However, the variation between the control and treatments were not significant (Fig. 4C, D). A similar trend was also evident in BCC wherein Bacteroidetes ( $1.16 \times 10^5$  copies  $\mu\text{L}^{-1}$ ), and Actinobacteria ( $6.80 \times 10^4$  copies  $\mu\text{L}^{-1}$ ) taxa were higher in brackish water (Table 2; Figs. 5B and 7A-C), and followed a similar trend in control as well as treatments (Figs. 5A-C and 7B-C). For instance, the relative

abundance of Bacteroidetes, Firmicutes and Actinobacteria taxa increased from day 0 to day 21, irrespective of the treatments (Fig. 7B, C). Although a significant difference in the abundance of Betaproteobacteria ( $F=13.92$ ,  $p < 0.001$ ) and Alphaproteobacteria ( $F=15.12$ ;  $p < 0.001$ ) taxa was evident when brackish inocula were translocated to fresh and marine waters, it did not influence the BGase activity as evidenced from RDA tri-plots well as species response to RDA axes 1 (Figs. 5A-C, 6A-B and 7A; Table 3A; Suppl. Table S1).

#### **3.2.4. Freshwater bacteria**

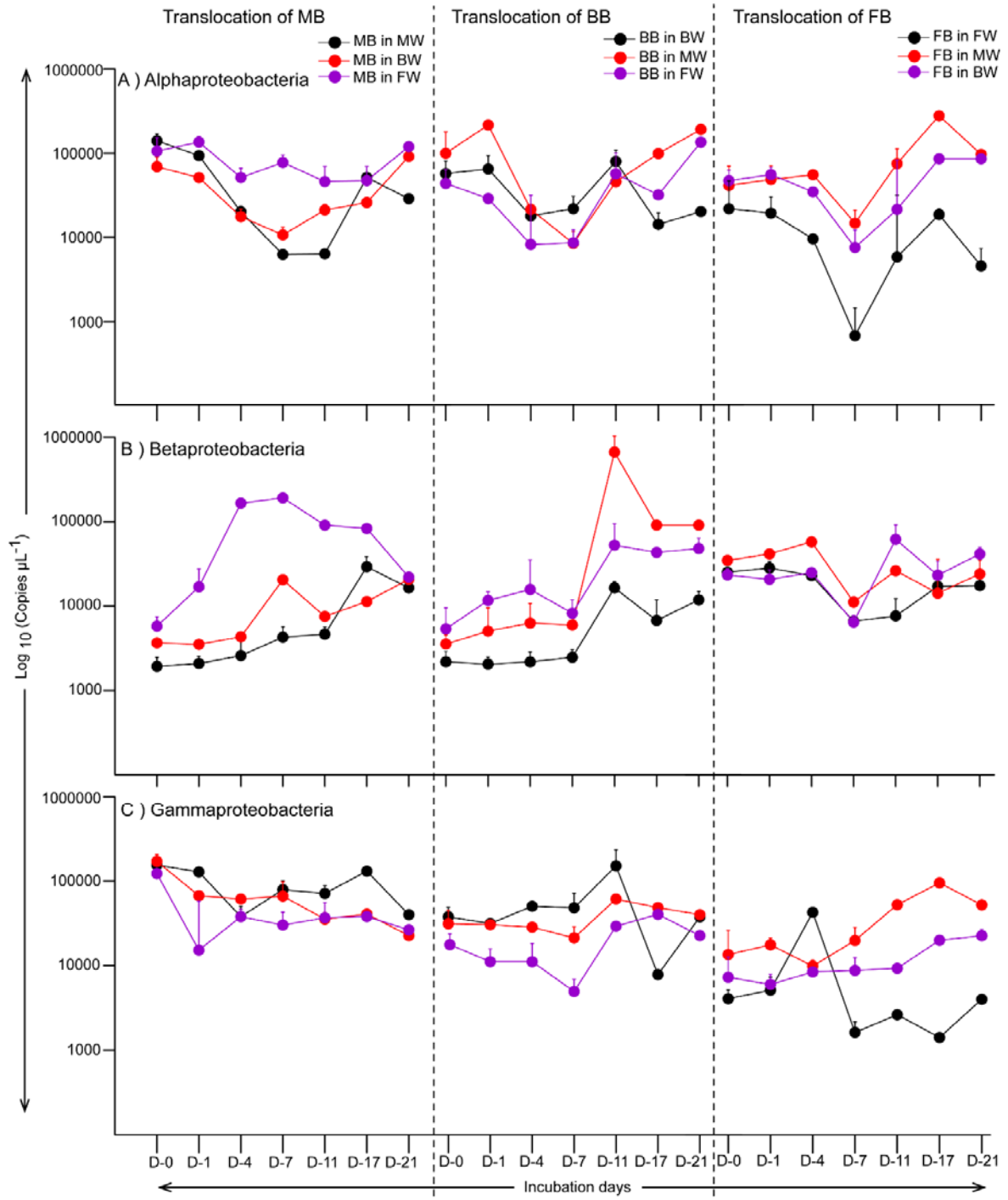
The translocation of freshwater bacteria to marine and brackish conditions resulted in a decrease in TBC ( $1.36 \times 10^6$  and  $7.04 \times 10^5$  cells  $\text{mL}^{-1}$ , respectively) and BP ( $0.5$  and  $5.35 \mu\text{g C L}^{-1} \text{h}^{-1}$  respectively), irrespective of the treatments (Fig. 4A, B). However, total BGase activity and the number of cells expressing BGase showed an opposite trend wherein the activity increased significantly irrespective of the treatments (ANOVA  $F=33.951$ ,  $P < 0.0001$  and  $F=10.17$ ;  $p < 0.01$ ; Fig. 4C, D). The increase was strongly related to the shift in the studied bacterial taxa, as observed in RDA (Fig. 5A-C). RDA axes 1 and 2 explained 77.08 % and 50.57 % of the variance in the studied bacterial taxa, when freshwater bacteria were translocated in marine and brackish water, respectively.

The qPCR analysis showed that the Betaproteobacteria was dominant taxa in freshwater (Table 2). When Betaproteobacteria rich freshwater inocula were translocated to brackish, an increase in the relative abundance of Actinobacteria, Bacteroidetes, and Firmicutes taxa was observed on day 21 (Figs. 5B and 7A-C). Their increase was negatively related to DOC, Chl $a$ , and TEP (Fig. 5B; Table. 3B; Suppl. Table S2). In the case of the marine condition, saline related taxa were dominant, i.e., Alphaproteobacteria, and Gammaproteobacteria, which showed a strong coupling with the total BGase activity and the number of cells expressing BGase (Figs. 5A, 6A, C; Table 3A; Suppl. Table S1).

