Biogeography of Free-living and Particle-Associated Bacteria in the St. Lawrence Estuary

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ABSTRACT

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The St. Lawrence Estuary (SLE) is a productive ecosystem characterized by steep gradients in salinity and dissolved oxygen (DO). The SLE has been well studied in terms of physical and chemical properties, however bacterial diversity and community structure have not been documented in this large estuarine system. Here, we used 16S rRNA gene surveys to assess the bacterial community structure of both the free-living (FL) and particle-associated (PA) fractions of the bacterial community. Non-metric multidimensional scaling (NMS) ordinations and ANOSIM analysis showed that bacterial communities inhabiting particles were distinct from those found free-living in the water column, and this structure held true for both surface communities and those inhabiting the bottom waters. Taxonomic assignment of 16S DNA sequences revealed a shift in the bacterial community structure along the surface salinity gradient of the SLE. In general, Proteobacteria were more common in the free-living fraction, while Bacteroidetes were more commonly associated with particles. Moreover, particle-associated bacterial communities had an abundance of Alteromonadales in the deep hypoxic waters and free-living bacteria exhibited a greater abundance of Marine Group A and Delta-proteobacteria. These findings are shedding light on the diversity of bacterial taxa inhabiting the SLE and further metagenomic analysis will elucidate the metabolic contribution of these bacterial communities.

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Introduction

Microbes mediate numerous processes that are essential to the maintenance of the biosphere and the biogeochemical cycles. They are responsible for the cycling of life's essential elements such as oxygen (O_2), nitrogen (N_2), carbon (C), sulfur (S) and hydrogen (H_2). Though invisible to the naked eye, they are an essential component of the earth's biota and they catalyze central and unique transformations that play major roles in the regulation of the earth's atmosphere. Most of the open ocean is devoid of observable life forms, but almost every milliliter of seawater is swarming with microbial life, In fact, aquatic microbes are not only abundant, but also highly diverse. Using molecular techniques, we can assess the identity abundance, and distribution of microbial species in the oceans, rivers and lakes. To understand the major role that microbes play on earth, we have to first understand the role they play in aquatic food webs.

1.1 Microbes and the aquatic food web

In aquatic ecosystems, primary productivity is driven mainly by the availability of nutrients and light. Phytoplankton are the main primary producers and are photoautotrophic microscopic organisms that harvest light energy to convert inorganic carbon (CO_2) to organic matter. Dissolved and particulate organic matter (DOM and POM) is released from phytoplankton by either extracellular discharge during growth, by predation or released during the viral lysis of cells (Anderson & Ducklow, 2001) (**Figure 1**). Heterotrophic bacteria then consume this marine DOM and it is this transfer of carbon from DOM to bacteria that structures the foundation of the microbial food web in the oceans (Azam et al., 1983). In addition, the degradation of organic matter releases other

mineral or inorganic nutrients like ammonium (NH_4^+) and phosphate (PO_4^{3-}). This process is called remineralization, which describes the uptake and the release of compounds that contain essential nutrients (e.g. N and P). Thus, the function that microbes play in the marine ecosystem carbon cycle is describes as the "microbial loop", coined by Azam et al (1983). It is important to distinguish the difference between the microbial loop and the classic aquatic food chain by noticing that bacteria remineralize the DOM and POM and then release the nutrients converting them into their respective inorganic form, thus making them accessible for uptake by phytoplankton and reintroducing them into the system (**Figure 1**).

1.2 Aquatic bacterial diversity

How are microbial bacterial communities structured in aquatic systems? What functional roles do these bacteria play in the ecosystems? And, how does bacterial metabolism relate to ecosystem functions such as biogeochemical cycling? It is the study of microbial ecology that intends to answer these fundamental questions and can shed light on marine microorganisms and their interactions with each other and with their environment. It was only within the last few decades that the abundance and diversity of bacteria in aquatic environments was fully recognized. Previously, bacterial abundance and species richness had been grossly under-estimated due to the dependence on conventional cultivation and staining techniques (Hobbie et al.,1977; Jannasch & Jones, 1959). The advent of nucleic acid staining technologies revealed that bacteria are found at an average concentration of 10^5-10^6 cells per milliliter of seawater instead of previous estimates of only a few 100 cells per milliliter (Whitman et al., 1998). An abundance of

biochemical and molecular methods have since been applied to assay the microbial community composition temporally and spatially in response to environmental changes. These have included Fluorescence in situ hybridization (FISH), Denaturing Gradient Gel Electrophoresis (DGGE), Terminal Fragment Length Polymorphism (T-RFLP), cloning-sequencing, and next generation sequencing (Ion Torrent).

1.3 Aquatic bacterial community composition

The use of molecular tools in microbial ecology has painted a better picture of the structure of bacterial communities. Phylogenetic analyses have shown a broad diversity of bacteria within the surface regions of lakes, which is usually dominated by Beta-proteobacteria and Actinobacteria (Glockner et al., 2000; Zwart et al., 2002). Other studies focusing on ocean bacterioplankton has shown that the SAR11 clade from the Alpha-proteobacteria class is ubiquitous in many oceanic regions (Rappé, Vergin, & Giovannoni, 2000). Furthermore, other studies have shown that a subgroup of the Bacteroidetes division, Cytophaga-Flavobacteria, seems to be abundant in both lakes and the oceans (Acinas et al., 2004; Reimann & Winding, 2001; Selje & Simon, 2003; Venter et al., 2004; Weiss et al., 1998). The high diversity of bacteria in aquatic environments can be partly explained by the high variety of ecological niches and the different substrates used for growth.

Typically, in aquatic environments, bacterioplankton are separated into two main groups: bacteria attached to aggregates and free-living bacteria. Here we refer to these groups as particle-associated (PA) bacteria and (FL) free-living bacteria. FL bacteria dominate in total numbers and total biomass (Kirchman & Mitchell, 1982; Simon et al.,

1990; Unanue et al., 1992), but in regions of high particulate material such as freshwater and estuaries, PA bacteria constitute an important proportion of the total bacterial numbers and are responsible for a large portion of the bacterial production and activity (Griffith et al., 1990; Kirchman & Mitchell, 1982). In fact, the particles in lakes and oceans, which are formed by the aggregation of organic matter, detritus, and fecal matter are "hot-spots" for microbial decomposition of organic matter (Simon et al., 2002). Recently, evidence supports that it is not only the aggregates but also their surroundings that are sites or "hot-spots" of microbial processes (Selje & Simon, 2003; Simon et al., 1990). Consequently, investigating the microheterogeneity of particles and the associated bacteria is important for understanding the spatial and temporal dynamics of organic-matter degradation in the environment.

1.4 Free-living and particle-associated bacteria in aquatic systems

Previous studies in the Columbia River estuary showed that the fraction of bacteria attached to particles accounted for approximately 90% of the heterotrophic bacterial activity in the water column and that these bacteria were 10 to 100 times more active than free-living bacteria (Crump and Baross 1996). Crump et al. (1999) also showed that particle-attached bacteria are responsible for most of the degradation of detrital organic matter (OM) in the estuary and are part of an estuarine food web in which they are taken up by detritivorous copepods. In an earlier study, DeLong et al. (1993) assessed the phylogenetic diversity of attached vs. free-living marine bacteria, co-occurring the same water mass. They found that free-living bacteria were closely related to Gamma-proteobacteria and that particle-associated bacteria were closely related to

Cytophaga and the Planctomycetes. More recently, Hollibaugh et al. (2000) have observed different traits that distinguish PA and FL bacterial assemblages in samples collected at different locations in San Francisco Bay (Hollibaugh et al., 2000) and they suggested that the level of phylogenetic overlap between PA and FL communities is dependent on the particle origin.

1.5. Importance of estuaries

Estuaries are partially enclosed bodies of water where freshwater from rivers and streams meet and mix with salt water from the ocean. They are places of transition and are influenced by the tides. Estuarine environments are characterized by the unique combination of physical, chemical and biological features, and are distinguished by exceptionally high productivity (Correll, 1978). They are thus ideal habitats to observe the shifts in bacterial assemblages along environmental gradients. In this section, I will describe some of the important physical, chemical and biological processes occurring in estuaries.

1.5.1 Physical processes of estuaries

Estuaries are generally wide at the mouth and narrow at the head and harbor the mixing of freshwater and seawater. The salinity gradient increases along the length of the estuary and it generally increases with depth. This salinity distribution results from the density difference between saltwater and freshwater. The outward flow of freshwater stimulate further saltwater to maneuver within the estuary and creates an outflow of freshwater towards the sea end at the surface and an inflow of salt water at the bottom

(Yáñez-Arancibia, & Crump, 2012; Telesh & Khlebovich, 2010). Water circulation and density stratification, which are controlled by river discharges, tides and shelf winds, facilitate the entrapment of suspended particulate matter (SPM) in the estuarine turbidity maxima (ETM) (Burchard & Baumert, 1998; De Nijs, Winterwerp, & Pietrzak, 2010; David L. Kirchman, 2008). The formation of an ETM could be due to a number of factors such as physical mechanisms like flocculation and deflocculation and ionic composition of water (Burchard et al., 2004; Michaelis, 1990; Prahl, Small, & Eversmeyer, 1997; Roman, Holliday, & Sanford, 2001). The turbidity maxima holds great interest due to the dramatic salinity and nutrient gradients that appear in these regions. Another physical process worth mentioning is called upwelling. The process of upwelling conveys the deep cold, nutrient-rich waters up to the surface. This promotes growth and phytoplankton blooms. Coastal upwelling could be influenced by wind speed and the upward flow of cold deep water, as warm surface water is taken away by offshore currents. Recent events in the U.S. Pacific Northwest have shown that wind-forced upwelling can cause naturally occurring low dissolved oxygen water to move onto the continental shelf which can lead to mortalities of benthic fish and invertebrates (Roegner, Needoba, & Bapista, 2011).

1.5.2 Chemical and biological characteristics of estuaries

Salinity is an important environmental factor in estuaries; it plays a key role in defining the structural and functional characteristics of aquatic biota. In addition to salinity, many other chemical and biological gradients, such as nutrient concentration and organic matter composition are thought to influence the composition of bacterioplankton communities. In fact, studies involving mesocosm and microcosm experiments

examining the changes in environmental conditions have shown shifts occurring in the phylogenetic composition of bacterioplankton communities (Gasol et al., 2002; Hannen et al.,1999; Lebaron et al., 2001). Thus it is rational to expect that similar shifts will take place in natural freshwater and marine bacterioplankton communities as soon as they come across estuarine gradients, perhaps leading to the establishment of an estuarine community.

1.5.3 Estuarine bacterial community composition

Several studies have already described estuarine microbial diversity and have established how freshwater and marine bacterioplankton communities mix along estuarine gradients and some have reported evidence of a unique estuarine bacterioplankton community (Bidle & Fletcher, 1995; Murray, Hollibaugh, & Orrego, 1996; Troussellier, Shafer, & Muyzer, 2002). In fact, Crump et al. (1999) identified putative estuarine bacteria associated with particles in the Columbia River estuarine turbidity maximum by comparing environmental clone libraries of PCR-amplified 16S ribosomal DNA from the river, the estuary, and the coastal ocean. Likewise, Hollibaugh et al. (2000) have demonstrated the mixing of bacterial communities in the ETM of the San Francisco Bay system and Selje and Simon (2003) concluded that a distinct microbial community resides in the brackish section of the Weser River estuary system. More recently, Herlemann et al. (2011) studied bacterial community structure along a 2000 km salinity gradient in the Baltic Sea. They identified a bacterial brackish water community, comprised of a diverse combination of freshwater and marine groups along with populations unique to the Baltic Sea environment. These studies confirmed the

occurrence of river and coastal ocean bacteria in estuaries and proposed that the development of unique estuarine bacterial communities could be correlated to the long residence time of the brackish water in the Baltic Sea.

Numerous studies around the world have demonstrated that with increasing salinity, the relative abundance of Beta-proteobacteria and Actinobacteria decreases, and that of the Alpha-proteobacteria and the Gamma-proteobacteria increases (Acinas et al., 2004; Barberan & Casamayor, 2010; Crump et al., 2004; Kirchman et al., 2005). In fact, the spatial distribution of Alpha and Beta-proteobacteria in estuarine environments is similar to what was observed in lakes and the oceans where Alpha-proteobacteria dominate the marine end of estuaries, however their abundance is low in low-salinity waters, contrary to the Beta-proteobacteria group as their abundance is high in low-salinity habitats. The Cytophaga-Flavobacteria group has been found coupled with the turbidity maximum of the Columbia River as well as in the Chesapeake Bay (Bouvier & Giorgio, 2002; Crump et al., 1999) and a variety of particle-associated groups comprising of Planctomycetes and Verrucomicrobia, occur in both freshwater and marine water habitats.

1.6 The St. Lawrence estuary

The St. Lawrence Estuary (SLE) is a transitional habitat flanked by the St. Lawrence River and the Northwest Atlantic Ocean (Dickie & Trites, 1983; Petrie et al., 1996). The Gulf of St. Lawrence is located geographically between 48° N, 61.5° W and it is the outlet of the Great Lakes of North America by way of the Saint Lawrence River into the Atlantic Ocean. Sharp stratification of water masses is characteristic of this

estuary and seasonal sea-ice cover establishes for several months harboring macrofauna which includes cold water species such as harp seals and beluga whales (Lesage, Hammill, & Kovacs, 2001; Saucier, Roy, & Gilbert, 2003). Its physical and chemical features are well studied, however the structure and function of the SLE bacterial community has yet to be explored.

1.6.1 SLE: Physical characteristics

The St. Lawrence River starts as the outflow of the Great Lakes and widens into an estuary located near Ile d'Orléans (**Figure 2**). This region is where the river's freshwater initially mixes with saltier waters from the ocean and the classic two-layer estuarine circulation commences. As the surface water of the St. Lawrence river moves downstream it becomes saltier, ultimately being completely marine at the gulf. The Gulf of St. Lawrence which is a semi–enclosed sea, covering an area of about 240 $x10^3$ km^2 and containing 3553 km^3 of water, that opens to the Atlantic Ocean through the Cabot Strait and the Strait of Belle Isle (Dickie & Trites, 1983; Dufour & Ouellet, 2007; Denis Gilbert & Pettigrew, 1997; Mucci et al., 2011; Saucier et al., 2003). The deep-waters of the St. Lawrence enter from the Atlantic through the Laurentian Channel and are advected by estuarine circulation towards the channel head, at the Saguenay River mouth, where strong mixing occurs with near–surface waters (El-Sabh & Silverberg, 2013; Keyte & Trites, 1976). The main sources of freshwater are the St. Lawrence River, the Saguenay River and three large rivers on the north shore ─ the Betsiamites, Aux Outardes and Manicouagan rivers (Dufour & Ouellet, 2007; Pinet et al., 2011).

The St. Lawrence estuary can be divided into sections depending on the salinity gradients and the depth. The Upper St. Lawrence estuary (USLE) is located from Ile d'Orleans to the Saguenay River/Ile aux Coudres and its depth ranges from 20 m at Quebec City, to 120 m near Tadoussac. The water is vertically homogeneous at the head of the estuary and well stratified at the eastern end near Tadoussac. The Lower St. Lawrence Estuary (LSLE) starts off at the Saguenay River and ends at Pointe–des–Monts. A prominent feature of the LSLE is the Laurentian Trough (Laurentian Channel), which is 1240 km long and can range from 250 m to 500 m depth. It is narrow at the Saguenay Fjord and expands through the gulf.

The St. Lawrence system also shows a seasonal vertical structure. During winter, a cold surface layer extends to an average depth of 75 m and up to 150 m in places at the end of March and displays temperatures near freezing. During the spring, the cold water is trapped underneath the newly formed summer surface layer and is partly isolated from the atmosphere, thus it becomes identified as the summer Cold Intermediate Layer (CIL) that will persevere until the next winter. Hence in the spring and summer there is a three layer structure: the surface layer, the cold intermediate layer (CIL) and the deeper water layer (Banks, 1966; Denis Gilbert & Pettigrew, 1997; Mucci et al., 2011; Saucier et al., 2003).

1.6.2 SLE: Chemical characteristics

The water composition in the estuary is influenced by dissolved oxygen, nutrients and organic carbon. Also, the distribution of suspended particular matter (SPM) is

significant in estuarine and coastal waters since particulate matter is transported both vertically and horizontally by currents and tidal action (Dufour & Ouellet, 2007; Uncles, 2002). Strong tidal forces in the USLE and upwelling due to the topography at the heads of the Laurentian and the Anticosti channels are inclined to move particles vertically inside the water column (Dufour, R., Ouellet, 2007; Gobeil, 2006; Hachey, 1961). The Estuarine Turbidity Maxima (ETM) of the SLE, where SPM concentrations range from 50 to 200 mg/L, is located between Ile d'Orleans and Ile aux Coudres in the upper estuary and in the lower estuary and SPM concentrations decrease at all depths as one moves away from the head of the Estuary (D'Anglejan & Smith, 1973; Dufour & Ouellet, 2007; J Painchaud, 1995).

The particle–rich deposit near the bottom is known as the nepheloid layer and it is well developed in the Laurentian Channel, where intensified turbidity is caused by the friction of tides above the seabed (Mulder & Alexander, 2001). The chemical composition of SPM is a complex mixture of organic and inorganic molecules and is maintained by the rate of primary production, the amount of terrigenous contribution and the sinking rate (D'Anglejan & Smith, 1973).

Lastly, oxygen is a critical chemical parameter in the SLE. The vertical distribution of dissolved oxygen concentrations present firstly as a surface layer where oxygen is nearby 100% saturation, then an intermediate layer where oxygen saturation decreases seasonally, and finally a low oxygen saturation level in the deep-waters. There is a gradual depletion of O_2 in the deep-waters from Cabot Strait towards the head of the

Laurentian Channel (Gilbert et al., 2005). Hypoxia occurs when the concentration of oxygen in the water column falls below the 2 mg/L or 62.5 μmol/L level, which is the minimal concentration necessary to maintain most animal life (Diaz & Rosenberg, 2008). Gilbert et al. (2005) have shown that the dissolved oxygen concentration of bottom waters in the Lower St. Lawrence Estuary declined two fold since the 1930s. This could be attributed to a 1.7ºC warming of the bottom waters and to the increasing nitrogen load from land sources as well as local oxygen consumption (Mucci et al., 2011).

1.7 Study objectives

In this study, our objective is to provide the first description of the bacterial community structure and diversity in the surface and deep environments of the SLE in May 2011. Here we focus on two aspects of the SLE community. First, we provide insight into how bacterial richness, community composition and taxonomic structure change along the salinity gradient in surface waters of the SLE. Second, we explore the diversity and the taxonomic bacterial community composition as well as the microenvironment structure (free-living or particle-associated) in the St. Lawrence's deep hypoxic waters. We selected and analyzed a total of 38 samples of microbial biomass, 19 samples from FL communities and 19 samples from PA communities, at 8 stations along the surface and deep oceanographic regions of the SLE.