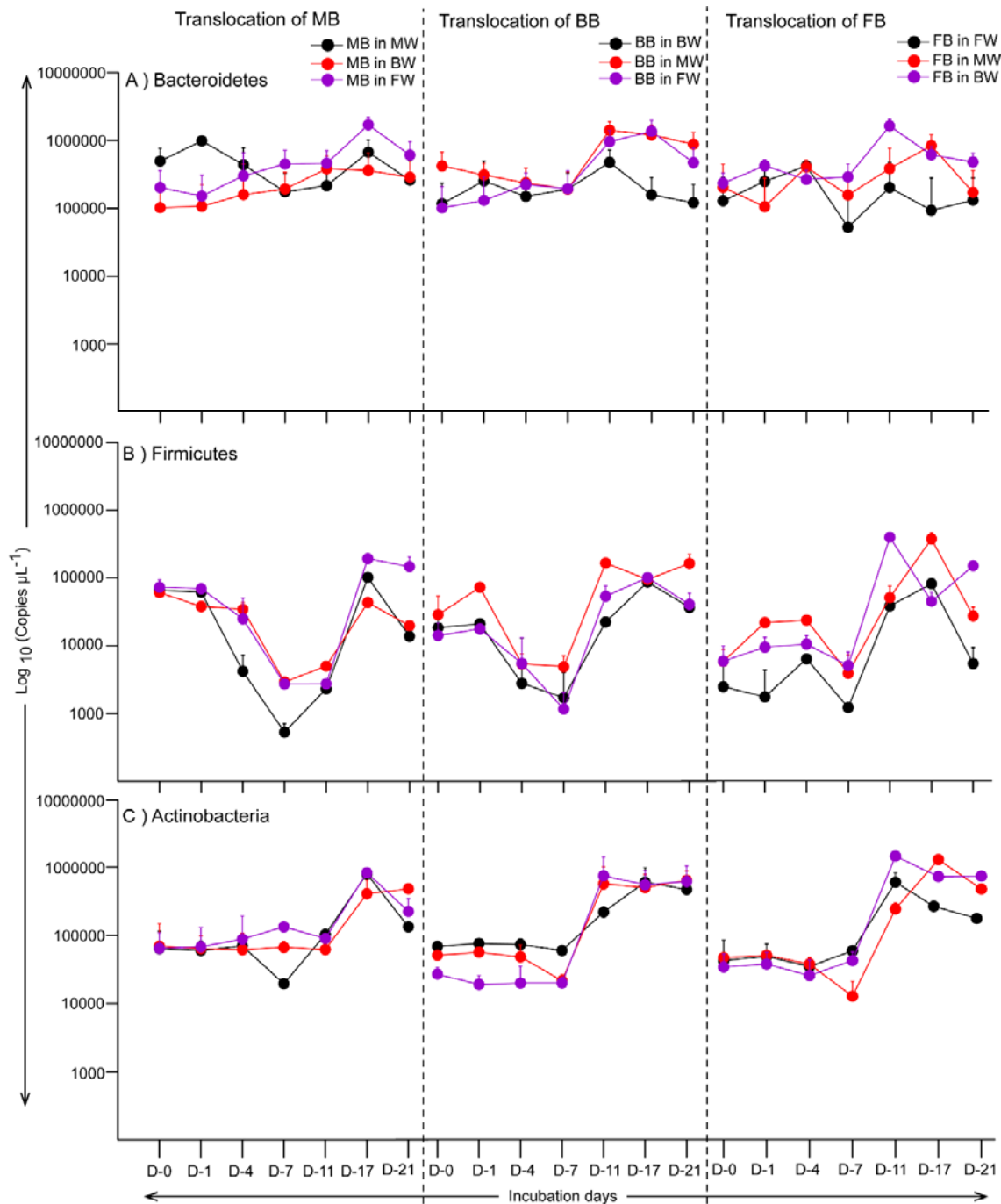
**Table 3** Species response on RDA environmental axes 1 and 2 using generalized linear model and F statistics; GLM – generalized linear model; NS – Not Significant

(A) Marine water						(B) Brackish water				(C) Freshwater			
S. No	Dependent	F	P	RDA 1	RDA 2	F	P	RDA 1	RDA 2	F	P	RDA 1	RDA 2
1	Alphaproteobacteria	58.14 <sup>b</sup>	0.00004	0.16	<b>-0.90</b>	NS	NS	-0.23	-0.24	16.39 <sup>a</sup>	0.001	<b>-0.86</b>	-0.07
2	Betaproteobacteria	4.89 <sup>a</sup>	0.04	<b>0.52</b>	-0.20	20.29 <sup>a</sup>	0.0005	<b>-0.85</b>	0.22	9.90 <sup>a</sup>	0.007	<b>-0.84</b>	0.04
3	Gammaproteobacteria	6.97 <sup>a</sup> /10.46 <sup>b</sup>	0.02 <sup>a</sup> /0.006 <sup>b</sup>	<b>-0.57</b>	<b>-0.65</b>	NS	NS	-0.30	-0.41	37.56 <sup>a</sup>	0.00003	<b>-0.93</b>	-0.05
4	Bacteroidetes	NS	NS	0.41	-0.31	5.81 <sup>b</sup>	0.04	-0.48	<b>0.53</b>	NS	NS	-0.33	-0.25
5	Firmicutes	8.19 <sup>b</sup>	0.01	0.38	<b>-0.62</b>	NS	NS	-0.30	0.39	9.38 <sup>a</sup>	0.009	<b>-0.77</b>	-0.49
6	Actinobacteria	6.95 <sup>a</sup>	0.02	<b>0.57</b>	-0.35	5.91 <sup>b</sup>	0.02	-0.33	<b>0.55</b>	8.74 <sup>b</sup>	0.01	-0.23	<b>-0.79</b>
7	BP	NS	NS	-0.25	0.42	4.73 <sup>a</sup>	0.04	<b>0.55</b>	-0.12	14.43 <sup>a</sup>	0.002	<b>0.64</b>	0.45
8	No. of cells exp BGase	17.75 <sup>b</sup>	0.001	0.07	<b>-0.76</b>	NS	NS	0.48	0.17	NS	NS	-0.48	0.12
9	TBC	NS	NS	-0.26	0.40	21.60 <sup>a</sup>	0.0004	<b>0.76</b>	-0.15	45.0 <sup>a</sup>	0.0001	<b>0.79</b>	-0.09
10	Total BGase activity	7.11 <sup>a</sup> /5.32 <sup>b</sup>	0.01 <sup>a</sup> /0.03 <sup>b</sup>	<b>-0.58</b>	<b>-0.51</b>	17.25 <sup>b</sup>	0.001	-0.27	<b>-0.86</b>	7.93 <sup>a</sup> /10.49 <sup>b</sup>	0.01/0.006	<b>-0.77</b>	<b>0.53</b>

<sup>a</sup> –significantly related to RDA 1; <sup>b</sup> -significant related to RDA 2



**Fig.6** Response of (A) Alphaproteobacteria, (B) Betaproteobacteria, and (C) Gammaproteobacteria during the translocation of MB, BB and FB to different waters. See Fig. 2 for additional details.



**Fig. 7** Response of (A) Bacteroidetes, (B) Firmicutes, and (C) Actinobacteria during the translocation of MB, BB and FB to different waters. See Fig. 2 for additional details.

#### 4. Discussion

The present study evaluated the effect of salinity stress on major bacterial taxa in a tropical estuary. The stations were well demarcated by salinity and nutrient composition

during the sampling. The bay was characterized by high salinity, nitrite, and phosphate, whereas silicate and nitrate were high at the upstream. In monsoonal estuaries, freshwater discharge and tidal circulation regulate the variations in nutrients and salinity in which the river run-off governs nitrate and silicate, while nitrite and phosphate via tide induced sedimentary release (Anand et al., 2014). Similar to physicochemical conditions, the studied bacterial taxa also varied significantly from the saline bay to the freshwater upstream in this study. The bay was dominated by Alphaproteobacteria, Gammaproteobacteria, Firmicutes, and Bacteroidetes, and these communities have also been reported earlier in Delaware estuary, Chesapeake Bay, and Columbia river estuary (Bouvier and del Giorgio, 2002; Cottrell and Kirchman, 2004; Crump et al., 1999), thus indicating their possible occurrence as autochthonous communities in the saline region of the estuaries. Meanwhile, many studies have also shown the presence and dominance of these taxa in different environmental settings such as rivers (Anderson-Glenna et al., 2008; Oshkin et al., 2014), sediment (Franco et al., 2017; Khandeparker et al., 2017), soil (Eilers et al., 2010), and biofilms (Anderson-Glenna et al., 2008; Edwards et al., 2010; Hede and Khandeparker, 2018), suggesting their ecological roles in other environments. Among the observed marine taxa, Gammaproteobacteria and Firmicutes taxa were strongly coupled with BGase activity and salinity in this study. Several studies have been reported that the Gammaproteobacteria could account for ~ 1 – 50% of total bacterial abundance in saline environments (Jiang et al., 2007; Wu et al., 2006), can degrade the organic matter which is rich in carbohydrates (Baker et al., 2015; Eswaran and Khandeparker, 2017 and 2019; Yu et al., 2011). Likewise, members of Firmicutes taxa are also reported as a reservoir of BGase expressing genes (Zang et al., 2017). Other than salinity, the relative abundance of Gammaproteobacteria taxa showed a strong positive correlation to DOC, Chl *a* (indicator of phytoplankton biomass), and TEP in this study. Members of Gammaproteobacteria taxa are often reported having a strong association with phytoplankton (Grossart et al., 2005). Although phytoplankton composition tends to vary with hydrography and environmental conditions, a few heterotrophic bacterial taxa are consistently reported to dominate as phytoplankton-associated communities. For example, members of Alteromonadaceae in Gammaproteobacteria taxa recurrently occurred as phytoplankton associated, which highlighted the role of substrate specificity driving the bacterial communities (Buchan et al., 2005; Teeling et al., 2012). Nevertheless, the response and proliferation of these autochthonous bacterial communities to different environmental conditions have not been addressed in monsoon influenced tropical estuaries. The translocation experiments conducted to elucidate this revealed that when the marine inocula

with high TBC, BP, and BGase activity were translocated to filtered and autoclaved brackish (19 salinity) and freshwaters (0.06 salinity), TBC decreased significantly, irrespective of the treatments. In the case of BP, the decrease was mainly in brackish water. Several field and experimental studies have reported that bacterial abundance and activities are adversely affected by salinity (Painchaud et al., 1995; Langenheder et al., 2003). A study by del Giorgio and Bouvier (2002) has suggested that changing salinity can be considered as a stress factor for bacterial physiological states and functional activities. Whereas, total BGase activity increased upon translocation of marine inoculum only in freshwater. Earlier, del Giorgio and Bouvier (2002) noticed sharp changes in the structure and function of bacterial taxa along a salinity gradient wherein salinity 'triggered' particular groups of bacteria, while it 'restrained' others. In the present study, an increase in the relative abundance of Betaproteobacteria and Firmicutes taxa possibly resulted in high BGase activity in freshwater translocation, which was decoupled in the marine water control. Moreover, an increase in DOC, nitrate, and silicate was observed after translocation to freshwater, which could be attributed to the breakdown of TEP by BGase-producing Betaproteobacteria and Firmicutes taxa. Also, cell lysis and disintegration of Chl *a* contributes to the release of dissolved organic matter (DOM), that can support the growth of heterotrophic bacterial communities (Morris et al., 1978). In Betaproteobacteria, members of Comamonadaceae (*Methylophilus*, *Sphingomonas*, and uncultured clones), Burkholderia (uncultured clones), and Oxalobacteraceae (uncultured clones) have been linked to degradation and utilization of DOM (Kisand et al., 2002; Kisand and Wikner, 2003; Lindh et al., 2015; Teira et al., 2009; Traving et al., 2017), and are prevalent in lacustrine (Boucher and Debroas, 2009) and freshwater/low-saline conditions (Kirchman et al., 2004; Pohlen et al., 2010). The other metabolic functions of Betaproteobacteria include denitrification (Gasol et al., 2002; Han et al., 2018; Heylen et al., 2006), ammonia oxidation (Molina et al., 2007), that influences bacterial carbon fluxes especially in coastal transition environments (Teira et al., 2009). Like Betaproteobacteria, the persistence of Firmicutes after translocation to freshwater indicates their ability to sustain in a wide range of salinity (Rath et al., 2019). Also, their persistence and proliferation could be attributed to species level change. For example, members of Firmicutes were detected during both high and low-saline conditions in this estuary in which marine originated strains were dominant in high saline condition. At the same time, it was terrestrial/freshwater strains during the low-saline condition (Eswaran and Khandeparker, 2019). It is known that the tide-induced saltwater intrusion and freshwater inflow are the two major factors that regulate the salinity in estuaries and indirectly affects the bacterial communities. During the saltwater



intrusion events, marine bacteria are relocated to freshwater systems, and vice versa, observed during high freshwater inflow. Such physical events not only drive the shifts in communities but also possibly affect the gene regulation and expression of the communities (Edmons et al., 2009). This could be another possible reason for the members of Betaproteobacteria, and Firmicutes taxa to adapt and express BGase activity in two distinct salinity conditions.

In the case of mid-estuary, which is brackish water (<20 salinity), Actinobacteria and Bacteroidetes were dominant and negatively related to Chl *a*, phosphate, and SPM. Members of Actinobacteria have been detected at less than 20 salinity, which is similar to mid-estuarine water, and utilize a narrow range of organic substrate for growth and metabolism (Sharp et al., 2009). Likewise, members of Bacteroidetes have developed a unique degradation mechanism that is generally encoded in polysaccharide utilization loci (PUL) (Fernández-Gómez et al., 2013). These PUL consist of genes that encode different hydrolytic enzymes such as sulfatases, glycosidases, and polysaccharide lyases (Kabisch et al., 2014; Tang et al., 2017). Our earlier study has also detected Actinobacteria and Bacteroidetes taxa in the upper middle region of this estuary (Eswaran and Khandeparker, 2017). After translocation of brackish inocula to marine, TBC and BP decreased when compared with control. In the case of freshwater, the decrease in TBC, BP, total BGase activity, and the number of cells expressing BGase was almost similar between control and treatments. Among the studied bacterial taxa, the relative abundance of Actinobacteria and Bacteroidetes taxa remained constant and gradually increased as that of brackish water control. It is known that the estuarine and brackish water communities are a mix of riverine and indigenous populations inhabiting the estuary but acclimatize to saline water, or originated from the freshwater sources (Crump et al., 1999). An experimental study by Langenheder et al. (2003) using Orean River water also reported similar findings and concluded that estuarine bacteria might better adapt to salinity disturbance than marine/riverine bacteria. Although there was a difference in the relative abundance of Alphaproteobacteria and Betaproteobacteria taxa after translocation to different waters, it did not influence the BGase activity during the study. It is possible that the species which emerged belonging to these taxa or inocula may not be expressing the BGase genes because bacteria tend to change the mineralization pathways under different environmental conditions. For example, a study by Edmons et al. (2009) reported that the artificial addition of saltwater in freshwater wetlands resulted in a decline in methanogens and the emergence of sulfate-reducing communities. Likewise, Bernhard et al.