2. Materials and Methods

2.1 Sampling

Water samples were collected along the SLE near Rimouski in Quebec, Canada during a 5-day ocean sampling expedition aboard the research vessel Coriolis II in May 2011. We selected 10 locations along this transect to study. Selected stations included stations B, D/E, F, I, K, 25, 23, 22, 21 and 20 (**Figure 3**). Stations B and D/E are representative of the freshwater region, Station 20 represents the high brackish end of the oceanographic transect and stations in between represent the brackish/estuarine waters. Samples were collected from different depths at these 10 stations along a geographic transect of the St. Lawrence Estuary (49°N, 68°W) using a conductivity-temperature-depth (CTD) rosette. With each CTD cast, depth profiles of salinity (PSU), temperature (°C), conductivity [mS/cm], oxygen [umol/Kg], PAR/Irradiance, beam transmission [%], density [Kg/m^3] and fluorescence were recorded. Water samples were collected using a Cole Parmer Masterflex 7553-70 Peristaltic Pump and were filtered for DNA analysis onto Sterivex and GF/D filters according to pore-size (2.7 um GF/D filter or 0.22 um Sterivex filters). Samples were collected at different depths along the water column at each station (surface, 3-5 m; CIL, 50-100 m; deep layer, 180-320m).

2.2 Bacterial production

Bacterial Production (BP) was measured using the ^{3}H-leucine incorporation method (Farooq Azam & Smith, 1992) by our collaborators (Roxane Maranger's Laboratory at University of Montreal). Water samples (1.2 ml) were dispensed, in triplicate, into clean 2-ml microcentrifuge tubes preloaded with 50μl ^{3}H-leucine (115.4

Ci/mmol, Amersham) to produce a final leucine concentration of 10 nM (Garneau et al., 2008). Samples were incubated in the dark in an ice-filled isothermic container (-1.8 to 0°C) for approximately 4h. Leucine incorporated into cell protein was collected after precipitation by trichloroacetic acid (TCA) and centrifugation. Tubes were filled with 1.25 ml liquid scintillation cocktail (ScintiVerse, Fisher Scientific), and radioactivity was measured using a Tri-Carb 2900 TR Packard Liquid Scintillation Analyzer. Rates of leucine incorporation were corrected for radioactivity adsorption using TCA-killed controls and converted to bacterial production (BP) using two conversion factors 0.9 and 1.5 kg C per mol-1 3H- leucine (Garneau et al., 2008).

2.3 Cell abundance

Paul A del Giorgio's laboratory from UQAM (Université du Québec à Montréal) performed the cell abundance analysis. The total number of bacteria was determined using the nucleic acid stain SYTO-13 and flow cytometry, as described in del Giorgio et al. 1996 (del Giorgio et al., 1996). Briefly, 2-ml aliquots of unfiltered water were dispensed into plastic cytometer tubes. Green 0.92-pm beads were dispensed from the stock solution into all the tubes, to a final concentration of 0.33 x 10^6 ml^{-1}, and SYTO13 was then dispensed to each tube to a final concentration of 2.5 μM. The tubes were vortexed for 5s, incubated for 10 min at room temperature, in the dark, and then analyzed by flow cytometry.

2.4 Environmental DNA extraction

DNA extraction was performed from 0.22 μm Sterivex Filters for free-living bacteria and from 2.7 μm GF/D filters for particle-associated bacteria using a modified

protocol from Zaikova E. et al. (Zaikova et al., 2010). Cell lysis and digestion were conducted by thawing and adding 100 µl of lysozyme (conc. 125 mg in 1000 µl) and 20 µl of RNase A (conc. 10 µg/ml) to the filters containing biomass followed by incubation in a rotating oven at 37°C for 1 hour. We then added 100 µl of Proteinase K (conc. 10mg/ml) and 100 µl of 20% SDS to the filter and rotate again for 2 hours more in the hybridization oven at 55°C. Using a 5cc Syringe (BD™ Syringes (without needles) from Fisher Scientific) we transferred the lysate from the filters into 2 ml centrifuge tubes. A 0.583 volume of MPC Protein Precipitation Solution (MPC) of MasterPure™ from Epicenter was added in order to precipitate the protein. A protein pellet was formed after centrifuging at a speed >10,000 g at 4°C for 10 min. The supernatant was carefully transferred into another tube and a 0.95 volume of isoproponal was added to precipitate the DNA which was then rinsed twice with 750 ul of 70% ethanol. Ethanol was removed and the pellet was suspended with 25 µl Tris buffer pH 7.5-8.0 from the QIAquick Gel Extraction Kit (Qiagen). DNA was quantified using PicoGreen .

2.5 Polymerase chain reaction (PCR) amplification of 16S rRNA gene sequences

Environmental DNA was PCR-amplified using primers targeting the bacterial 16S ribosomal RNA genes. Each sample was assigned a uniquely barcoded reverse primer and amplified (Hamady et al., 2008). Primers used for amplification were bacteria-specific primers DW786F (5'-GATTAGATACCCTSGTAG-3') and DW926R (5'-CCGTCAATTCMTTTRAGT-3') modified from Baker et al. (2003) (Baker et al., 2003) that targeted the hyper-variable region 5 (V5 region). Reactions were performed in 50-µl volumes of which 10 µL is of 5X Phire Reaction Buffer (conc. final 1.5mM $MgCl_2$), 1 µL of 10 mM dNTPs (conc. final 0.20 mM of dNTPs) and 1.0 µL of Phire Hot Start II DNA

Polymerase (Finnzymes Thermofisher Scientific) and 2.5 μL was the primers (conc. final 0.5 μM) each primer. Cycling conditions started with an initial 3 min denaturing step at 98°C, followed by 30 cycles of 5 s at 98°C, 5s at 49°C, and 10 s at 72°C, and a final elongation step of 1 min at 72°C. Each reverse primer was barcoded with a specific sequence to identify samples. DNA was purified using QIAquick Gel Extraction Kit (QAIGEN). Quantification of PCR product was conducted using Quantifluor dsDNA System (Promega), a fluorescence DNA-binding dye enabling sensitive quantification of small amounts of dsDNA. DNA was then pooled together in equimolar concentration of 16 pM.

2.6 Ion Torrent sequencing

DNA was subsequently sequenced on an IonTorrent PGM using the 200 base pair kit. A total of 3.50 x 10^7 molecules were used in an emulsion PCR using an Ion OneTouch 200 template kit (Life Technologies) and OneTouch ES instruments (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. The sequencing of the pooled library was performed using an Ion Torrent Personal Genome Machine (PGM) system and a 316 chip (Bacteria) with the Ion Sequencing 200 kit according to the manufacturer's protocol.

2.7 Sequence processing and quality control

The 16S rDNA sequence-processing pipeline used for this study is composed of a selection of bioinformatics tools proven to be accurate, robust and fast (Fouts et al., 2012). Initially, the SFF file (Standard Flowgram Format), output from the sequencer, was converted into fasta and qual files. Subsequently, the trim.seqs function in

MOTHUR (mothur v.1.30.0) was used for subsequent sequence processing (Schloss et al., 2009). Sequences having an average quality < 25, not exactly matching the IonXpress sequence or PCR reverse and forward primer sequence, or that were shorter than 100 bp were discarded. Unique sequences were aligned and clustered into operational taxonomic units (OTUs) using MOTHUR in order to reduce the size of the dataset. Sequences were trimmed, aligned to the reference SILVA database, and a distance matrix was made on the aligned sequences. Subsequently, we clustered the sequences at 90 and 97% sequence similarity using the furthest neighbor algorithm to achieve OTU definitions at 0.10 and 0.03 distances. Accounting for possible sequence error, we removed sequences that were only observed once in a sample.

2.8 Diversity and taxonomic analysis

Alpha diversity measures on rarefied samples (~7500 sequences), to control for uneven sample size, were conducted using MOTHUR (Schloss et al., 2009). Chao1 index (Chao et al., 2000) was used for estimating species richness. Using MOTHUR, the chao calculator returns the Chao1 richness estimate for an OTU definition. It is a non-parametric estimator for species richness in the formula: $S_{chao1}=S_{obs}+ n_1(n_1-1)/2(n_2+1)$, in which S_{chao1} is the estimated richness, S_{obs} is the observed number of species, n_1 is the number of OTUs with only one sequence (singletons) and n_2 is the number of OTUs with only two sequences (doubletons). Bray-Curtis index (Bray & Curtis, 1957) was used to determine dissimilarity between samples and this index accounts for richness by calculating the minimum times a phylotype is commonly found between two samples and dividing it by the total number of both libraries. Bray-Curtis uses the formula $D_{Bray\text{-}Curtis} = 1\text{-}2\ \sum\min(S_{A,i,}\ S_{B,\ i})/\ \sum S_{A,\ I} + \sum S_{B,\ I}$. Intra- versus inter-sample diversity comparisons

were analyzed using analysis of similarity statistics (ANOSIM) on Bray-Curtis. ANOSIM results in a value between 0 and 1 when comparing two communities. Zero indicates that there is as much variation within each community as between them, therefore the lower the number the less distinguishable the communities are from each other in terms of variation. The value 1 indicates that the communities have less variation within the community then between communities, therefore indicating distinct communities that can be distinguished from each other. Quality-filtered sequences were then assigned to taxonomic groups using the Wang method implemented in MOTHUR and a bootstrap value cutoff of >60.

2.9 Bacterial community structure analysis

Relationships between bacterial communities and environmental parameters were assessed using multivariate Non-metric Multidimensional Scale ordination (NMS Ordination) using Bray-Curtis distances using PC-ORD statistical program (McCUNE & Mefford, 1999). The NMS avoids distortions originating from the non-normal distribution of the species data. Analyses were performed using Sørensen distance measures (also known as Bray-Curtis). For the identification of correlations between environmental variables and the sample profiles, Pearson and Kendall's correlations were calculated for the significant axes of the NMS ordinations. Pearson and Kendall's correlations were performed separately for free-living and particle-associated bacteria of each sample. To test the significance of differences between the different samples, ANOSIM (Clarke & Green, 1988) was applied. ANOSIM generates a test statistic (R), which is an indication of the degree of separation between groups. A score of 1 indicates complete separation whereas a score of 0 indicates no separation.

3. Results

3.1 The physicochemical environment of the SLE

3.1.1 Salinity

At the time of sampling a pronounced salinity gradient was observed. Surface water salinity increased from 0 PSU at Station B and D/E to 12.8 PSU at Station F and then progressively increased reaching 28.6 PSU at Station 20 with a slight decrease at Station 22 (**Figure 4, Table 1**). The general vertical profile of salinity showed an increase in salinity in the first 50 m of the water column, consistent with estuarine circulation, reaching to a salinity level of 34.6 PSU in deeper waters. Station 22 held great interest due to the lowering of the surface salinity value of 22.16 PSU that is similar to Station 25 (21.2 PSU). This could possibly be due to the influx of several northern rivers, mainly the Manicouagan River and the Outardes Rivers. Station 20 was the approximate boundary separating the LSLE and the Gulf of St. Lawrence; its surface salinity was the highest observed (28.7 PSU) and increased to 34.5 PSU at lower depths.

3.1.2 Temperature

Surface water temperature was consistent along the transect and ranged between 4-6ºC. We observed the typical three layer system that characterizes the LSLE in spring and summer, namely warm surface water, the cold intermediate layer (CIL), and deep North Atlantic waters (**Figure 4**). In fact, the general surface layer was characterized by relatively warm temperatures (around 5°C) over a depth of about 20 m, the CIL had temperatures between -1 and 2°C over a depth of around 100 m and finally, the water layer below 200 m was distinguished by temperatures ~5°C, which was similar to the

temperature of water in the Northwest Atlantic. The cold intermediate layer at Station 20 seemed to be higher in the water column and more compressed than any other LSLE stations starting from 6.2°C at the surface (3 m) and immediately dropping to 0°C in the span of 20 m. It then increased progressively reaching 5°C at 315 m.

3.1.3 Oxygen

A defining feature of the SLE is a pronounced vertical O_2 gradient and an objective of this study was to characterize the bacterial communities residing in the deep hypoxic waters. At the time of sampling, surface waters (3-5 m) as well as CIL waters (80m and 90m) held O_2 concentration values close to saturation levels ranging from 339.6 μmol/kg and 344.0 μmol/kg at the surface stations (Stations B and D/E) to 329.4 μmol/kg and 309.2 μmol/kg at Station I (3-145m) (**Figure 4**, **Table 1**). O_2 concentrations decreased with depth at all LSLE stations, from between 412.6 μmol/kg and 309.2 μmol/kg at the photic zone to between 67.3 μmol/kg to 62.2 μmol/kg at the bottom nepheloid layer (**Figure 4, Table 1**). The deep-water O_2 concentrations in the LSLE has been previously described to be hypoxic (Gilbert et al., 2005) and this region is highly interesting in terms of elucidating bacterial community composition and the possible metabolic activity that could potentially exist in this hypoxic environment.

3.1.4 Water column turbidity

We used beam transmission (BT) to measure water column turbidity, which is determined by particle load in the water column. Low beam transmission values correspond to high turbidity and particle load. The maximum turbidity zone of the SLE is

located between Île d'Orléans and Île aux Coudres in the Upper Estuary (USLE). The water turbidity at the freshwater stations in the USLE was high (BT between 29.9% and 4.3%) and in addition, located near Îles-aux-Coudres, Station F also showed high concentrations of SPM, as proven by the beam transmission data ranging from 23.2% at the surface (3m) to 0.6% at 45m (**Figure 4, Table 1**). Station 25 was an interesting station due to its location in the upwelling regions of the SLE. Located at the Esquiman channel, turbidity was high at the surface (78.6% beam transmission) and it was also high at the nepheloid layer at 320m (74.7% beam transmission). Although not as high as USLE samples, this value was relatively high for the LSLE regions. In fact, it was this high BT value, representing the resuspension of the bottom sediments near the head of the estuary, which helped us uncover the fact that we had successfully sampled the nepheloid layer.

3.2 Cell abundance and bacterial production

Cell abundance and bacterial production (BP) were variable in the surface salinity transect of the SLE (**Figure 5**). Cell abundance decreased steadily from 6.48 x 10^5 cells/ml at the freshwater Station B to 2.55 x 10^5 cells/ml at the brackish Station K. The same decrease is observed in BP, showing a decrease from 21.64 μgC/l/d at Station B to 4.73 μgC/l/d at Station K. Cell abundance and BP then abruptly increased to 4.24 x 10^5 cells/ml and 15.90 μgC/l/d respectively at Station 25 in the span of 21.08 km and kept on steadily increasing reaching a peak at Stations 22 with 6.92 x10^5 cells/ml and a BP value of 59.12 μgC/l/d in the span of 121.64 km. Both datasets then decreased to 3.64 x 10^5

cells/ml and 26.03 μgC/l/d at Station 21 in the span of 56.93 km and then decreased slowly to 3.36 x 10^5 cells/ml and 21.22 μgC/l/d.

Bacterial production was correlated with water mass distribution along the vertical profile of the SLE water column in both the USLE and the LSLE (**Figure 6**). In fact, BP is highest in the top 30 m of the water column and followed the same step-wise trend as the temperature profile. Lowest BP was thus observed in the deepest region of the SLE.

3.3 Summary of the 16S rRNA gene sequence data

To study bacterial communities in the SLE, we performed PCR amplification and sequencing of the variable region 5 (V5) of the 16S rRNA gene. Using barcoded semi-conductor sequencing technology (Hamady et al., 2008), we generated a total of 1,080,991 V5 16S rRNA gene sequences. After quality filtering, trimming, and clustering of sequences into operational taxonomic units (OTUs), the dataset consisted of 121,127 unique sequences and 50,718 OTUs defined at 97% sequence identity and 12,635 OTUs defined at 90% sequence identity (**Table 2**). A 97% and 90% identity in 16S rRNA gene sequence was the criterion used to identify a taxon at the species and family levels, respectively.

3.4 Estuarine bacterial community richness

The majority of multicellular organisms prefer either a freshwater or a marine environment and only several brackish species have a wide range of salinity tolerance (Cognetti & Maltagliati, 2000; Ysebaert et al., 2003).Therefore, estuarine and brackish

waters are characterized by reduced diversity in faunal and floral communities. Here, we estimated bacterial richness using the Chao 1 richness estimator at 90% sequence identity (Chao, 1984). In the SLE, we observed a higher diversity in the particle-associated (PA) bacterioplankton fraction than its free-living (FL) counterparts along the surface salinity gradient (**Figure 7a**). Within the FL fraction, bacterial richness ranged between 492 and 1,420 estimated phylotypes, with no apparent relationship with salinity. However, within the PA fraction, bacterial richness ranged between 874 and 2,813 and appeared to exhibit an overall decrease along the salinity gradient. More specifically, PA community richness steadily increased from the freshwater stations (Stations B and DE) with a peak at Station I and then progressively decreased until Station 20 passing by an observable slight increase in diversity in the particle-associated fraction at Station 22. Bacterial richness in the CIL of Station 23 and 21 **(Figure 7b**) was lower than richness observed in surface communities from the same station, suggesting that the less productive bacterial communities associated with icy cold water harbour less taxa.

The bacterial community richness in deep hypoxic water was variable depending on the location where samples were collected. Richness within the FL fraction ranged between 962 and 1,241 and was similar between locations. However, we observed a range in richness between PA bacterial communities. In fact, the highest estimated richness (3,244) observed in the whole of the SLE was from the PA bacteria sampled from the deepest point (320 m) at Station 25. Station 25 is located in the middle of the estuary, characterized by strong upwelling, and high loads of particles were located in this region. A similar level of richness (3,113) was also observed in the particle rich

bottom waters sampled at Station I, suggesting that physical mixing of water at the head of the Laurentian Channel is leading to high bacterial richness.

3.5 Comparison of SLE bacterial community structure

Owing to the distinct origin and environmental conditions of surface and deep water in the SLE, we expected the bacterial communities residing in these different regions of the water column to be taxonomically distinct. Ordination analysis using Non-metric Multidimensional scaling (NMS) demonstrated that this was indeed the case, and that both the free-living and particle associated bacterial communities were distinct in the surface and deep layers (**Figure 8**). In the ordination plots, samples collected from the deep layer (>150 m) were clearly separated from samples collected from the surface layer along ordination axis 2 (**Figure 8**). ANOSIM results (using Bray Curtis at 97%) also confirmed this high difference in surface and deep samples with an R-value of 0.670 (p-value < 0.001) (**Figure 8a, Table 5**) for free-living samples and an R-value of 0.860 (p-value =0.001) for particle-associated samples (**Figure 8b Table 5**). High variation exists in surface (3-5 m) samples originating from the USLE and LSLE, hence we performed a further ANOSIM only comparing the LSLE surface and deep communities. Indeed, ANOSIM values show a higher degree of dissimilarity between surface and deep samples, with an R-value of 0.993 (p-value = 0.001) for the FL fraction and an R-value of 0.905 (p-value = 0.001) for the PA fraction (**Table 5**).

Next, we asked which environmental variables correlated with the separation of surface and deep communities along axis 2 of the ordinations. Not surprisingly, depth exhibited a strong negative correlation with axis 2 (**Figure 8**). Moreover, oxygen

exhibited a strong positive correlation with axis, which reflects the clear vertical oxygen gradient that exists in the LSLE (**Figure 8**).