(2005) observed a decrease in ammonia-oxidizing bacteria in response to increasing salinity at Plum Island Sound Estuary, north-east Massachusetts.

The upstream freshwater station showed relatively high numbers of Betaproteobacteria ( $1.26 \times 10^5$  copies  $\mu\text{L}^{-1}$ ) as compared to other studied taxa during the study. Although these taxa are present in other environmental conditions, they contribute significantly to ecological processes that occur in the freshwater systems (Kan et al., 2006; Li et al., 2012). Li et al. (2012) and Ruiz-González et al. (2013) observed a positive relationship between the relative abundance of Betaproteobacteria and DOC, which emphasized their significant role in the processing of riverine DOC. Besides, they contribute up to 60 – 70 % of total bacteria in epilimnetic waters (Newton et al., 2011). In our study, when freshwater inocula with high numbers of Betaproteobacteria taxa were translocated to marine and brackish conditions, their abundance decreased significantly at the end of incubation. Whereas, an increase in the relative abundance of Alphaproteobacteria and Gammaproteobacteria taxa were detected in marine waters and showed a positive relationship with BGase activity, salinity, and DOC. The variations in salinity and source of DOC may have contributed to this response because the availability of marine-derived DOC and increased salinity favors the proliferation of specific marine species, which are inactive at low salinity conditions. For instance, within the Alphaproteobacteria, members of *Novosphingobium*, and *Sphingomonas*, *Nitratireductor* have been detected in mid-salinities with carbohydrate degradation ability (Soares Júnior et al., 2013). Whereas, metabolically versatile *SAR 11* and *Roseobacter* clade was abundant in high saline condition (Brinkhoff et al., 2008; Wagner-Döbler and Biebl, 2006). Likewise, Gammaproteobacteria are fast-growing opportunists and adapt quickly to salinity stress (Langenheder et al., 2003; Soto-Ramírez et al., 2007), and changing substrate availability (Pernthaler and Amann, 2005). Within Gammaproteobacteria, genus such as *Serratia*, *Citrobacter*, *Enterobacter*, *Pantoea* has been detected abundantly in low-saline waters, while *Pseudomonas*, *Vibrio*, and *Acinetobacter* in high-saline water with carbohydrate degrading ability (Arora et al., 2012; Eswaran and Khandeparker, 2019; Khandeparker et al., 2011; Langenheder et al., 2003). In the case of freshwater bacteria when translocated to brackish, an increase in the relative abundance of Actinobacteria, Firmicutes, and Bacteroidetes were negatively related to DOC, Chl *a*, and TEP. During this time, silicate and nitrite levels also increased significantly at the end of the incubation, which could be attributed to the disintegration of freshwater phytoplankton communities in different salinity conditions (Morris et al., 1978; Khandeparker et al., 2017). Thus, it seems that salinity and availability of

labile DOC contribute to the proliferation and emergence of species belonging to specific bacterial taxa, which in turn produce an array of enzymes to breakdown complex molecules/polymers and play a significant role in the estuarine food web dynamics.

The use of group-specific primers, coupled with qPCR, helped in the enumeration of major bacterial taxa in response to non-ambient salinity conditions during the study. However, there could be other minor or rare bacterial taxa, which may show a similar response to changing salinity and other environmental variables. Thus, further use of modern molecular tools can manifest such species-level changes in the prokaryotic communities and is need of the hour, especially in such monsoon influenced tropical estuarine systems.

## **5. Conclusions**

Overall, the results of the present experiment revealed a clear shift in major bacterial taxa on translocation to different salinities wherein marine and freshwater bacteria showed pronounced changes in the studied bacterial taxa and BGase activity. While brackish water bacteria showed a strong persistence to changing salinity in this tropical estuary, especially Actinobacteria and Bacteroidetes. The persistence or the changes in the studied bacterial groups may also relate to the species level shifts in particular bacterial taxa, and unravelling the same using different functional gene markers would ascertain their role in organic matter processing and is way forward.

## **Acknowledgments**

The authors would like to thank the Director of the CSIR-National Institute of Oceanography, Goa, for his support. We also thank Biofouling and Bioinvasion Lab members for their support during the course of work. This work was supported by CSIR funded Ocean Finder Program (PSC0105) and Ballast Water Management Program (GAP 2429), India (Ministry of Shipping and DG shipping). The first author RE acknowledges CSIR for the award of Senior Research Fellowship (SRF award no: 31/26(282)/2015EMRI) and is a registered Ph.D. student in the School of Earth, Ocean and Atmospheric Sciences, Goa University. This is NIO contribution #####.

## References

- Anderson-Glenna, M.J., Bakkestuen, V., Clipson, N.J., 2008. Spatial and temporal variability in epilithic biofilm bacterial communities along an upland river gradient. *FEMS Microbiol. Ecol.* 64, 407-418.
- Anand, S.S., Sardessai, S., Muthukumar, C., Mangalaa, K.R., Sundar, D., Parab, S.G., Kumar, M.D., 2014. Intra-and inter-seasonal variability of nutrients in a tropical monsoonal estuary (Zuari, India). *Cont. Shelf. Res.* 82, 9-30.
- Arora, M., Anil, A.C., Delany, J., Rajarajan, N., Emami, K., Mesbahi, E., 2012. Carbohydrate-degrading bacteria closely associated with *Tetraselmis indica*: influence on algal growth. *Aquat. Biol.* 15, 61-71.
- Baker, B.J., Lazar, C.S., Teske, A.P., Dick, G.J., 2015. Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria. *Microbiome* 3,14.
- Bernhard, A.E., Donn, T., Giblin, A.E., Stahl, D.A., 2005. Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. *Environ. Microbiol.* 7, 1289-1297.
- Boucher, D., Debroas, D., 2009. Impact of environmental factors on couplings between bacterial community composition and ectoenzymatic activities in a lacustrine ecosystem. *FEMS Microbiol.Ecol.* 70, 66-78.
- Bouvier, T.C., del Giorgio, P.A., 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnol. Oceanogr.* 47, 453-470.
- Brinkhoff, T., Giebel, H.A., Simon, M., 2008. Diversity, ecology, and genomics of the Roseobacter clade: a short overview. *Arch. Microbiol.* 189, 531-539.
- Buchan, A., González, J.M. and Moran, M.A., 2005. Overview of the marine Roseobacter lineage. *Appl. Environ. Microbiol.* 71, 5665-5677.
- Bullock, A., Ziervogel, K., Ghobrial, S., Smith, S., McKee, B., Arnosti, C., 2017. A multi-season investigation of microbial extracellular enzyme activities in two temperate coastal North Carolina rivers: evidence of spatial but not seasonal patterns. *Front. Microbiol.* 8, 2589.
- Campbell, B.J., Kirchman, D.L., 2013. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *The ISME J.* 7, 210.
- Cottrell, M.T., Kirchman, D.L., 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 66, 5116-5122.
- Cottrell, Matthew, T., Kirchman, L.D., 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. *Aquat. Microb. Ecol.* 34,139-149.

- Crump, B.C., Armbrust, E.V., Baross, J.A., 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. *Appl. Environ. Microbiol.* 65, 3192-3204.
- Crump, B.C., Hopkinson, C.S., Sogin, M.L., Hobbie, J.E., 2004. Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. *Appl. Environ. Microbiol.* 70, 1494-1505.
- Cunha, M.A., Almeida, M.A., Alcântara, F., 2000. Patterns of ectoenzymatic and heterotrophic bacterial activities along a salinity gradient in a shallow tidal estuary. *Mar. Ecol. Prog. Ser.* 204, 1-12.
- Cunha, M.A., Almeida, M.A., Alcântara, F., 2001. Short-term responses of the natural planktonic bacterial community to the changing water properties in an estuarine environment: ectoenzymatic activity, glucose incorporation, and biomass production. *Microb.Ecol.* 42, 9-79.
- Divya, B., Fernandes, S.O., Sheelu, G., Nair, S., Bharathi, P.L., Chandramohan, D., 2009. Limno-tolerant bacteria govern nitrate concentration in Mandovi Estuary, India. *Estuar. Coast. Shelf Sci.* 82, 29-34.
- Edmonds, J.W., Weston, N.B., Joye, S.B., Mou, X., Moran, M.A., 2009. Microbial community response to seawater amendment in low-salinity tidal sediments. *Microb.Ecol.* 58,558-568.
- Edwards, J.L., Smith, D.L., Connolly, J., McDonald, J.E., Cox, M.J., Joint, I., Edwards, C., McCarthy, A.J., 2010. Identification of carbohydrate metabolism genes in the metagenome of a marine biofilm community shown to be dominated by Gammaproteobacteria and Bacteroidetes. *Genes* 1, 371-384.
- Eilers, K.G., Lauber, C.L., Knight, R., Fierer, N., 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol. Biochem.* 42, 896-903.
- Eswaran, R., Khandeparker, L., 2017. Seasonal variation in bacterial community composition and  $\beta$ -glucosidase expression in a tropical monsoon-influenced estuary. *Aquat. Microb. Ecol.* 80, 273-287.
- Eswaran, R., Khandeparker, L., 2019. Seasonal variation in  $\beta$ -glucosidase-producing culturable bacterial diversity in a monsoon-influenced tropical estuary. *Environ.Monit.Assess.* 191, 662.
- Fernández-Gomez, B., Richter, M., Schüller, M., Pinhassi, J., Acinas, S.G., González, J.M., Pedros-Alio, C., 2013. Ecology of marine Bacteroidetes: a comparative genomics approach. *The ISME J.* 7, 1026.
- Franco, D.C., Signori, C.N., Duarte, R.T., Nakayama, C.R., Campos, L.S., Pellizari, V.H., 2017. High prevalence of gammaproteobacteria in the sediments of admiralty bay and north bransfield Basin, Northwestern Antarctic Peninsula. *Front. Microbiol.* 8, 153.
- Garrison, D.L., Gowing, M.M., Hughes, M.P., Campbell, L., Caron, D.A., Dennett, M.R., Shalapyonok, A., Olson, R.J., Landry, M.R., Brown, S.L., Liu, H.B., 2000. Microbial food

web structure in the Arabian Sea: a US JGOFS study. *Deep. Sea. Res. Part II: Top. Stud. Oceanogr.* 47, 1387-1422.

Gasol, J.M., Comerma, M., García, J.C., Armengol, J., Casamayor, E.O., Kojdecká, P., Šimek, K., 2002. A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir. *Limnol. Oceanogr.* 47,62-77.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. *Methods of Seawater Analysis*, vol. 419, Verlag Chemie, New York, 159-223.

Grossart, H.P., Levold, F., Allgaier, M., Simon, M., Brinkhoff, T., 2005. Marine diatom species harbour distinct bacterial communities. *Environ. Microbiol.* 7, 860-873.

Han, F., Wei, D., Ngo, H.H., Guo, W., Xu, W., Du, B., Wei, Q., 2018. Performance, microbial community and fluorescent characteristic of microbial products in a solid-phase denitrification biofilm reactor for WWTP effluent treatment. *J. Environ.Manage.* 227,375-385.

Hede, N., Khandeparker, L., 2018. Influence of darkness and aging on marine and freshwater biofilm microbial communities using microcosm experiments. *Microb. Ecol.* 76, 314-327.

Herfort, L., Crump, B.C., Fortunato, C.S., McCue, L.A., Campbell, V., Simon, H.M., Baptista, A.M. and Zuber, P., 2017. Factors affecting the bacterial community composition and heterotrophic production of Columbia River estuarine turbidity maxima. *MicrobiologyOpen* 6, e00522.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME J.* 5, 1571.

Herlemann, D.P., Woelk, J., Labrenz, M., Jürgens, K., 2014. Diversity and abundance of “*Pelagibacterales*”(SAR11) in the Baltic Sea salinity gradient. *Syst.Appl.Microbiol.* 37, 601-604.

Heylen, K., Gevers, D., Vanparys, B., Wittebolle, L., Geets, J., Boon, N., De Vos, P., 2006. The incidence of nirS and nirK and their genetic heterogeneity in cultivated denitrifiers. *Environ.Microbiol.* 8, 2012-2021.

Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar.Ecol.Prog.Ser.* 299-308.

Hu, Y., Wang, L., Tang, Y., Li, Y., Chen, J., Xi, X., Zhang, Y., Fu, X., Wu, J., Sun, Y., 2014. Variability in soil microbial community and activity between coastal and riparian wetlands in the Yangtze River estuary–Potential impacts on carbon sequestration. *Soil. Biol. Biochem.* 70, 221-228.

Jiang, H., Dong, H., Yu, B., Liu, X., Li, Y., Ji, S., Zhang, C.L., 2007. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. *Environ. Microbiol.* 9, 2603-2621.