3.6 Comparison of FL and PA bacterial communities in SLE

Owing to the distinct nature of bacterial communities in surface and deep layers of the SLE, we separated our samples into two datasets for further analysis. The first dataset consisted of samples from the surface layer and CIL and was used to explore bacterial community dynamics across the surface salinity gradient. The second dataset consisted of samples from the deep hypoxic region, encompassing the bottom nepheloid layer, and was used to explore the differences between FL and PA bacteria in the hypoxic bottom water.

Ordination analysis was then performed on communities sampled from the upper water column regions. FL communities were clearly separated from PA communities for all samples along axis 2 (**Figure 9a**), indicating distinct bacterial composition in the FL and PA communities Moreover, communities were clearly separated by geographic location along the estuarine salinity gradient along axis 1 (**Figure 9a**). Moreover, comparison between the surface PA group and the surface FL bacterial groups showed an R-value of 0.435 (Bray-Curtis) and P-value of 0.005, suggesting distinct, yet variable, community structure between and within these two groups.

A different pattern was observed in ordination analysis of deep layer samples (**Figure 9b**). PA and FL communities were strongly separated from each other along axis

1, however unlike PA and FL bacteria in the surface samples, deep samples did not exhibited a similar structure along the geographic transect. In fact, the variation within the free-living community appeared to be considerably less than the variation within the PA communities.

This different community structures observed in the surface and deep water becomes clearer when the average similarity is plotted for FL and PA communities. In **Figure 10**, I present the box plots from 3 different comparisons: FL vs. FL, PA vs. PA and FL vs. PA from the same location. These comparisons indicate the degree of dissimilarity between the various groups as measured by the Bray-Curtis dissimilarity index. Panel a) and b) of Figure 10, showed the dissimilarity between surface a) and deep b) samples at 97% sequence similarity. For the surface communities, comparisons between FL communities or between PA communities displayed higher dissimilarly index suggesting the clear geographical range in different samples from the freshwater section (samples from Stations B and D/E) towards the marine stations. However, the FL-PA comparisons, in which we compared the dissimilarity between a single free-living sample and its particle-associated counterpart, showed that there was not as high a dissimilarly between them. ANOVA (analysis of variance) was used to determine the similarity between surface groups were not statistically different from each other (ANOVA, $p=0.402$ at 97%, $p= 0.307$ at 90% sequence similarity).

In contrast to the surface samples, the deep samples (**Figure 10b**) showed a higher dissimilarity between the pairwise comparison of free-living and its particle-

associated counterparts (FL-PA). FL-FL pairwise comparison showed high similarity among them suggesting that the observations among the free-living groups tend to be similar to each other. This could also be observed in the NMS (**Figure 8**) that showed the free-living community clustered together. The PA comparison (PA-PA) showed higher dissimilarity values suggesting higher variability among this group. However, pairwise comparisons from FL and its PA counterpart in the deep regions show significant high dissimilarity values suggesting a clear distinction between free-living and particle-associated bacterioplankton communities in the deep-waters of the St. Lawrence Estuary. Indeed, ANOVA did find a significant difference between the deep groups. In fact a p-value of 2.6×10^{-12} at 97% and p-value of 1.25×10^{-12} at 90% was determined for the deep sample groups.

3.7 Distance decay

The distance–decay relationship reflects how diversity is spatially distributed and provides a broad perception of community structure. In **Figure 11**, we observed that geographical distance was mainly affecting community composition at the surface (**Figure 11a**) rather than the deep (**Figure 11b**).

Figure 11a showed the pairwise comparison between each sample and the distance between them in the surface (3-5m) section of the SLE. For example, we compared Station B with Station D/E and subsequently Station B with Station 20. As Station B was located in the freshwater section of the SLE and Station 20 was located at the high-brackish region, the geographical distance between them was significant. We then established the relationship between geographical distance (in kilometers) and

community similarity as measured by the Bray Curtis Similarity Index. Both FL and PA fractions showed a gradual decay in community similarity as the distance between sampling stations increased. In fact, it was well known that many ecological phenomena incorporate the pattern of decreasing community similarity with geographical distance (Nekola & White, 1999) and our dataset suggested that in the surface region of the SLE, the bacterial community similarity decayed with distance because of decreasing similarity in environmental features. In fact, bacterial species could differ in their ability to grow under distinctive environmental conditions such as the pronounced salinity gradient that existed in the surface region of the SLE.

Figure 11b showed the relationship between distance and bacterial community structure of the deep nepheloid layer (280m and 320m) of the LSLE. In contrast to the surface SLE, a different pattern was observed for FL and PA fractions. The FL deep nepheloid samples did not show great variation with distance, however the PA fraction showed higher variability. This suggests that higher variation could be found on the particles, as the free-living pairwise comparison did not vary with geographical distance.

In all, these analyses showed that in the surface waters of the SLE, local differences (differences between FL and PA) are trumped by higher geographical differences on a macro scale. Furthermore, in the deep nepheloid layer of the SLE, PA bacterial fractions show higher variability than FL bacteria and the bacterial community from the FL fraction was not affected by geographical distance as opposed to the PA fraction.

3.8 Taxonomy of bacterial communities in the SLE

While ordination analysis supplied clear evidence that bacterial communities varied in the surface waters, the identity of the bacterial groups remained unknown. Therefore, we next performed a taxonomic analysis of the 16S rRNA gene sequences to investigate how taxonomic groups varied in SLE surface waters.

3.8.1 Taxonomy of surface water (3-5m) bacterial communities

In agreement with the ordination, bacterial community composition in surface samples (3-5 m) changed considerably along the estuary salinity gradient. The relative abundance of 16S rRNA gene sequences from Beta-proteobacteria and Actinobacteria showed a negative relationship with salinity. In contrast, the relative abundance of Alpha-proteobacteria and Gamma-proteobacteria increased along the SLE salinity gradient in the surface. Several bacterial groups were found throughout the salinity gradient such as Bacteroidetes. Since this is the first taxonomic assessment of the bacterial community in the SLE system, we will provide information in detail about each of the main phyla we identified

Beta-proteobacteria. The relative abundance of FL Beta-proteobacteria decreased from 43% at the freshwater station (Station B) to less than 2% at the high brackish station (Station 20) (**Figure 12**). At higher taxonomic resolution, we observed that the Beta-proteobacteria at the surface (3-5m) in the SLE was comprised mostly of the orders Comamonadaceae, Burkholderiales and Methylophilales (**Figure 12**). The Beta-proteobacteria showed a greater abundance in the free-living fraction compared to the particle-associated fraction. In addition, we observed a decrease in the group Comamonas from the freshwater section (Stations B and D/E) of the estuary and a subsequent increase

in the group Methylophilus in the estuarine section of the estuary (from Stations I to Station 22). There was also a signal for the group Polynucleobacter necessarius belonging to the order of Burkholderiales in the USLE up until Station 25.

Actinobacteria. Actinobacteria was mostly found in the freshwater regions of the SLE, mainly at Stations B, DE and I (**Figure 13**). These Actinobacteria were mainly comprised of Actinobacteridae (ACK-M1). Free-living Actinobacteria were significantly more abundant than their PA counterparts at the freshwater region (station B and D/E). However, starting at the brackish regions at Station I, both free-living and particle-associated fractions did not differ substantially in regards to their 16S rRNA relative abundance as both of the fractions decrease as salinity increases.

Alpha-proteobacteria. The FL fractions of the Alpha-proteobacteria increased from 3.67% at Station B to 25.87% at Station 20 (**Figure 14**). We observed an abundance of the SAR11 clade and the genus Rhodobacter in the free-living fraction between the estuarine sections of the SLE (**Figure 14**). In general, Rhodobacterales, mainly the Arctic96A-1 clade, seemed to peak at the estuarine section between salinities of 21.2 and 22.2 PSU and decreased in relative abundance at Station 21 with a salinity value of 27.1, but then increased again in abundance at Station 20 with 28.7 PSU (**Figure 14**). The other abundant Rhodobacter, the OM42 clade, seemed to steadily increase from the freshwater section towards the marine section from a relative abundance value of less than 1% to 12% at Station 20 and this clade seemed to dominate the marine end of the estuary. Similar to previous studies, we have also detected the OM42 clade, belonging to the SAR83 cluster (Allgaier et al., 2003; Gonzalez et al., 2000; Rappé et al., 1997; Suzuki et al., 1997).

Gamma-proteobacteria. The relative abundance of Gamma-proteobacteria increased along the salinity gradient in the same manner as the Alpha-proteobacteria group (**Figure 15**). The clade Arctic96B-16, nested under ZA2333c, part of the order of the Oceanospirillales, was abundant with 8% at Station 23 3 m free-living and Station 20 3 m free-living (**Figure 15**). SAR86 also displayed the same trend with 5% relative abundance at station 23 and 22. The estuarine sections (Stations 25 to 22 with salinities between 21 and 24 PSU) were dominated by the groups ZA2333c (Arctic96B-16), GSO clade and SAR86 while the marine section is dominated by Arctic96B-16, HTCC2207 which is known as SAR92 group and the group Alteromonadales in particle-associated fractions.

Bacteroidetes. The relative abundance of the phylum Bacteroidetes (mainly comprised of the class Flavobacteria) varied over the estuarine gradient and shifted in regards to the relative abundance of its particular groups (**Figure 16**). The Psychroserpens burtonensis group was abundant at the high-brackish end of the SLE (**Figure 16**). On the other side of the salinity profile, the freshwater regions (Stations B, D/E and I) showed the presence of Sporocytophaga, Saprospirales and other Flavobacteria possibly originating from terrestrial and soil environments. In fact, we do observe in the freshwater section the abundance of the Sporocytophaga group (8%), Saprospirales (4%) and an assortment of unclassified Flavobacteria (8%) as well as unclassified Bacteroidetes (15%).

3.8.2 Taxonomy of deep-water (>150m) bacterial communities

We then analyzed the taxonomic composition of deep samples in the SLE. The phylum Marine Group A (MGA) was present in all LSLE deep samples with the free-living fraction much more pronounced that its particle-associated counterparts (**Figure**

17). The relative abundance of MGA in the nepheloid layers of the deep LSLE samples varied from 16% at station 25 280m to 14% at station 20 280m (**Figure 17**). The MGA phylum consisted mostly of the subgroups Arctic96B-7, SAR406 and ZA3312c. Another prominent class in the deep-waters of the SLE is Delta-proteobacteria with relative abundances of 8% in all LSLE deep samples (**Figure 18**). The main subgroups belonging to the Delta-proteobacteria are the SAR324 clade and the Nitrospina group which are mainly found on the free-living samples.

3.8.3 Phylotype variation in the nepheloid layer

Comparison of phylotype distributions among the selected samples as shown by the venn diagrams (**Figure 22**) was able to compare the observed richness shared among 4 groups, these groups originated from the nepheloid layer at the estuarine end and at the seaward end of the SLE transect. We chose the samples at Station 25 280m and 320m (FL and PA) as representatives of the estuarine region. We then chose the Station 20 280m and 315m as representatives of the marine environment. Here we use the term phylotype to describe bacterial species since the term phylotype is taxon-neutral and we can choose the phylogenetic level at which the phylotype is described, in this case it is at 97% sequence similarity.

Figure 22a showed us that overall there were 357 phylotypes common to the fours samples. 721 phylotypes were common to only the FL fractions of Station 25 deep nepheloid layer depths and 1,277 phylotypes were common to only the PA fractions from the same depths. It is interesting to note that we identified many more phylotypes found

only in the set of samples representing the PA fraction (1,277), which supports our diversity estimates presented in **Figure 7**.

In fact, we investigated the taxonomic assignment of these 1,277 shared phylotypes and we have found a diverse assembly of bacteria, mainly in the Gamma-proteobacteria group (**Figure 22 and 23**). We focused on Station 25 nepheloid layer because this region is located at the head of the Laurentian Channel, where particulate matter is carried vertically by upwelling processes and high diversity was measured at this location. Of those 1277 phylotypes, 24 could not be taxonomically assigned, 139 phylotypes were found to be associated with the Planctomycetes phylum, 97 phylotypes belonged to the Alpha-proteobacteria class, of which 4 phylotypes were assigned to the Consistiales order (1 belonged to Pelagibacter SAR11 and 3 to the Rickettsiales), 25 phylotypes were assigned to the Rhodobacterales order, of which 12 phylotypes were assigned to Rhodobacter and 1 phylotype, OTU283, belonged to the OM42 clade.

Furthermore, 188 phylotypes belonged to the Delta-proteobacteria class of which 39 belonged to the Myxococcales order, 34 belonged to the Japan trench sediment clone NB1-j and 3 phylotypes assigned to Nitrospina were found. Then, 72 Flavobacteriales were assigned, of those 72 phylotypes, 34 were assigned to the Cytophaga order that were mainly comprised of Psychroserpens burtonensis (12 phylotypes) and 10 phylotypes belonged to the Sporocytophaga order.

Moreover, 339 phylotypes were assigned to the Gamma-proteobacteria class, of which 75 were unclassified, 12 were Alteromonadales, 47 were assigned to the BD1-1 group (an oligotrophic marine Gammaproteobacteria group), 5 were assigned to the HTCC2207 or SAR92 clade, 2 were assigned to GSO clade, 4 phylotypes belonged to the

SAR86 clade, 16 phylotypes were assigned to the Oceanospirillales order, 36 were assigned to the OM60 clade, 6 phylotypes were assigned to Arctic96B-16 cluster and 9 phylotypes were assigned to the Thiomicrospira, which are marine sulfur oxidizing bacteria.

In addition, Figure 22a also showed us that there were 721 phylotypes that were common to only the FL fractions of the nepheloid layer at Station 25. Of those 721 phylotypes, 54 could not be assigned, 111 phylotypes were assigned to the Marine Group A phylum and 137 phylotypes were assigned to the Alpha-proteobacteria class of which 80 phylotypes were Pelagibacter SAR11 (**Figure 23**). Subsequently, 135 phylotypes were assigned to the Delta-proteobacteria, of which 95 were assigned to the SAR324 clade and 34 were assigned to the Nitrospina order. Finally, 185 phylotypes belonged to the Gamma-proteobacteria class, of which 90 phylotypes were assigned to the oligotrophic marine Gammaproteobacteria (OMG) group SUP05.

In all, the taxonomic distribution of phylotypes varied when it came to preferred microenvironments. In fact, the samples belonging to the PA fraction seemed to harbor an array of taxonomic diversity greater than the FL fraction. FL phylotypes belonged mainly to the Marine Group A phylum, the SAR324 clade of Delta-proteobacteria and SAR11 of the Alpha-proteobacteria. In contrast, PA phylotypes showed a preference for the Gamma-proteobacteria class, the Flavobacteria class (Cytophaga and Sporocytophaga) and the Planctomycetes phylum.

We then we wanted to uncover which phylotypes were distinct from the nepheloid region of Station 25, a region with upwelling, as opposed to the nepheloid layer at Station 20, which could host autochthonous marine phylotypes. In other words, which phylotypes

are present at Station 25 and not at Station 20 on the particle-associated fraction. Figure 22c showed that there were 562 phylotypes only common to the PA fraction from Station 20 and there are 932 phylotypes common to the PA fraction from Station 25. Most of the 562 phylotypes from Station 20 are assigned to the Alteromonadales order of the Gamma-proteobacteria class (190 phylotypes) while most of the 932 phylotypes from Station 25 were assigned to an array of diverse taxonomic groups varying from OM60, Cytophaga, Delta-proteobacteria (Myxococcales, NB1-j), Oceanospirillales, to uncultured gamma proteobacteria. We further explored the diversity of the nepheloid layer at Station 25 with emphasis on the action of upwelling. Upwelling created physical mixing of the waters which lead to possible taxonomic groups that could be sinking with the particles to the nepheloid region. We have found the OTU284 assigned to the ACK-M1 clade of the Actinobacteria phylum which is also present at the Upper Estuary at Station I 40m on the particles. Similarly, OTU101 corresponding to the Methylophilales of the Beta-proteobacteria class was also found at the nepheloid layer at station 25 and was present at the USLE Station I 3m FL and 40m PA. OTU2601 of the Cytophaga Psychroserpens burtonensis, OTU3656 and OTU213 belonging to OM60 and the Sporocytophaga order all were found both in the nepheloid layer at Station 25 and the USLE Station I 40m PA.

The overall abundant groups shared in the deep hypoxic SLE nepheloid layer consisted of Flavobacteria, Marine Group A, Alpha, Delta and Gamma-proteobacteria. The GSO of the Gamma-proteobacteria class was shared among all FL deep samples (Station 25 and 20). Alteromonadales were found mainly in PA marine water samples at Station 20 and Marine Group A was only shared between free-living deep samples at Station 20.

Discussion

In this study, we provide the first description of the community structure and diversity of bacterioplankton inhabiting the free-living (FL) and particle-associated (PA) surface and deep environments of the SLE. We first provided insight into how bacterial richness, community composition and taxonomic structure changes along the salinity gradient in the surface waters of the SLE. We then explore the diversity and the taxonomic bacterial community composition in the LSLE deep hypoxic waters.

4.1 Bacterial activity in the SLE

Previous studies in the SLE by Vincent et al. (1996) found that there was a large increase in the contribution of bacteria attached to particles in the frontal zone of increasing salinity and turbidity in the SLE. This zone was then described to be the estuarine turbidity maxima (ETM) located between Île d'Orléans and Île aux Coudres in the Upper Estuary. In May 2011, we did not observe highest bacterial production in the ETM, but instead we did observe a peak in bacterial production and cell abundance in surface water at Station 22. This station is located near Pointe-des-Monts and it is influenced by the influx the Manicouagan River and the Rivière aux Outardes. High bacterial activity could be due to a number of factors that can limit or augment bacterial production over different spatial scales. These limitations could be the quality of dissolved organic matter, inorganic nutrients, grazing, or physical mixing (Kirchman, 1990; Rivkin & Anderson, 1997). Previous studies in the Mackenzie River have shown that glucose addition increased total bacterial production (Vallières et al., 2008). This suggests that bacterial metabolism was limited by the lability of available organic carbon

and this could be one of the factors affecting the high bacterial production at Station 22. In fact, estuaries are known to receive OM inputs from multiple sources, including allochthonous terrigenous materials exported from land by rivers (Goñi et al., 2003) and in the SLE it could be that the influx of the Manicouagan River and the Rivière aux Outardes could lead to higher bacterial production by introducing more OM into the SLE system. Moreover, earlier studies conducted in the SLE have shown that there were considerable differences in phytoplankton assemblages in different parts of the Estuary and the Gulf. Therriault et al. (1990) showed that one of the regions of high phytoplankton assemblage is the "plume" region dominated by outflows from the Manicouagan and Aux–Outardes Rivers. Station 22 is located at the Manicouagan and Aux–Outardes Rivers and phytoplankton assemblages could also be involved in the elevated bacterial production.