- Kabisch, A., Otto, A., König, S., Becher, D., Albrecht, D., Schüler, M., Teeling, H., Amann, R.I., Schweder, T., 2014. Functional characterization of polysaccharide utilization loci in the marine Bacteroidetes '*Gramella forsetii*' KT0803. *The ISME J.* 8,1492.
- Kan, J., Crump, B.C., Wang, K., Chen, F., 2006. Bacterioplankton community in Chesapeake Bay: predictable or random assemblages. *Limnol. Oceanogr.* 51, 2157-2169.
- Khandeparker, L., Eswaran, R., Gardade, L., Kuchi, N., Mapari, K., Naik, S.D., Anil, A.C., 2017. Elucidation of the tidal influence on bacterial populations in a monsoon influenced estuary through simultaneous observations. *Environ. Monit. Assess.* 189, 1-17.
- Khandeparker, L., Kuchi, N., Kale, D., Anil, A.C., 2017. Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing. *Ecol. Indic.* 74, 172-181.
- Khandeparker, L., Eswaran, R., Hede, N., Anil, A.C., 2018. Significance of metabolically active bacterioplankton in the frontal regions of the Northeastern Arabian Sea. *Aquat. Sci.* 80, 38.
- Khandeparker, R., Verma, P., Meena, R.M., Deobagkar, D.D., 2011. Phylogenetic diversity of carbohydrate degrading culturable bacteria from Mandovi and Zuari estuaries, Goa, west coast of India. *Estuar. Coast. Shelf. Sci.* 95, 359-366.
- Khodse, V.B., Bhosle, N.B., Matondkar, S.P., 2010. Distribution of dissolved carbohydrates and uronic acids in a tropical estuary, India. *J. Eart. Syst. Sci.* 119, 519-530.
- Kirchman, D.L., Dittel, A.I., Findlay, S.E., Fischer, D., 2004. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquat. Microb. Ecol.* 35, 243-257.
- Kisand, V., Wikner, J., 2003. Combining culture-dependent and-independent methodologies for estimation of richness of estuarine bacterioplankton consuming riverine dissolved organic matter. *Appl. Environ. Microbiol.* 69, 3607-3616.
- Kisand, V., Cuadros, R., Wikner, J., 2002. Phylogeny of culturable estuarine bacteria catabolizing riverine organic matter in the northern Baltic Sea. *Appl. Environ. Microbiol.* 68, 379-388.
- Klein, C., Claquin, P., Pannard, A., Napoléon, C., Le Roy, B., Véron, B., 2011. Dynamics of soluble extracellular polymeric substances and transparent exopolymer particle pools in coastal ecosystems. *Mar. Ecol. Prog. Ser.* 427, 13-27.
- Langenheder, S., Kisand, V., Wikner, J., Tranvik, L.J., 2003. Salinity as a structuring factor for the composition and performance of bacterioplankton degrading riverine DOC. *FEMS Microbiol. Ecol.* 45, 189-202.
- Li, D., Sharp, J.O., Saikaly, P.E., Ali, S., Alidina, M., Alarawi, M.S., Keller, S., Hoppe-Jones, C., Drewes, J.E., 2012. Dissolved organic carbon influences microbial community composition and diversity in managed aquifer recharge systems. *Appl. Environ. Microbiol.* 78, 6819-6828.

- Lindh, M.V., Lefébure, R., Degerman, R., Lundin, D., Andersson, A., Pinhassi, J., 2015. Consequences of increased terrestrial dissolved organic matter and temperature on bacterioplankton community composition during a Baltic Sea mesocosm experiment. *Ambio*. 44, 402-412.
- Molina, V., Ulloa, O., Farías, L., Urrutia, H., Ramírez, S., Junier, P., Witzel, K.P., 2007. Ammonia-oxidizing  $\beta$ -proteobacteria from the oxygen minimum zone off northern Chile. *Appl. Environ. Microbiol.* 73, 3547-3555.
- Morris, A.W., Mantoura, R.F.C., Bale, A.J., Howland, R.J.M., 1978. Very low salinity regions of estuaries: important sites for chemical and biological reactions. *Nature* 274, 678.
- Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., Bertilsson, S., 2011. A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol. Biol. Rev.* 75, 14-49.
- Oshkin, I.Y., Wegner, C.E., Lüke, C., Glagolev, M.V., Filippov, I.V., Pimenov, N.V., Liesack, W., Dedysh, S.N., 2014. Gammaproteobacterial methanotrophs dominate cold methane seeps in floodplains of West Siberian rivers. *Appl. Environ. Microbiol.* 80, 5944-5954.
- Painchaud, J., Therriault, J.C., Legendre, L., 1995. Assessment of salinity-related mortality of freshwater bacteria in the Saint Lawrence Estuary. *Appl. Environ. Microbiol.* 61, 205-208.
- Pernthaler, J., Amann, R., 2005. Fate of heterotrophic microbes in pelagic habitats: focus on populations. *Microbiol. Mol. Biol. Rev.* 69, 440-461.
- Pohlen, E., Marxsen, J., Küsel, K., 2010. Pioneering bacterial and algal communities and potential extracellular enzyme activities of stream biofilms. *FEMS Microbiol Ecol.* 71, 364-373.
- Rath, K.M., Fierer, N., Murphy, D.V., Rousk, J., 2019. Linking bacterial community composition to soil salinity along environmental gradients. *The ISME J.* 13, 836.
- Ruiz-González, C., Proia, L., Ferrera, I., Gasol, J.M., Sabater, S., 2013. Effects of large river dam regulation on bacterioplankton community structure. *FEMS Microbiol. Ecol.* 84, 316-331.
- Santos, A.L., Mendes, C., Gomes, N.C., Henriques, I., Correia, A., Almeida, A., Cunha, Â., 2009. Short-term variability of abundance, diversity and activity of estuarine bacterioneuston and bacterioplankton. *J. Plankton Res.* 31, 1545-1555.
- Santos, L., Vaz, L., Gomes, N.C.M., Vaz, N., Dias, J.M., Cunha, Â., Almeida, A., 2014. Impact of freshwater inflow on bacterial abundance and activity in the estuarine system Ria de Aveiro. *Estuar. Coast. Shelf Sci.* 138, 107-120.
- Sharp, J.H., Yoshiyama, K., Parker, A.E., Schwartz, M.C., Curless, S.E., Bearegard, A.Y., Ossolinski, J.E., Davis, A.R., 2009. A biogeochemical view of estuarine eutrophication: seasonal and spatial trends and correlations in the Delaware Estuary. *Estuar. Coast.* 32, 1023-1043.



- Shetye, S.R., Shankar, D., Neetu, S., Suprit, K., Michael, G.S., Chandramohan, P., 2007. The environment that conditions the Mandovi and Zuari estuaries. National Institute of Oceanography, India.
- Soares Júnior, F.L., Dias, A.C.F., Fasanella, C.C., Taketani, R.G., Lima, A.O.D.S., Melo, I.S., Andreote, F.D., 2013. Endo-and exoglucanase activities in bacteria from mangrove sediment. *Braz. J. Microbiol.* 44, 969-976.
- Soto-Ramirez, N., Sanchez-Porro, C., Rosas, S., Gonzalez, W., Quinones, M., Ventosa, A., Montalvo-Rodriguez, R., 2007. *Halomonas avicenniae* sp. nov., isolated from the salty leaves of the black mangrove *Avicennia germinans* in Puerto Rico. *Int. J. Syst. Evol. Microbiol.* 57, 900-905.
- Tang, K., Lin, Y., Han, Y., Jiao, N., 2017. Characterization of potential polysaccharide utilization systems in the marine Bacteroidetes *Gramella flava* JLT2011 using a multi-omics approach. *Front. Microbiol.* 8, 220.
- Tang, X., Xie, G., Shao, K., Chen, Y., Gao, G., 2012. Influence of salinity on the bacterial community composition in Lake Bosten, a large oligosaline lake in arid northwestern China. *Appl. Environ. Microbiol.*, 78, 4748-4751.
- Teira, E., Martínez-García, S., Lønborg, C., Álvarez-Salgado, X.A., 2009. Growth rates of different phylogenetic bacterioplankton groups in a coastal upwelling system. *Environ. Microbiol. Rep.* 1, 545-554.
- Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M., Kassabgy, M., Huang, S., Mann, A.J., Waldmann, J., Weber, M., 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336, 608-611.
- ter Braak, C.J., Smilauer, P., 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination version 4.5. Microcomputer Power, Ithaca, NY.
- Traving, S.J., Rowe, O., Jakobsen, N.M., Sørensen, H., Dinasquet, J., Stedmon, C.A., Andersson, A., Riemann, L., 2017. The effect of increased loads of dissolved organic matter on estuarine microbial community composition and function. *Front. Microbiol.* 8, 351.
- Vijith, V., Sundar, D., Shetye, S.R., 2009. Time-dependence of salinity in monsoonal estuaries. *Estuar., Coast. Shelf Sci.* 85, 601-608.
- Wagner-Döbler, I., Biebl, H., 2006. Environmental biology of the marine *Roseobacter* lineage. *Annu. Rev. Microbiol.* 60, 255-280.
- Wu, Q.L., Zwart, G., Schauer, M., Kamst-van Agterveld, M.P., Hahn, M.W., 2006. Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. *Appl. Environ. Microbiol.* 72, 5478-5485.
- Yu, Y., Li, H.R., Zeng, Y.X., Chen, B., 2011. Bacterial diversity and bioprospecting for cold-active hydrolytic enzymes from culturable bacteria associated with sediment from Nella Fjord, Eastern Antarctica. *Mar. drugs* 9, 184-195.