Bacterial production along the vertical profile of the LSLE differed between water masses. It is well known that bacteria convert existing organic molecule into their own biomass and oxidize some of these to final metabolic end products thus contributing to the total community respiration (Cole & Pace, 1995). This process tends to take place in the upper euphotic zone of the water column where there are more resources available. Thus, heterotrophic bacteria are highly active in the top regions of the water column and are less productive in the deeper areas.

4.2 Bacterial community richness in the SLE

It is well known that most organisms prefer freshwater or marine habitats and that only a few brackish species can tolerate the transition zones between marine and

freshwater environments (Cognetti & Maltagliati, 2000; Ysebaert et al., 2003). A reduced diversity in faunal and floral communities is observed in brackish regions such as coastal and estuarine areas (Telesh & Khlebovich, 2010). A number of studies have investigated bacterial diversity in marine-freshwater transitions zones such as estuaries and have shown that the pattern observed in flora and fauna does not hold for bacteria. For example Herlemann et al. (2011) could not establish that brackish waters had reduced bacterial diversity, and they suggest that rapid evolution by bacteria have allowed them to adapt to brackish environments (Herlemann et al., 2011). In our study, we examined bacterial richness in FL and PA bacteria along a surface salinity gradient in the SLE from freshwater to high brackish water (0.08-28.67 PSU). The study by Herlemann et al. (2011) was restricted to the FL component of the Baltic Sea and in support of their finding, we do not find a decline in bacterial richness from fresh to high brackish regions in the free-living SLE community. However, particle-associated communities show a different pattern, characterized by a decline in diversity from freshwater to brackish locations.

Numerous other studies have compared FL and PA bacterial communities. In fact, Hollibaugh et al. 2000 have shown that the FL community tended to be richer than the PA community in the San Francisco Bay, but this difference was not statistically significant (Hollibaugh et al., 2000). Additional studies have shown that marine bacteria show a preference for the microenvironment offered by particles (Acinas et al 1999; Bidle & Fletcher, 1995; Moesenederet al 2001). In fact, the variability and richness of the PA bacterial community in aquatic habitats could be explained by the differences in the concentration of particulate organic matter. An estuary system receives a large amount of

allochthonous organic matter that will be subsequently transformed and decomposed leading to diverse microhabitats on particles. Thus, taken into account these factors, the high bacterial richness observed in the SLE could further indicate that the PA bacterial fraction could be influenced by this microenvironment offered by particles from the inflow of terrestrial material.

We have also examined bacterial richness in FL and PA bacteria in the deep hypoxic regions of the SLE. The second highest level of richness was observed in the particle rich bottom waters sampled at Station I. This region is located at the head of the Laurentian Channel and is subject to high physical mixing that could lead to high bacterial richness. The bacterial richness exhibited in the hypoxic deep waters of the SLE show variation depending on the sampled location. The highest richness was in areas of high particle load at the head of the Laurentian channel located at Station 25. The particle–rich layer close to the bottom nepheloid is well developed in the Laurentian Channel and this region is portrayed by increasing turbidity caused by the friction of tides at the sediment–water interface. It could be that the higher number and quality of particles in the deep regions can explain the higher richness of the particle-associated fraction as well as the higher richness of the whole bacterial assemblage at those sites. In fact, the analysis concerning phylotype variation in the nepheloid layer has shown that the PA fraction from Station 25 (estuarine) differs in taxonomic identity from the saline nepheloid region originating from Station 20. We will discuss in detail the taxonomic assignment of phylotypes belonging to the nepheloid layer further on.

4.3 Allochthonous versus autochthonous bacteria in the SLE

We have explored the bacterial richness in PA bacteria, however the question still arises whether or not there exists a resident brackish/estuarine bacterial community on these particles? Recently, a study on the Baltic Sea showed the existence of a brackish bacterial group within the Verrucomicrobia, (Herlemann et al., 2011). We do not observe an abundance of this group in the SLE. However, since the water residence times in the Baltic Sea is more than 5 years long (Reimann et al., 2008), the established brackish bacterial community is not the result of mixing of fresh water and saltwater, but it is instead an autochthonous brackish microbiome (Herlemann et al., 2011).

Another study suggesting the existence of an estuarine bacterial community was conducted in the Parker River estuary and Plum Island Sound, in northeastern Massachusetts, where they found a native estuarine bacterioplankton community that is phylogenetically distinct from allochthonous communities advected into the estuary by tidal action and river flow (Crump et al 2004). They suggested that shifts from a mixture of allochthonous communities to a native estuarine community requires sufficient bacterial growth rates and a comparatively long water residence time. Water residence time is the average time it takes for a parcel of water in a section of an estuary to leave that section (Crump et al., 2004). Given that the SLE has variable water layers (surface, CIL, deep), the residence time of a parcel of water is also variable. In the SLE, the saline bottom water flows slowly (~0.5 km/day) landward at the bottom of the estuary, while freshwater moves rapidly (~10–20 km/day) seaward at the surface (Pinet et al., 2011). The mean water residence time in the upper estuary is of the order of 10-20 days (D'Anglejan & Smith, 1973; Lucotte, 1989) and a parcel of water that enters the mouth

of the Laurentian Channel at 250 m would reach the head of the Laurentian Channel in about 3 to 4 years (Gilbert et al., 2005; Gilbert, 2004). Given the rapid water movement in the surface layer of the SLE, it is questionable that this system could support the development of a unique bacterial community. However, in order to maintain a resident estuarine bacterial community in a system like the SLE, one has to take into account the generation time of the bacteria. It could be possible for Station 22 to harbor a resident bacterial community since bacterial production was especially high at this location. Estimating the generation time of bacteria is complex due to numerous biological and physical factors affecting estuaries such as grazing, predation and hydrodynamics. Previous studies on bacterial dynamics in the SLE using a steady state box model has shown that bacterial growth and removal are closely balanced, and this suggests that bacteria tended toward a state of trophodynamic equilibrium where growth was relatively equal to grazing (Jean Painchaud et al., 1996). Therefore, if adequate bacterial growth rates could not be established in the SLE, a shift from a mixture of allochthonous communities to a native estuarine community would be observed. Given the high bacterial production and cell abundance at Station 22, we could theorize that this region can be a "hot spot" for the temporally the development of an estuarine group.

4.4 Shifts in bacterial communities along the salinity gradient

The surface (3-5m) area of the SLE holds great variations in salinity, ranging from freshwater to marine waters. Since salinity enforces selective pressures on aquatic microorganisms, it is interesting to ask how this change in salinity affects bacteria community structure in a large estuarine system such as the SLE.

In our dataset, we observe longitudinal partitioning in the surface SLE that is characterized by a shift from Actinobacteria and Beta-proteobacteria usually found in the freshwater to taxa such as Flavobacteria, Alpha- and Gamma-proteobacteria that are often associated with more saline regions. Thus, the major taxa found in the surface region of the SLE in May 2011 comprise of a mixture of freshwater and marine bacterioplankton. These findings have also been found in previous studies regarding estuarine systems (Allgailer and Grossart 2006; Crump et al., 2004; Glockner et al., 2000; Henriques et al., 2006; Rappé et al., 1999; Wu et al., 2011). Moreover, some of these bacteria, such as the OM42 clade are shown to have the capability for aerobic anoxygenic photosynthesis (Allgaier et al., 2003). Aerobic anoxygenic phototrophic (AAP) bacteria are photoheterotrophic organisms able to use both light and organic substrates for energy production and are widely distributed in coastal and oceanic environments (Beatty, 2002; Suzuki & Béjà, 2007; Yurkov, 1998). Previous study by Waidner and Kirchman have shown that AAP bacteria have a preference for particles in the Delaware estuary (Waidner & Kirchman, 2007). In our study, we observed Alpha and Gamma-proteobacteria groups that have been shown to have the capability for aerobic anoxygenic photosynthesis and we do observed the presence of both the OM42 clade and the OM60 clade on particles but also on the free-living fraction. This observation suggests that AAP bacteria appear to vary with environmental conditions in different habitats.

Furthermore, an array of different taxa within the Gamma-proteobacteria was identified in SLE surface waters. The more abundant are the SAR92 clade (HTCC2207) and the ZA2333c group. The SAR92 clade has been shown to possess genes for proteorhodopsin (PR), a light-dependent proton pump (Stingl et al., 2007). PR has also

been found in the SAR86 clade of Gamma-proteobacteria, which is also found in our dataset. PR phototrophy has been demonstrated to grant a fitness advantage to marine bacteria and can be a novel mechanism for bacterioplankton to endure frequent periods of resource deprivation at the ocean's surface (Gómez-Consarnau et al., 2010). In all, PR bacteria are not only abundant in the oligotrophic open ocean, but are also present in highly productive systems such as estuaries.

Moreover, Polaribacter and Cytophaga are also found in the surface waters of the SLE and their abundance increases as salinity increased. These Flavobacteria are traditionally known to degrade polysaccharides (Gonzalez et al., 2008; Kirchman, 2002). It is interesting to observe the presence of a particular group of Cytophoga, the Psychroserpens burtonensis, which was shown to be in high abundance in high-brackish regions of the surface waters in the SLE. This psychrophilic bacterium has been shown to prefer organic acids as carbon sources and does not in general utilize carbohydrates, alcohols, aromatics, or amino acids (Krieg et al., 1984).

4.5 Depth and bacterial community composition in deep hypoxic nepheloid layer of the SLE.

Depth is another environmental factor to consider. There are a number of variables that co-vary with depth in aquatic environments. Consequently, bacterial community composition can be influenced by these gradients that could result in diverse bacterial communities inhabiting diverse niches (Wu et al., 2011). It has been previously found that specific phylogenetic groups have only been detected in the photic zone of surface waters while other bacterial groups display maximal abundances at lower depths

of the water-column (Agogué et al., 2011). In fact, we do observe vertical partitioning in the SLE since the bacterial groups shift from Actinobacteria, Bacteroidetes, Alpha and Beta-proteobacteria in the surface to a bacterial community composition mostly comprised of Gamma, Delta-proteobacteria and Marine Group A in the deep hypoxic habitat.

Despite the separation of water masses in the SLE, the level to which these water masses are divided depends on the degree of mixing by river flow, tides, upwelling and other physical factors (Dufour and Ouellet 2007). This mixing could lead to the development of communities that consist of bacterial populations from various water masses. In fact, the bathymetry profile of the SLE off the coast of Tadoussac shows an interesting pattern: the Laurentian Channel ends abruptly at the confluence of the Saguenay Fjord located near Tadoussac and this results in an upwelling event in which deep cold, nutrient-rich coastal waters rise into the estuary. This upwelling process also moves particles vertically within the water column. Moreover, in May 2011, during an upwelling event, similar freshwater phylotypes were found in the nepheloid bottom waters at the head of the Laurentian Channel in the upwelling zone. This could indicate a possible exchange between surface and deep environments. In fact, Fortunato and Crump (2011) have also describe a similar upwelling mixture in the coastal zone of the Pacific Northwest in which they have also observed the mixing of communities from bottom to surface and found that the most abundant estuary-classified phylotypes were also found in some shelf bottom samples (Fortunato and Crump, 2011).

To examine the possible effects of upwelling leading to the exchange between surface and deep bacterial communities, we compared phylotype distributions among

groups from the nepheloid layer (**Figure 22**). To compare if deep particle associated phylotypes are originating from the upper estuary region or are autochthonous to the LSLE, we chose stations 25 and 20 as our estuarine and marine extremities. We observed a higher number of phylotypes residing on the particle-associated (PA) fraction of the low depths of Station 25 than in the free-living (FL) fraction, suggesting that higher bacterial richness are linked to high particle loads. The FL phylotypes from both stations were mainly assigned to the SAR11 clade of the Alpha-proteobacteria class, the Marine Group A phylum, the GSO clade of Gamma-proteobacteria and the SAR324 clade of Delta-proteobacteria. In contrast, the PA phylotypes differs from these two stations, in fact the presence of Alteromonadales make up the majority of the taxonomic identity in the saline deep nepheloid region at Station 20, while most of the phylotypes from Station 25 were assigned to an array of diverse taxonomic groups. This shows that some of these phylotypes originate from the sediments, such as the Delta-proteobacteria clones NB1-j (Yanagibayashi et al.,1999), suggesting resuspension of the sediments rich in SPM (suspended particulate matter). However, other phylotypes found are assigned to the Sporocytophaga order that could originate from the freshwater section at Station I due to upwelling and mixing of the waters. In fact, we have found several phylotypes that were both present in the upper St. Lawrence estuary as well as in the nepheloid layer region. Examples of such phylotypes are OTU284 assigned to the ACK-M1 clade of the Actinobacteria phylum, which is present at the Upper Estuary (USLE) at Station I 40m on the particles. Similarly, OTU101 corresponding to the Methylophilales of the Beta-proteobacteria class was also found at the nepheloid layer at station 25 and was present at the USLE Station I 3m FL and 40m PA. OTU2601 assigned to the Cytophaga

Psychroserpens burtonensis and OTU213 belonging to the Sporocytophaga order all were found both in the nepheloid layer at Station 25 and the USLE Station I 40m PA. OTU45, corresponding to the OM60 clade of the Gamma-proteobacteria class is another example. In fact, Station I (located upstream of Station 25), had an abundance of OM60 in its particle-associated fractions. The OM60 clade could be on sinking particles from the upper estuary given that we saw a signal for this clade in the nepheloid sample at the estuarine Station 25. Thus, particle input from the USLE can lead to sinking particles that are highly diverse, which is what we can ascertain from data extracted from Station 25 particles.

To explore this effect, we have to observe the bathymetry of the SLE. In fact, Station I and Station 25 are located close to one another (73.1 km apart) and the bathymetry of the SLE shows that Station 25 is located at the head of the Laurentian channel where particulate matter is carried vertically by upwelling effects. Furthermore, extreme tidal forces in the Upper Estuary (USLE) (Station I) all contribute in moving the particles vertically within the water column. It is then possible that the physical circulation patterns could of drive the particles originating from the USLE to the LSLE. Therefore by the action of upwelling, there will be physical mixing of the waters leading to possible taxonomic groups that could be sinking with the particles to the nepheloid region.

Another environmental occurrence that renders the St. Lawrence Estuary system appealing is the hypoxic regions in the LSLE. As mentioned previously, hypoxia is ongoing in the bottom waters of the LSLE. In fact, the release of nitrates and phosphates into the SLE from land sources can support the development and increase of

phytoplankton which can die off and sink into the ocean floor where its decomposition would cause a decrease in the oxygen levels. The development of hypoxic regions in the LSLE can thus be explained by a combination of physical and biogeochemical processes and we examined the taxonomic identity of deep hypoxic waters. We have found that the most prominent taxonomic groups found in hypoxic regions belong to the Gamma, Delta-proteobacteria and Marine Group A. More precisely, in the FL fractions of the LSLE, the Delta-proteobacteria cluster SAR324, which possesses genes for both carbon fixation and sulfur oxidation (Swan et al., 2011) has been found. This could suggest that chemolithotrophy is a metabolic lifestyle for bacterial communities within the hypoxic zone of the LSLE. Moreover, Nitrospina, a lineage that converts nitrite to nitrate is also found in the LSLE hypoxic waters and they have also been shown by previous study to exist in the deep ocean (Mincer et al., 2007). Other deep ocean bacterial groups found in the LSLE are members of the GSO, SAR11 and Alteromonadales groups. The GSO clade is commonly found in oxygen-deficient waters and this group may be involved in autotrophic sulfur oxidation. Indeed, recent work has revealed that the SUP05 (part of the GSO clade) metagenome harbors a versatile repertoire of genes mediating autotrophic carbon assimilation, sulfur oxidation, and nitrate respiration responsive to a wide range of water-column redox states (Walsh et al., 2009). In the SLE, the abundance of the SUP05 clade in the hypoxic waters of the SLE has been shown to be higher in FL fractions, whereas the PA fractions are shifting to the Alteromonadales groups. Traditionally, Alteromonadales are known to degrade high molecular dissolved organic matter (DOM), and have been also shown to contain TonB-dependent transporter (TBDT) genes for the uptake of DOM (Tang et al., 2012). An interesting recent paper has shown that a survival

strategy in many marine bacteria is the ability to undergo shifts in metabolism to adapt to a changing nutritional environment that can cause heterotrophic bacteria to utilize alternative pathways of carbon uptake such as inorganic carbon assimilation (DeLorenzo et al., 2012). In fact, Alteromonadales have been shown to be prevalent among the heterotrophic DIC-assimilating community and this could suggest that bacterial DIC assimilation operates in the deep hypoxic regions of the SLE, however further metagenomic works should be conducted to clearly examine the metabolic potential within the bacteria inhabiting these deep hypoxic regions.

Given that bacterial species can exist in an array of metabolic states, it would be highly interesting to explore whether they are dormant, active or growing. One interesting question that could be investigated following this study could be the metabolic state of the assigned taxonomic groups, Could the bacterial communities found in the hypoxic environments of the LSLE be metabolic active or dormant? Could the most abundant groups be the least metabolically active? Could the rare taxa exhibit high metabolic potential when facing hypoxic events? In this study, we only observed the DNA fraction of the dataset, however studying the product of active transcription of the ribosomal gene, the RNA fraction, can be useful to explore the metabolic activity and potential growth rate of specific taxa. It would be thus highly interesting to perform the same analysis but to employ the use of RNA analysis to show which groups can be more metabolically active.

5. Conclusion

The St. Lawrence Estuary has been well studied in terms of physical and chemical properties, however bacterioplankton community structure and diversity has not been well documented. This study has shed light on the bacterial community composition exiting in the surface and deep hypoxic waters of the SLE with differences between the taxa commonly found in the free-living fraction different from the particle-fraction. The particle-associated community differs at some stations with higher particle load than free-living counterparts. The most remarkable differences were found between the particle-associated and the free-living communities, with respect to community composition, OTU richness, and spatial dynamics. We conclude that the particle-associated community was more heterogeneous and more dependent on changes in the environmental conditions, such as algal blooms or inflow of terrestrial organic matter, than the free-living bacterial community. Next-generation sequencing has radically changed the scene of microbial ecology and in-depth diversity examinations are now broadly accessible. Future developments regarding the improvement of conceptual and methodological models would help in improve our understanding of spatial and temporal patterns of microbial life in different habitats. Metagenomics and proteomics are both powerful approaches as they provide the opportunity of exciting novel findings that motivates the advancement of microbial community analysis. These tools can also help unlock the expansive uncultured microbial diversity existing in the environment and could further supply new molecules for therapeutic and biotechnological applications.

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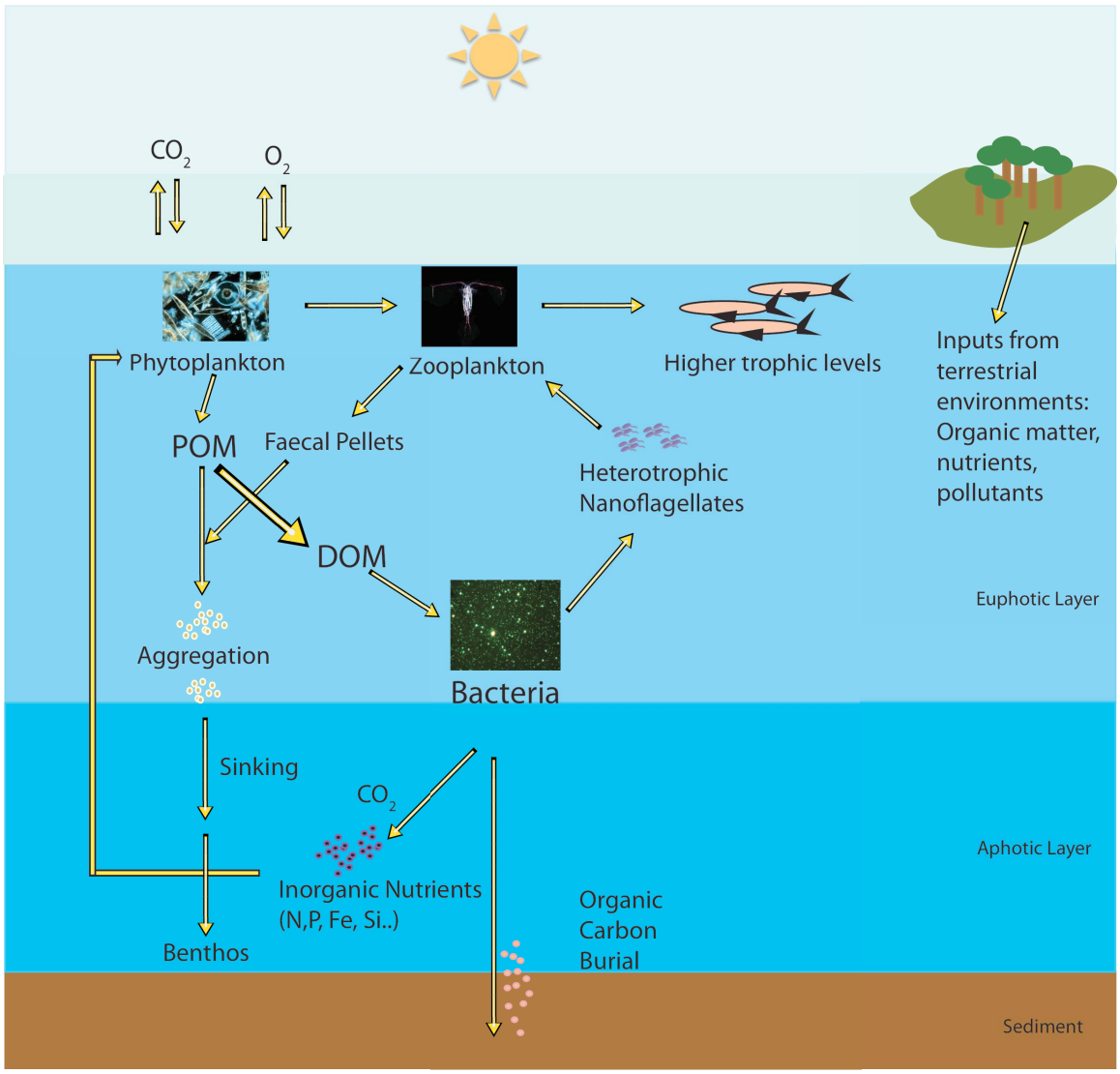

Figure 1) The classical and microbial food webs. This figure depicts the various biological processes occurring in aquatic habitats comprising several interplays between phytoplankton, higher trophic predators, dissolved organic matter (DOM), particulate organic matter (POM) and bacteria.

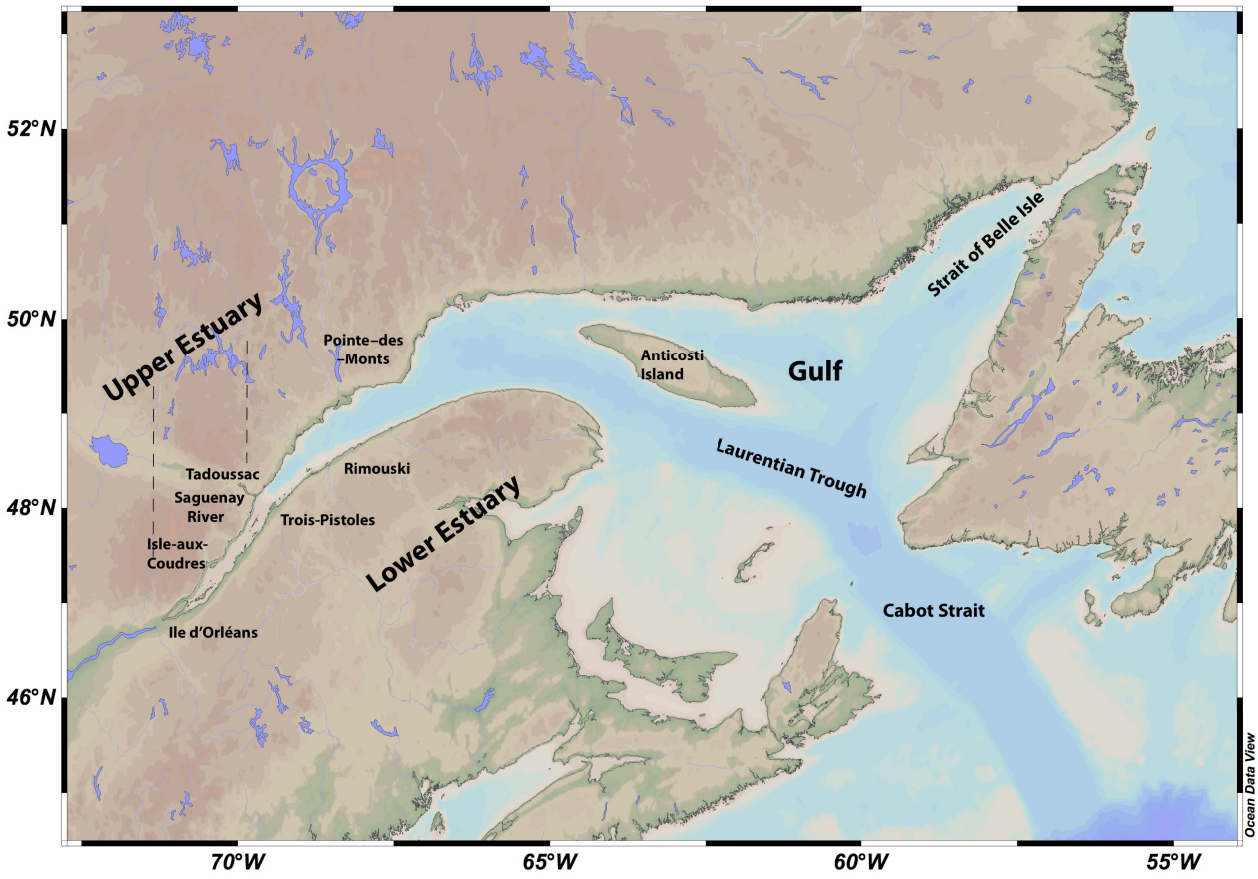

Figure 2) Map of the Estuary and Gulf of St. Lawrence system. This map shows the geographical location of the St. Lawrence Estuary with the Upper Estuary and Saguenay River covering an area from the eastern tip of Ile d'Orléans to the mouth of the Saguenay River. Highly turbid water is pronounced in the area between Ile d'Orléans and Ile aux Coudres. The Laurentian Channel of Trough is an underwater valley of more than 350 m in depth, which begins in the mouth of the Saguenay River and continues on to Gulf. Intense upwelling events happen at the confluence of the Saguenay River and the lower estuary. Figure generated using Ocean Data View (ODV) version 4.5 (Schlitzer, 2002).

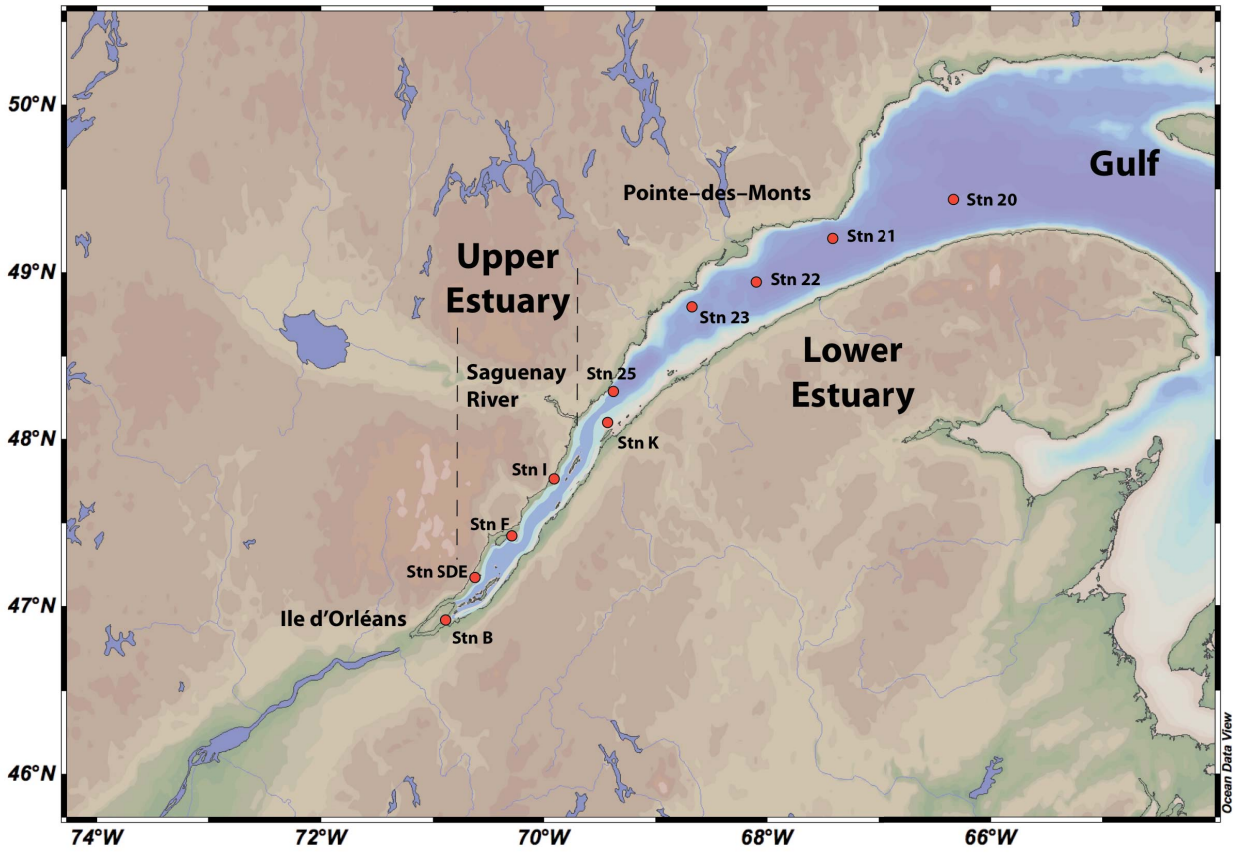

Figure 3) Map of the St. Lawrence Estuary sampling sites. Samples of microbial communities were collected from different depths at 10 Stations (Station B to Station 20, with the head of the Laurentian Channel at Station 25) along an oceanographic transect of the St. Lawrence Estuary (49°N, 68°W) located in Eastern Canada. Figure generated using Ocean Data View (ODV) version 4.5 (Schlitzer, 2002).

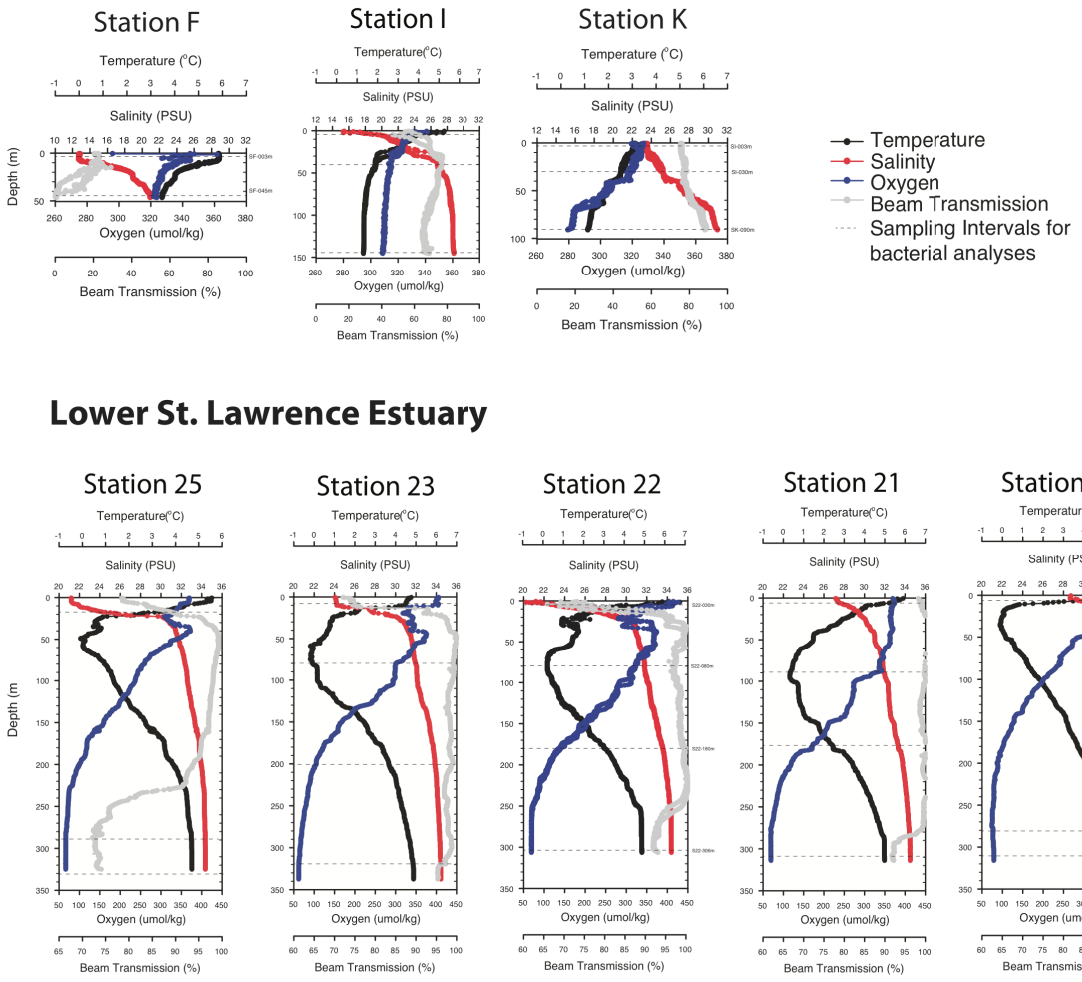

Figure 4) Depth distribution of oxygen, salinity and temperature from the St. Lawrence Estuary cruise during May 16-21 2011. CTD (Conductivity, Temperature and Depth) data was collected including full water column profiles for salinity, oxygen, temperature, density, and beam transmission at different depths (shown by horizontal dotted lines) in 8 stations along the St. Lawrence Estuary transect. The figure was generated using SigmaPlot version 11.2, from Systat Software, Inc., San Jose California USA.

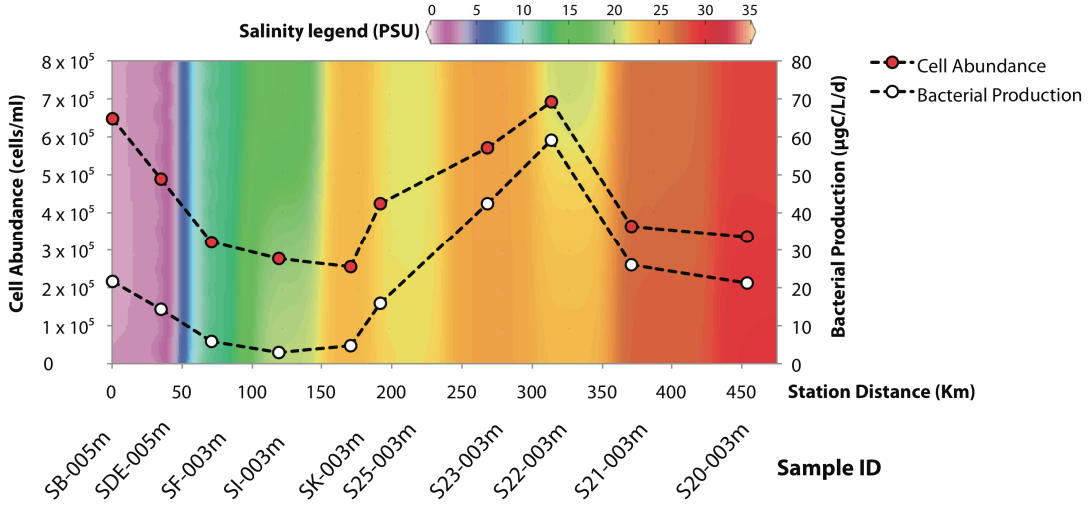

Figure 5) Cell abundance and bacterial production along the surface salinity gradient of the St. Lawrence Estuary transect. Cell abundance is measured in cells/ml and Bacterial production is measured in µgC/l/d. The salinity profile of the first 5m of the water column is shown in the background. Sample ID is depicted according to the distance between each station. The salinity figure was generated using Ocean Data View (ODV) version 4.5 (Schlitzer, 2002).