Zang, X., Liu, M., Wang, H., Fan, Y., Zhang, H., Liu, J., Xing, E., Xu, X., Li, H., 2017. The distribution of active  $\beta$ -glucosidase-producing microbial communities in composting. *Can. J. Microbiol.* 63, 998-1008.

Supplementary Table S1 Correlation analysis between biotic and abiotic variables during translocation of MB, BB, and FB in marine water.

	Salinity	SPM	Chl <i>a</i>	DOC	TEP	Phosphate	Nitrite	Nitrate	Silicate	$\alpha$	$\beta$	$\gamma$	Bac	Firmi	Actino	BP	Cells exp BGase	TBC	Total BGase	
Salinity	1.00																			
SPM	-0.25	1.00																		
Chl	<b>0.69**</b>	0.04	1.00																	
DOC	0.16	0.05	<b>0.48**</b>	1.00																
TEP	0.03	0.24	<b>0.56**</b>	0.15	1.00															
Phosphate	-0.08	0.02	0.11	0.08	<b>0.29*</b>	1.00														
Nitrite	<b>0.44**</b>	<b>-0.39**</b>	<b>0.33*</b>	-0.11	0.08	<b>0.35**</b>	1.00													
Nitrate	0.19	<b>-0.27*</b>	0.17	<b>0.32*</b>	0.09	0.07	<b>0.40**</b>	1.00												
Silicate	-0.21	-0.06	-0.05	-0.27	<b>.300*</b>	<b>0.67**</b>	<b>0.55**</b>	-0.01	1.00											
$\alpha$	-0.24	0.10	0.04	-0.07	<b>0.27*</b>	0.10	0.09	-0.14	0.22	1.00										
$\beta$	<b>-0.60**</b>	-0.01	<b>-0.67**</b>	-0.18	-0.23	-0.17	-0.07	0.09	0.14	0.16	1.00									
$\gamma$	<b>0.35*</b>	0.16	<b>0.39**</b>	<b>0.41**</b>	<b>0.39**</b>	-0.04	0.24	0.22	0.19	<b>0.46**</b>	0.25	1.00								
Bac	<b>0.34*</b>	<b>-0.40**</b>	0.14	-0.12	0.03	-0.07	<b>0.34*</b>	-0.15	0.20	<b>0.46**</b>	0.05	0.07	1.00							
Firmi	-0.16	<b>-0.27*</b>	-0.18	-0.25	-0.05	-0.27	0.10	-0.15	0.20	<b>0.80**</b>	<b>0.28*</b>	0.23	<b>0.71**</b>	1.00						
Actino	0.03	-0.03	-0.18	<b>-0.31*</b>	<b>-0.31*</b>	-0.21	0.06	<b>-0.38**</b>	0.14	<b>0.43**</b>	0.05	-0.14	<b>0.59**</b>	<b>0.67**</b>	1.00					
BP	<b>0.37**</b>	0.22	<b>0.64**</b>	0.10	<b>0.55**</b>	<b>0.36**</b>	<b>0.34*</b>	0.05	<b>0.37**</b>	-0.05	<b>-0.38**</b>	0.16	0.16	-0.23	-0.15	1.00				
cells exp BGase	<b>-0.27*</b>	<b>0.29*</b>	0.14	<b>0.38**</b>	0.12	-0.16	-0.05	0.22	-0.15	<b>0.66**</b>	0.15	<b>0.54**</b>	-0.08	<b>0.36**</b>	0.16	-0.22	1.00			
TBC	-0.03	0.24	<b>0.36**</b>	0.06	<b>0.46**</b>	-0.10	-0.05	0.21	0.08	0.10	-0.11	<b>0.33*</b>	<b>-0.34*</b>	-0.15	-0.20	<b>0.47**</b>	0.21	1.00		
Total BGase	<b>-0.35**</b>	<b>0.34*</b>	0.22	<b>0.39**</b>	<b>0.48**</b>	-0.10	0.10	0.11	0.02	<b>.467**</b>	0.24	<b>0.57**</b>	-0.15	0.12	-0.22	0.08	<b>0.69**</b>	0.23	1.00	

\*\*Correlation is significant at the 0.01 level, \*Correlation is significant at the 0.05 level. SPM – Suspended particulate matter; Chl *a* – Chlorophyll *a*; DOC – Dissolved organic carbon; TEP – Transparent exopolysaccharides;  $\alpha$  – Alphaproteobacteria;  $\beta$  – Betaproteobacteria;  $\gamma$  – Gammaproteobacteria; Bac – Bacteroidetes; Firmi-Firmicutes; Actino – Actinobacteria; BP – Bacterial Production; Cells exp BGase – Cells expressing  $\beta$  – glucosidase; TBC – Total bacterial count; Total BGase – Total  $\beta$  – glucosidase activity

**Supplementary Table S2** Correlation analysis between biotic and abiotic variables during translocation of MB, BB, and FB in brackish water.