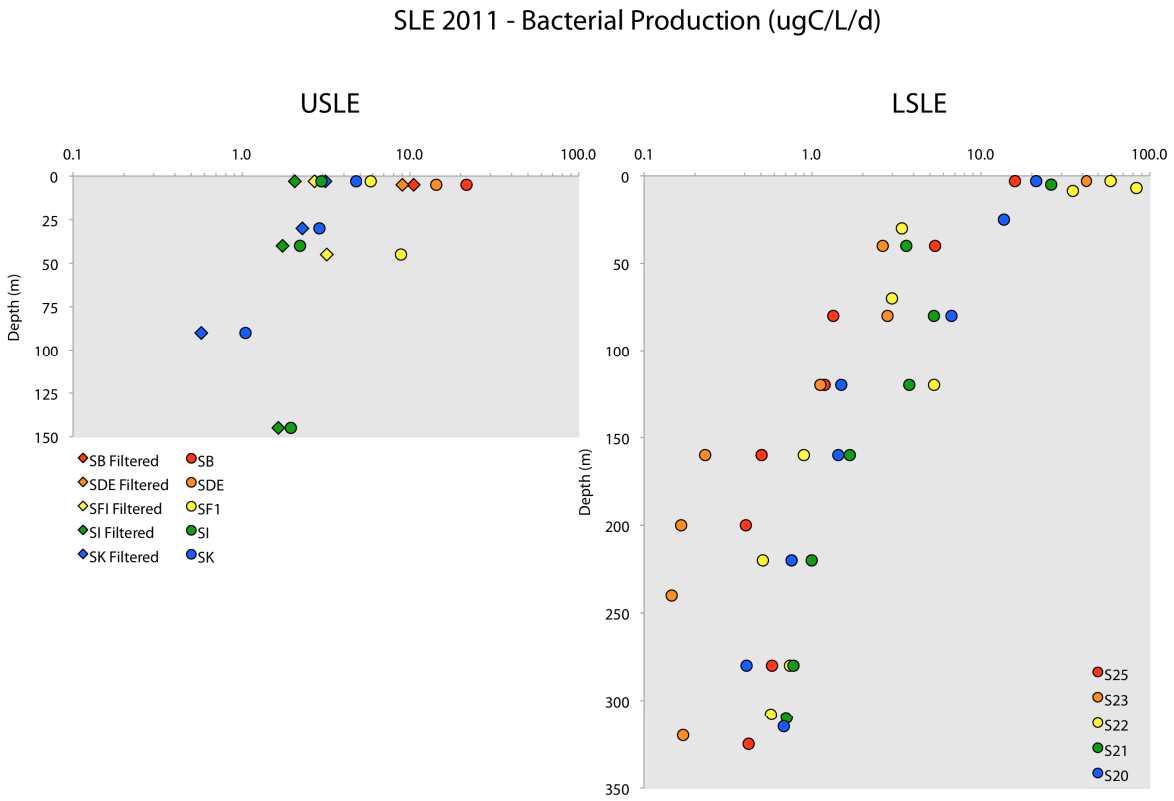

Figure 6) Bacterial production in the Upper St. Lawrence Estuary (USLE) and Lower St. Lawrence Estuary (LSLE). Bacterial production as measured in µgC/L/d is plotted against depth (m). Diamond shaped dots in the USLE represent the filtered portion of the collected samples, symbolizing the free-living bacterial fraction while circle dots (USLE and LSLE) represent the complete unfiltered bacterial samples, symbolizing particle-associated and free-living bacterial fractions. Different colors are shown to represent different sampling stations.

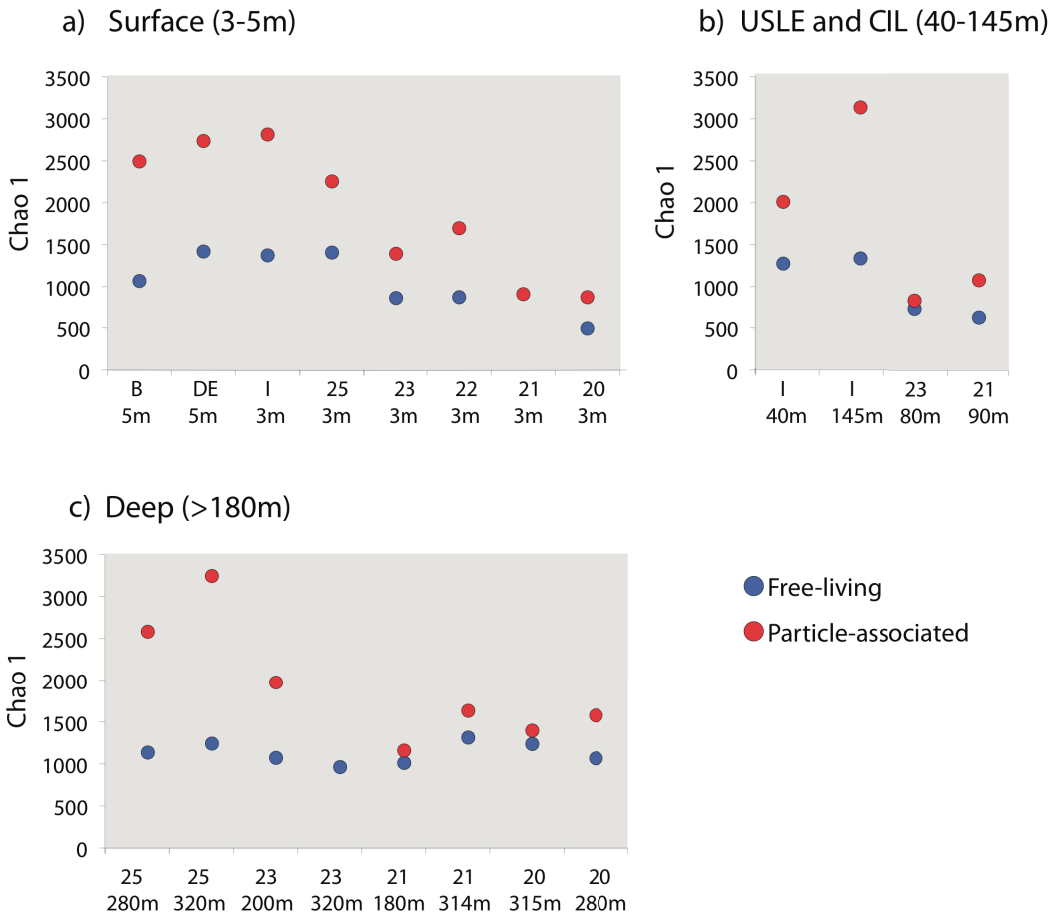

Figure 7) Alpha diversity (as measured with the Chao 1 estimator) is described for surface, cold intermediate layer (CIL) and deep samples of the St. Lawrence Estuary. Alpha diversity measures using Mothur (Schloss et al., 2009) were performed on rarefied samples (~7500 sequences), to control for uneven sample size. The Chao1 index (Chao et al., 2000) was used for estimating species richness. Chao 1 was calculated for both free-living (shown in blue) and particle-associated (shown in red) bacteria.

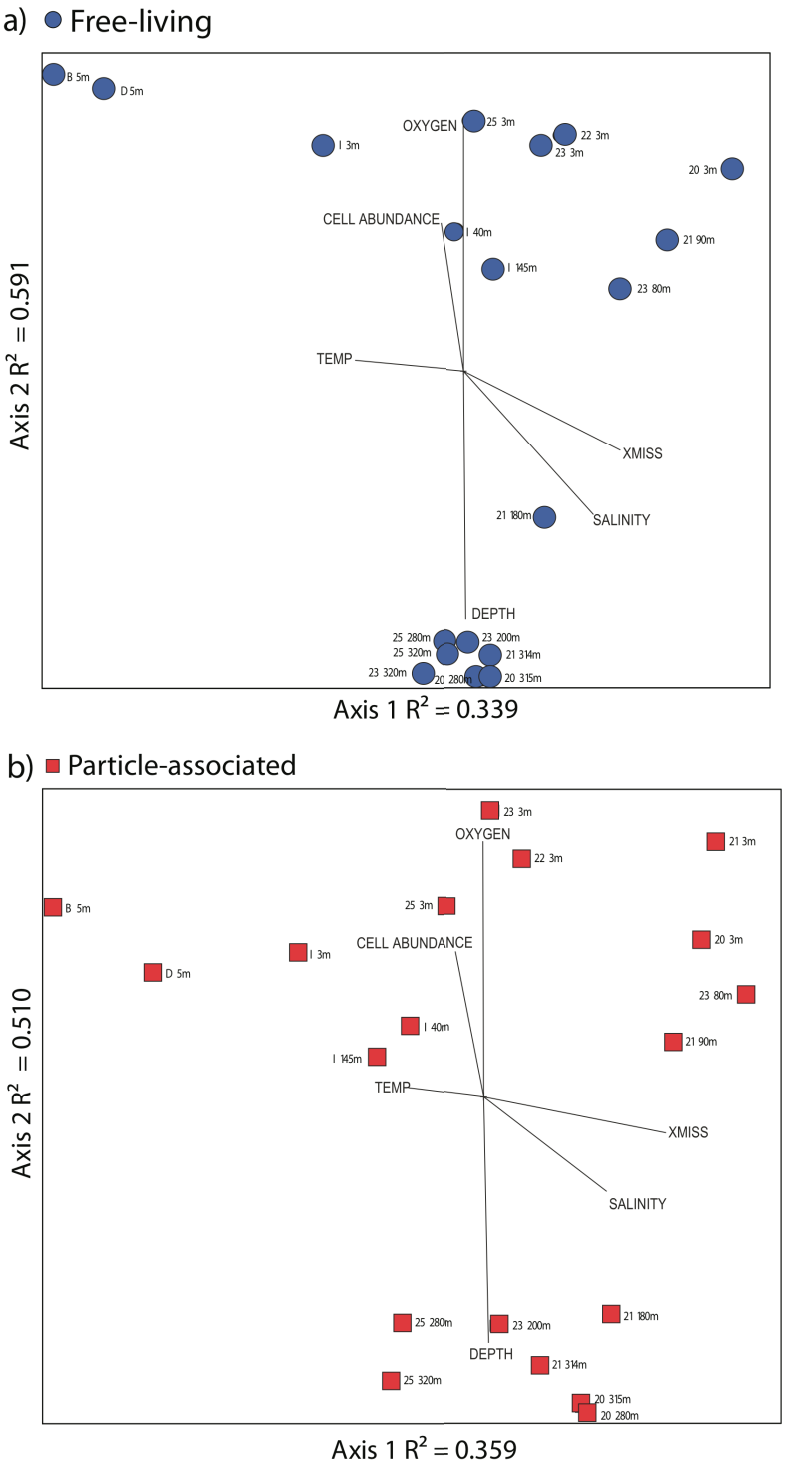

Figure 8) Non-metric multidimensional scaling (NMS) showing the comparison between free-living (FL) and particle-associated (PA) groups at the 90% sequence clusters. Panel a) shows the NMS ordination for all Free-living (FL) data collected and panel b) shows the ordination for particle-associated (PA) bacteria from surface and deep regions of the SLE. The environmental parameters (oxygen, depth, salinity, cell abundance, temperature and transmissivity (xmiss) have been plotted separately for both free-living and particle-associated samples.

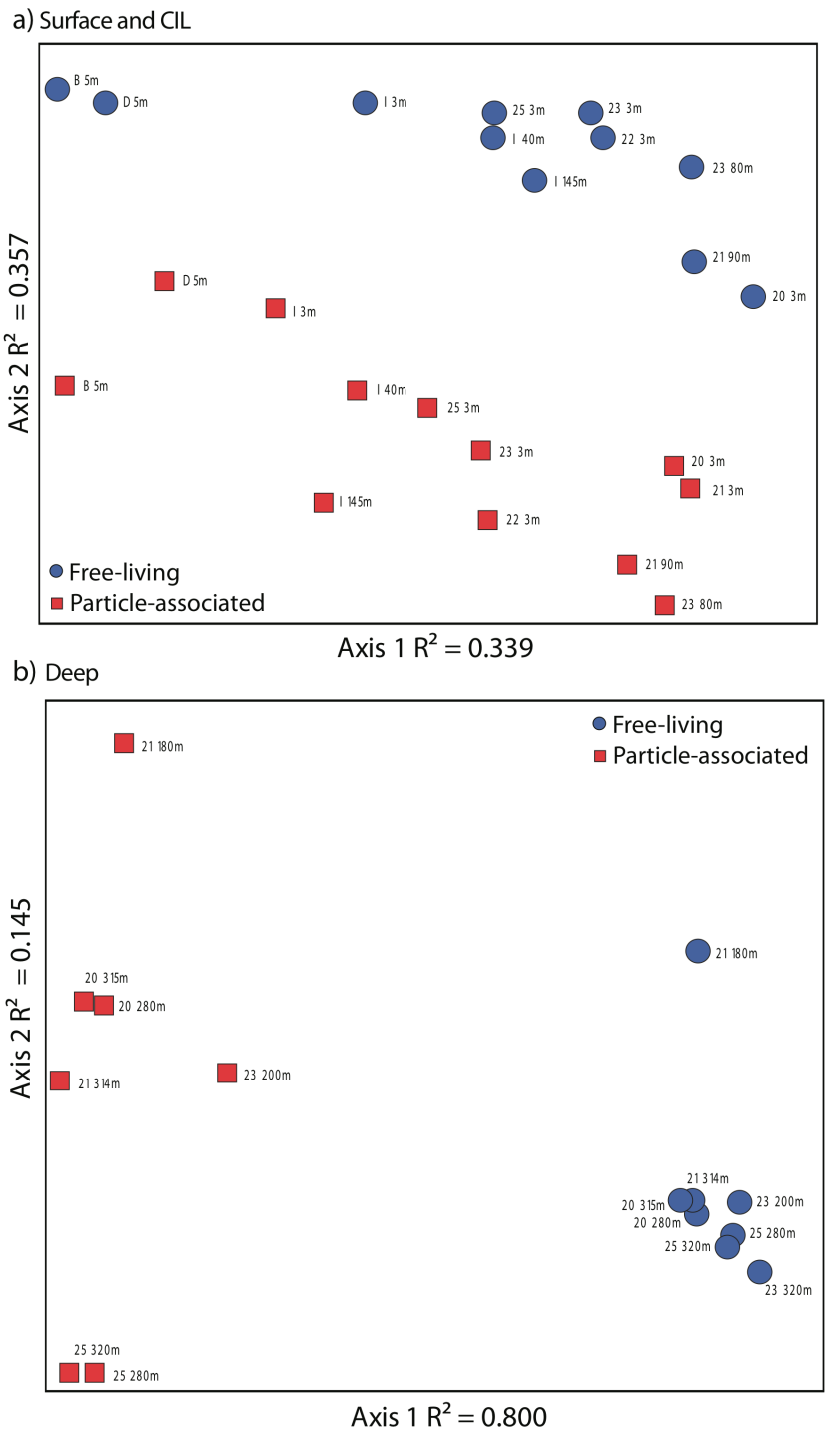

Figure 9) Non-metric multidimensional scaling (NMS) showing the comparison between surface vs. deep groups at 90% sequence clusters. Panel a) shows the NMS ordination for all surface and cold intermediate layer (CIL) data collected and panel b) shows the ordination for deep samples bacteria. Free-living bacteria are shown in blue circles and particle-associated bacteria are shown in red squares.

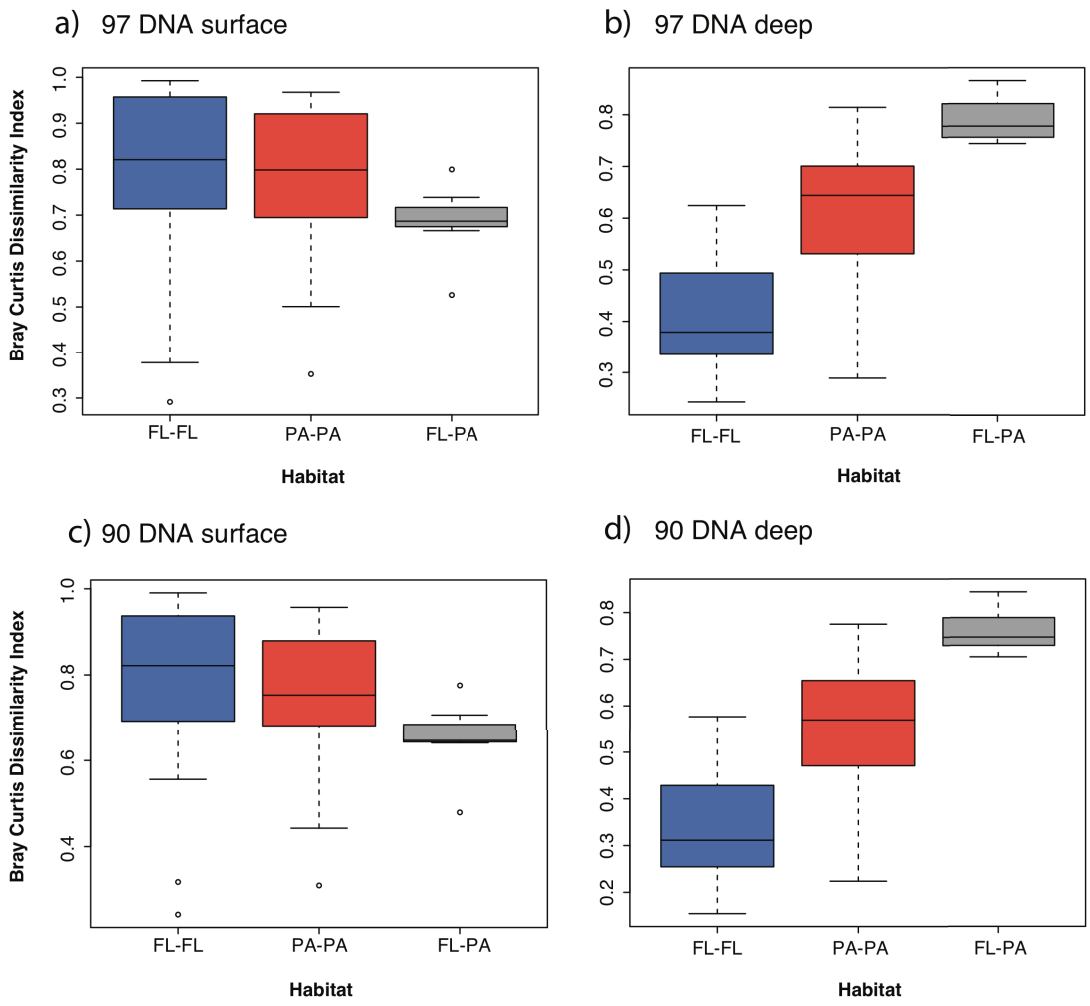

Figure 10) Box plots depicting the Bray-Curtis dissimilarity against pairwise comparison between different groups. Panel a) and b) show Bray-Curtis dissimilarity measure against pairwise comparisons at 97% sequence similarity while panel c) and d) show 90% sequence similarity. FL signifies the free-living fraction and PA signifies the particle-associated fraction. The comparisons were performed using MOTHUR in which FL communities from surface encompasses samples at 3-5m while deep communities comprise of samples of more than 180m. Box plots groups for each comparison were done using R (www.r-project.org).