	Salinity	SPM	Chl <i>a</i>	DOC	TEP	Phosphate	Nitrite	Nitrate	Silicate	$\alpha$	$\beta$	$\gamma$	Bac	Firmi	Actino	BP	Cells exp BGase	TBC	Total BGase
Salinity	1.00																		
SPM	<b>-0.74**</b>	1.00																	
Chl	<b>-0.62**</b>	<b>0.57**</b>	1.00																
DOC	-0.05	0.13	0.26	1.00															
TEP	-0.07	0.07	<b>0.32*</b>	-0.08	1.00														
Phosphate	-0.26	0.22	<b>0.53**</b>	0.20	0.09	1.00													
Nitrite	0.29	0.09	<b>-0.40**</b>	0.02	-0.04	<b>-0.38**</b>	1.00												
Nitrate	-0.01	0.08	0.24	-0.28	0.06	<b>0.44**</b>	-0.22	1.00											
Silicate	-0.20	0.26	<b>0.34*</b>	0.06	0.15	<b>0.76**</b>	-0.24	0.18	1.00										
$\alpha$	-0.13	<b>0.49**</b>	0.20	0.10	<b>0.42**</b>	-0.26	<b>0.40**</b>	0.10	-0.23	1.00									
$\beta$	<b>0.38**</b>	<b>-0.30*</b>	<b>-0.74**</b>	0.08	-0.18	<b>-0.47**</b>	0.29	-0.09	-0.16	0.21	1.00								
$\gamma$	0.23	-0.05	0.09	<b>0.50**</b>	0.12	0.11	-0.14	0.21	-0.08	<b>0.34*</b>	<b>0.45**</b>	1.00							
Bac	-0.02	0.06	<b>-0.63**</b>	-0.18	<b>-0.29*</b>	<b>-0.55**</b>	<b>0.44**</b>	-0.13	0.13	0.12	<b>0.57**</b>	-0.28	1.00						
Firmi	<b>0.40**</b>	-0.06	-0.08	0.02	0.21	<b>-0.56**</b>	<b>0.55**</b>	-0.24	0.11	<b>0.53**</b>	0.05	0.06	-0.05	1.00					
Actino	<b>-0.61**</b>	<b>0.43**</b>	0.08	<b>0.31*</b>	-0.21	-0.10	0.14	-0.06	-0.22	0.09	-0.12	<b>0.53**</b>	<b>0.44**</b>	-0.06	1.00				
BP	<b>-0.54**</b>	<b>0.56**</b>	<b>0.33*</b>	0.21	-0.09	<b>0.43**</b>	<b>-0.37*</b>	0.18	<b>0.48**</b>	0.04	-0.18	-0.07	0.19	<b>-0.60**</b>	0.29	1.00			
cells exp BGase	-0.09	<b>0.37*</b>	0.14	<b>0.37*</b>	-0.09	<b>-0.35*</b>	<b>0.43**</b>	-0.03	<b>-0.48**</b>	0.01	0.15	0.28	0.22	<b>0.61**</b>	0.19	-0.05	1.00		
TBC	<b>-0.72**</b>	<b>0.56**</b>	<b>0.62**</b>	0.16	0.14	<b>0.42**</b>	<b>0.54**</b>	0.16	<b>0.48**</b>	0.04	<b>0.43**</b>	-0.10	-0.12	<b>-0.52**</b>	<b>0.32*</b>	<b>0.76**</b>	-0.17	1.00	
Total BGase	0.04	0.08	0.06	<b>0.49**</b>	<b>0.43**</b>	-0.17	<b>0.36*</b>	-0.09	<b>-0.35*</b>	0.01	0.10	<b>0.53**</b>	-0.04	<b>0.40**</b>	-0.27	<b>-0.33*</b>	<b>0.48**</b>	<b>-0.36*</b>	1.00

\*\*Correlation is significant at the 0.01 level, \*Correlation is significant at the 0.05 level. See suppl. Table S1 for abbreviations

**Supplementary Table S3** Correlation analysis between biotic and abiotic variables during translocation of MB, BB, and FB in freshwater.

	Salinity	SPM	Chl $\alpha$	DOC	TEP	Phosphate	Nitrite	Nitrate	Silicate	$\alpha$	$\beta$	$\gamma$	Bac	Firmi	Actino	BP	Cells exp BGase	TBC	Total BGase	
Salinity	1.00																			
SPM	<b>-0.55**</b>	1.00																		
Chl	<b>-0.61**</b>	<b>0.50**</b>	1.00																	
DOC	0.20	-0.12	-0.10	1.00																
TEP	0.06	0.12	<b>0.55**</b>	0.09	1.00															
Phosphate	0.22	<b>-0.29*</b>	0.10	<b>0.58**</b>	<b>0.34**</b>	1.00														
Nitrite	0.05	-0.05	0.13	0.19	0.09	0.22	1.00													
Nitrate	0.02	-0.13	-0.07	0.12	0.22	<b>0.30*</b>	-0.22	1.00												
Silicate	0.14	-0.24	0.15	<b>0.63**</b>	<b>0.34**</b>	<b>0.78**</b>	0.22	<b>0.29*</b>	1.00											
$\alpha$	<b>0.78**</b>	<b>-0.28*</b>	<b>-0.44**</b>	0.19	0.02	0.10	<b>0.30*</b>	-0.17	0.07	1.00										
$\beta$	<b>0.83**</b>	<b>-0.38**</b>	<b>-0.51**</b>	<b>0.32*</b>	0.06	0.16	-0.03	0.01	0.10	<b>0.78**</b>	1.00									
$\gamma$	<b>0.61**</b>	<b>-0.28*</b>	<b>-0.49**</b>	<b>.258*</b>	-0.08	0.15	0.05	-0.12	0.11	<b>0.55**</b>	<b>0.66**</b>	1.00								
Bac	0.16	-0.16	<b>-0.61**</b>	0.03	<b>-0.37**</b>	-0.10	-0.03	-0.02	-0.17	0.15	0.19	0.18	1.00							
Firmi	<b>0.38**</b>	-0.03	<b>-0.24*</b>	<b>0.34*</b>	<b>-0.30*</b>	<b>-0.42**</b>	0.03	<b>-0.55**</b>	<b>-0.44**</b>	<b>0.53**</b>	<b>0.39**</b>	<b>0.37**</b>	<b>0.35**</b>	1.00						
Actino	0.03	0.18	<b>-0.29*</b>	<b>-0.383**</b>	<b>-0.54**</b>	<b>-0.64**</b>	0.13	<b>-0.25*</b>	<b>-0.70**</b>	0.21	0.17	0.20	<b>0.55**</b>	<b>0.44**</b>	1.00					
BP	<b>-0.56**</b>	-0.01	<b>0.49**</b>	-0.06	<b>0.27*</b>	<b>0.27*</b>	-0.20	0.22	<b>0.31*</b>	<b>-0.61**</b>	<b>-0.53**</b>	<b>-0.33**</b>	<b>-0.31*</b>	<b>-0.51**</b>	<b>-0.42**</b>	1.00				
cells exp BGase	0.22	0.21	-0.03	<b>.349**</b>	0.25	0.21	<b>-0.29*</b>	-0.11	0.24	0.07	<b>0.28*</b>	<b>0.53**</b>	0.18	0.22	<b>-0.14</b>	-0.10	1.00			
TBC	<b>-0.76**</b>	<b>0.46**</b>	<b>0.51**</b>	-0.16	0.07	<b>-0.29*</b>	0.06	-0.11	-0.23	<b>-0.50**</b>	<b>-0.52**</b>	<b>-0.70**</b>	<b>-0.57**</b>	-0.19	0.02	<b>0.43**</b>	<b>-0.30*</b>	1.00		
Total BGase	<b>0.59**</b>	<b>-0.38**</b>	<b>-0.40**</b>	0.09	0.24	<b>0.27*</b>	<b>-0.42**</b>	<b>0.35**</b>	0.24	<b>0.36**</b>	<b>0.46**</b>	<b>0.35**</b>	0.23	<b>0.39*</b>	<b>-0.34**</b>	-0.07	<b>0.40**</b>	<b>-0.50**</b>	1.00	

\*\*Correlation is significant at the 0.01 level, \*Correlation is significant at the 0.05 level. See suppl. Table S1 for abbreviations