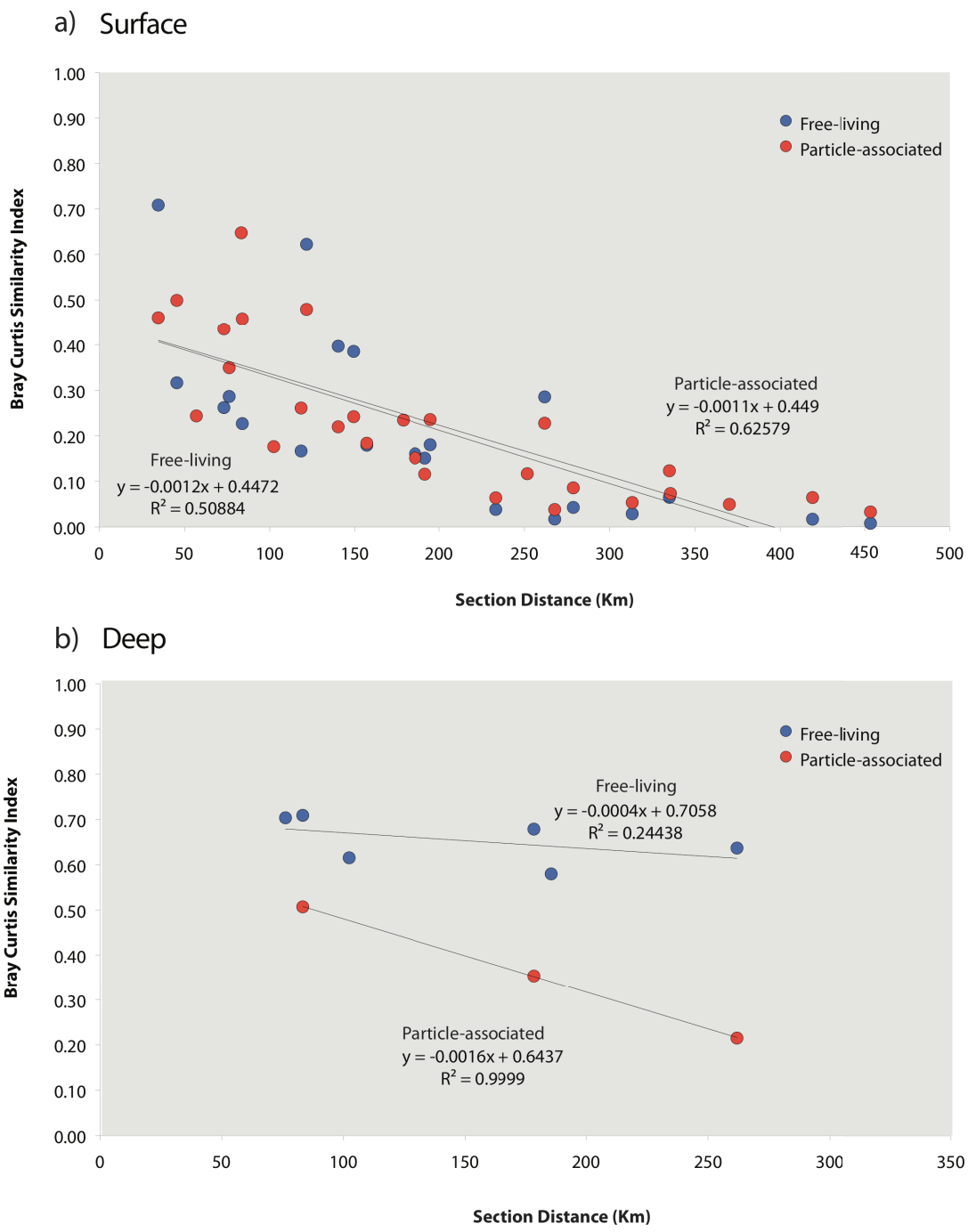

Figure 11) Distance decay analysis on surface and deep samples of the SLE at 97% sequence similarity. A negative relationship between distance (km) and similarity is depicted for pairwise comparisons of surface (3-5m) samples (a). While deep samples (b) do not share the same negative trend. The best-fit linear regression line is shown and the coefficient of determination (R-square) is also shown. Free-living bacteria are shown in blue circles while the particle-associated bacteria are shown in red circles.

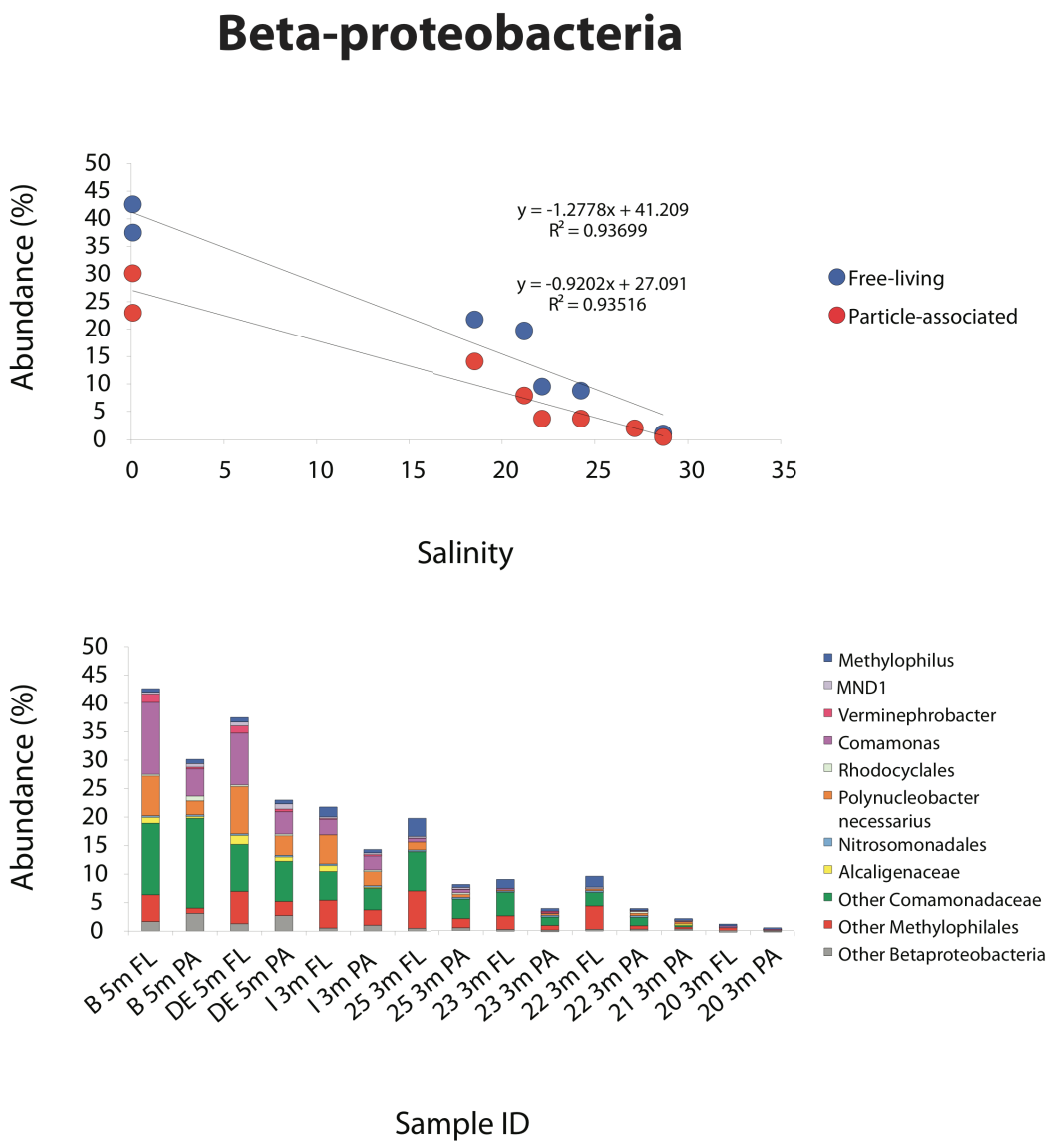

Figure 12) Relative abundance of the Beta-proteobacteria class belonging to surface sample (3-5m) versus the salinity gradient of the SLE. The relative abundance is also shown in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Beta-proteobacteria class

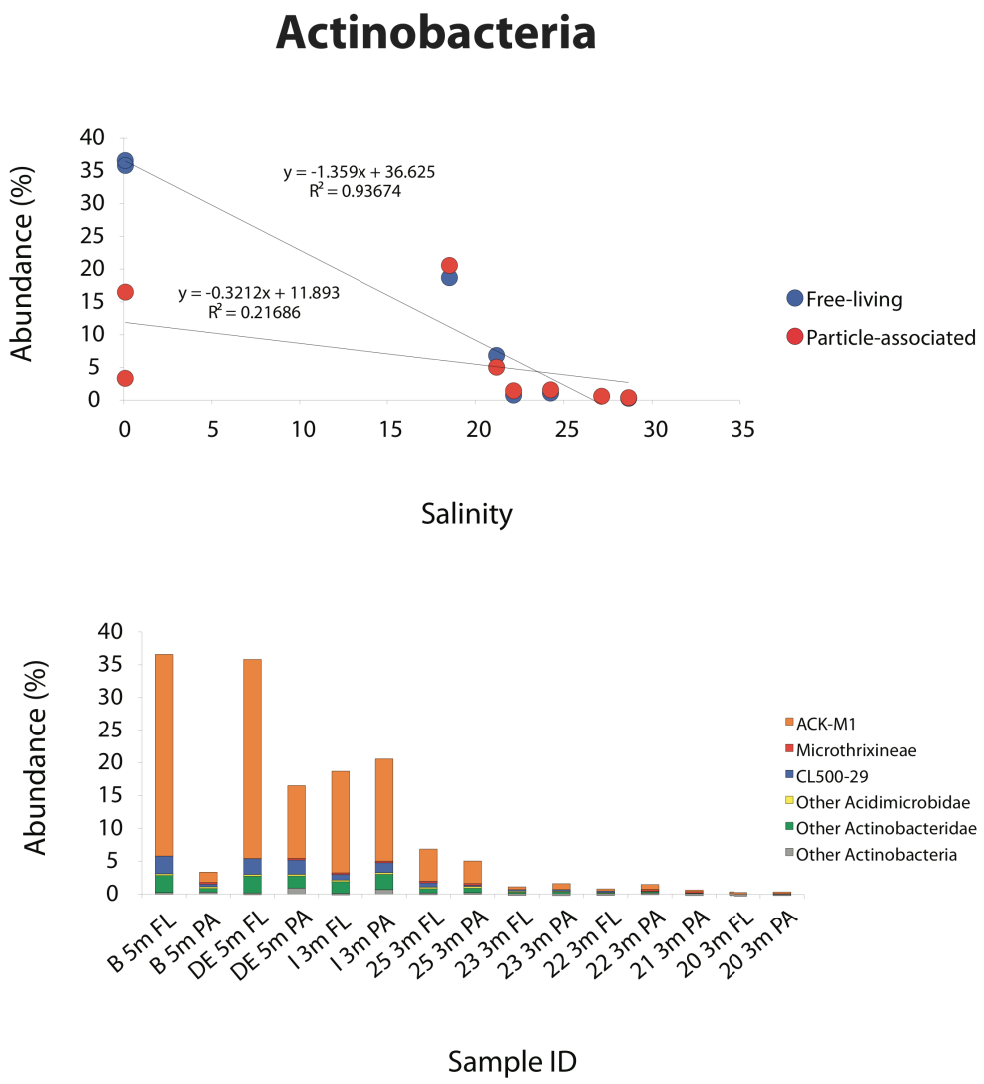

Figure 13) Relative abundance of the Actinobacteria phylum belonging to surface sample (3-5m) versus the salinity gradient of the SLE. The relative abundance is also shown in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Actinobacteria phylum.

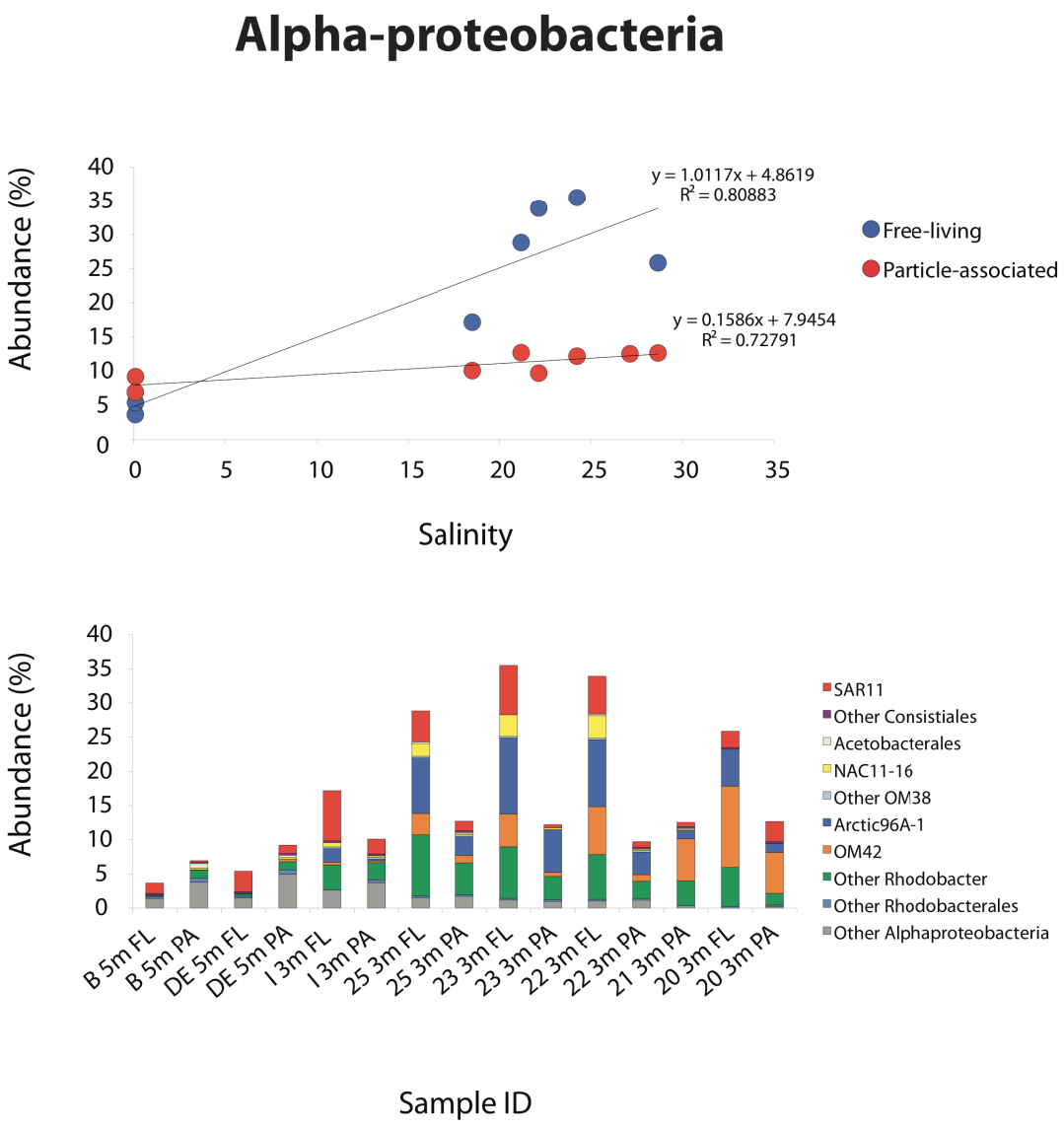

Figure 14) Relative abundance of the Alpha-proteobacteria class belonging to surface sample (3-5m) versus the salinity gradient of the SLE. The relative abundance is also shown in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Alpha-proteobacteria class.

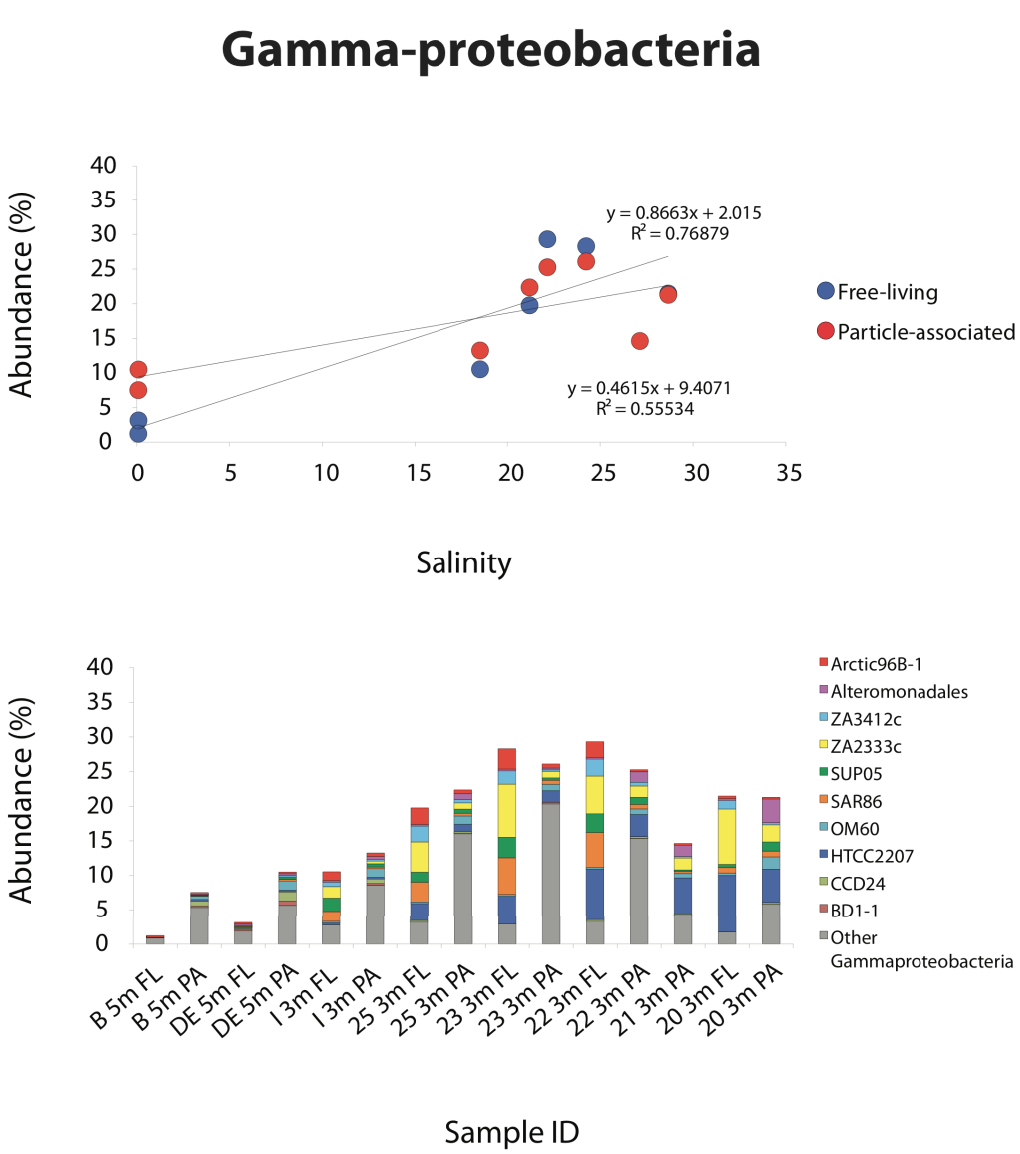

Figure 15) Relative abundance of the Gamma-proteobacteria class belonging to surface sample (3-5m) versus the salinity gradient of the SLE. The relative abundance is also shown in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Gamma-proteobacteria class.

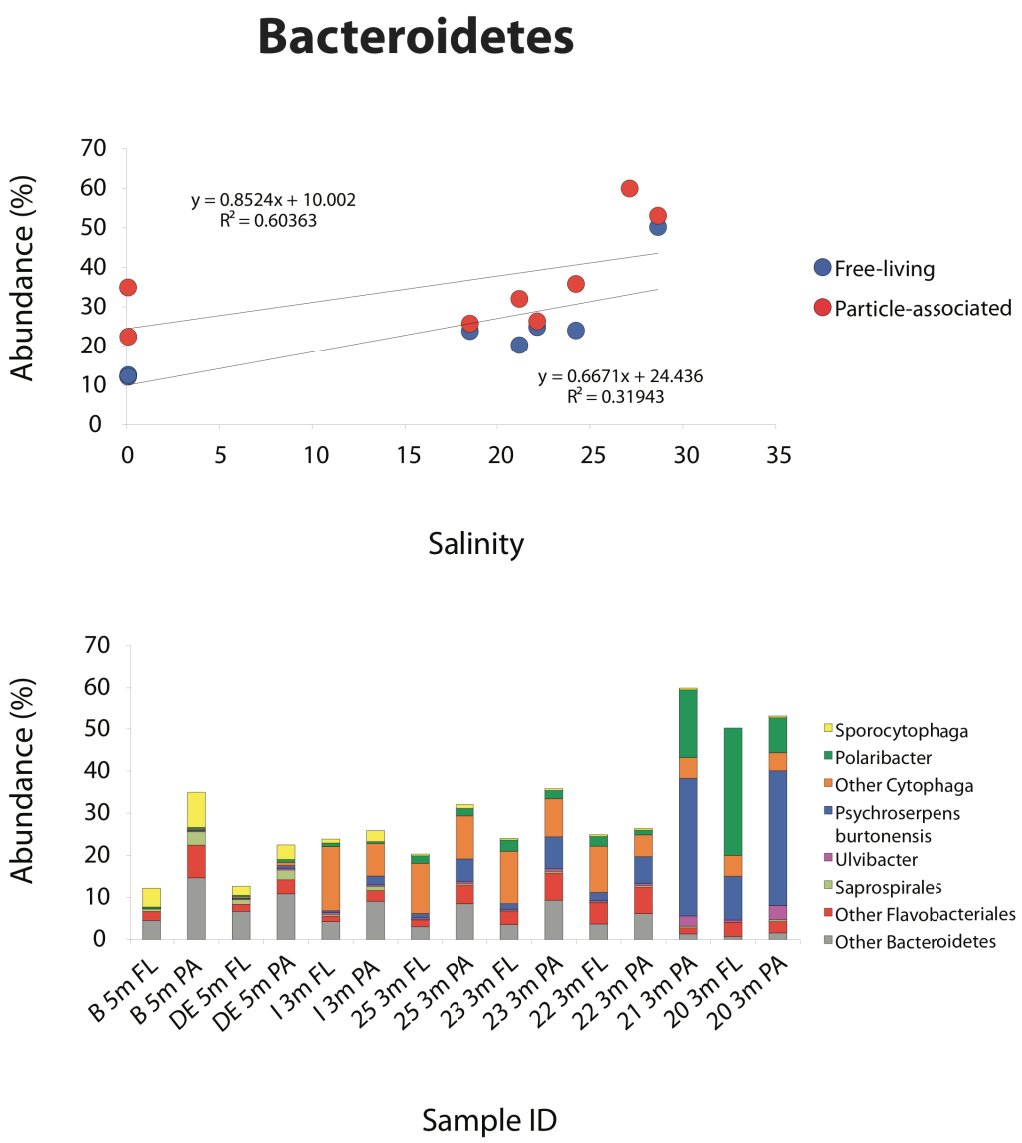

Figure 16) Relative abundance of the Bacteroidetes phylum belonging to surface sample (3-5m) versus the salinity gradient of the SLE. The relative abundance is also shown in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Bacteroidetes phylum.

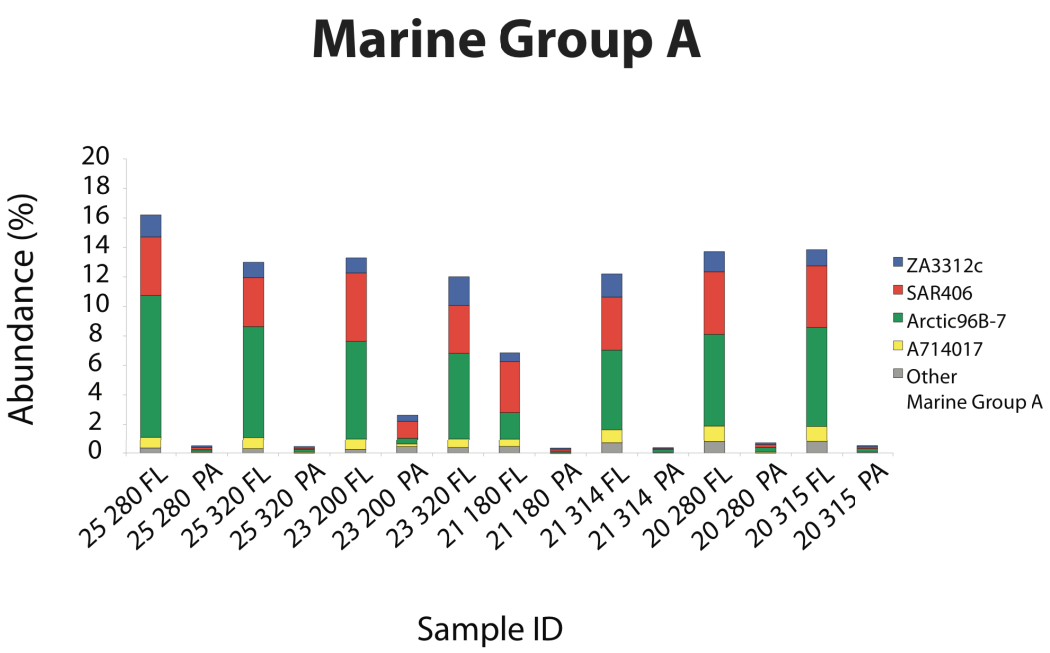

Figure 17) Relative abundance of the Marine Group A phylum belonging to deep samples (>150m) in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Marine Group A phylum.

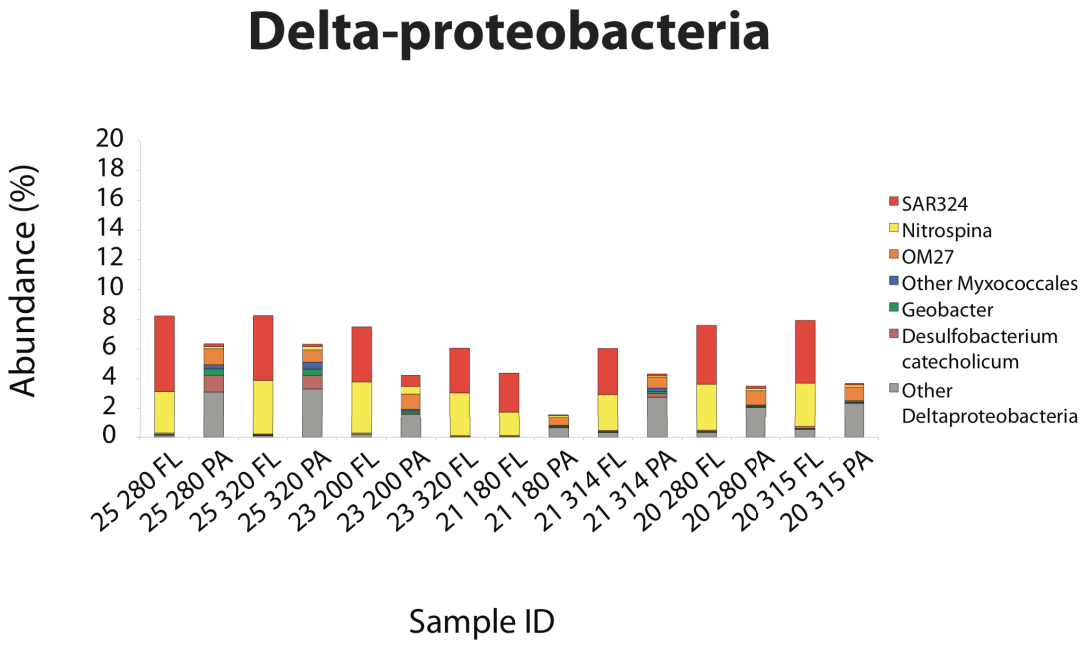

Figure 18) Relative abundance of the Delta-proteobacteria class belonging to deep samples (>150m) in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Delta-proteobacteria class.

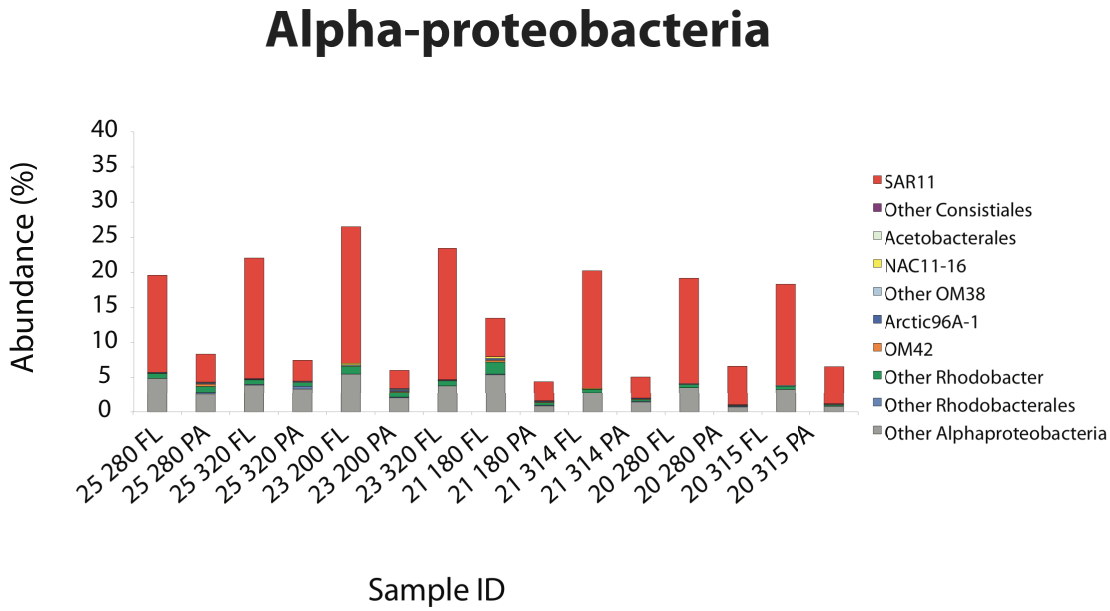

Figure 19) Relative abundance of the Alpha-proteobacteria class belonging to deep samples (>150m) in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Alpha-proteobacteria class.

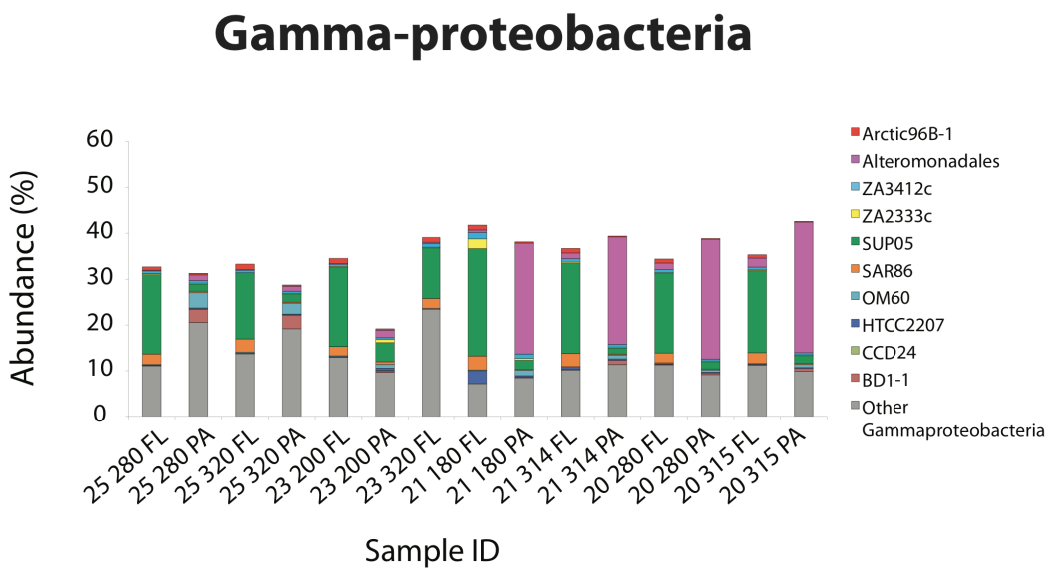

Figure 20) Relative abundance of the Gamma-proteobacteria class belonging to deep samples (>150m) in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Gamma-proteobacteria class.

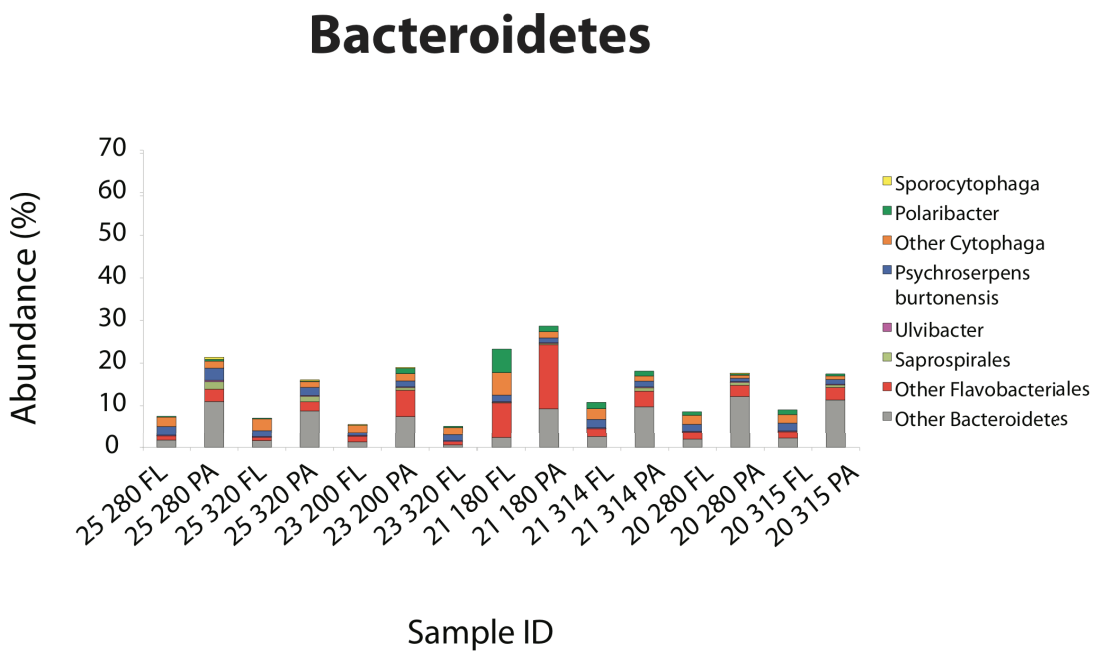

Figure 21) Relative abundance of the Bacteroidetes phylum belonging to deep samples (>150m) in accordance with the taxonomic identity assigned to the 16S rRNA gene sequences belonging to the Bacteroidetes phylum.

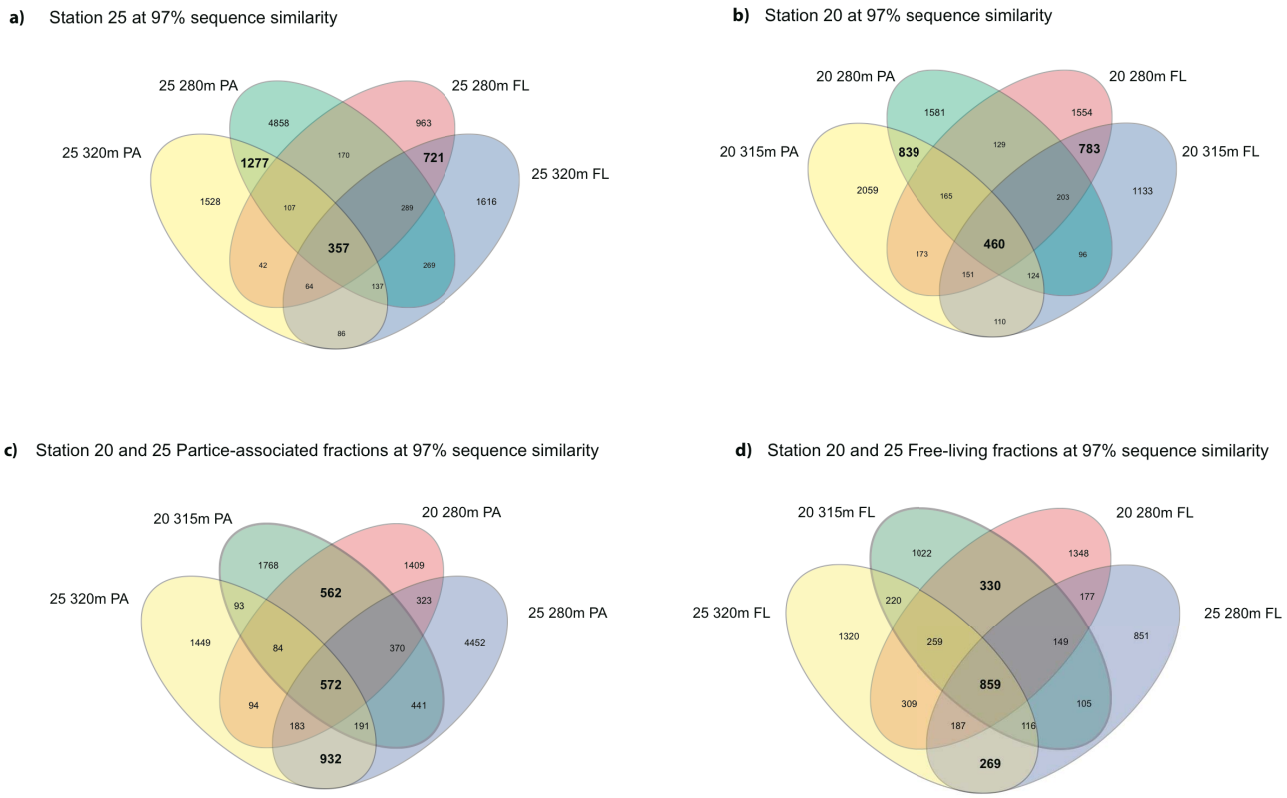

Figure 22) Comparison of phylotype distributions among the selected samples as shown by the venn diagrams depicting the observed shared OTUs between free-living (FL) and particle-associated (PA) groups of the nepheloid layer of the SLE. Panel a) shows the phylotype distribution of the deep nepheloid layer depths (280m and 320m) of Station 25. Panel b) shows the phylotype distribution of the deep nepheloid layer depths (280m and 315m) of Station 20. Panel c) shows the phylotype distribution of particle-associated (PA) deep nepheloid samples and panel d) shows the phylotype distribution of free-living (FL) deep nepheloid samples. The phylotype assignments used the 97% sequence similarity cutoff using MOTHUR.

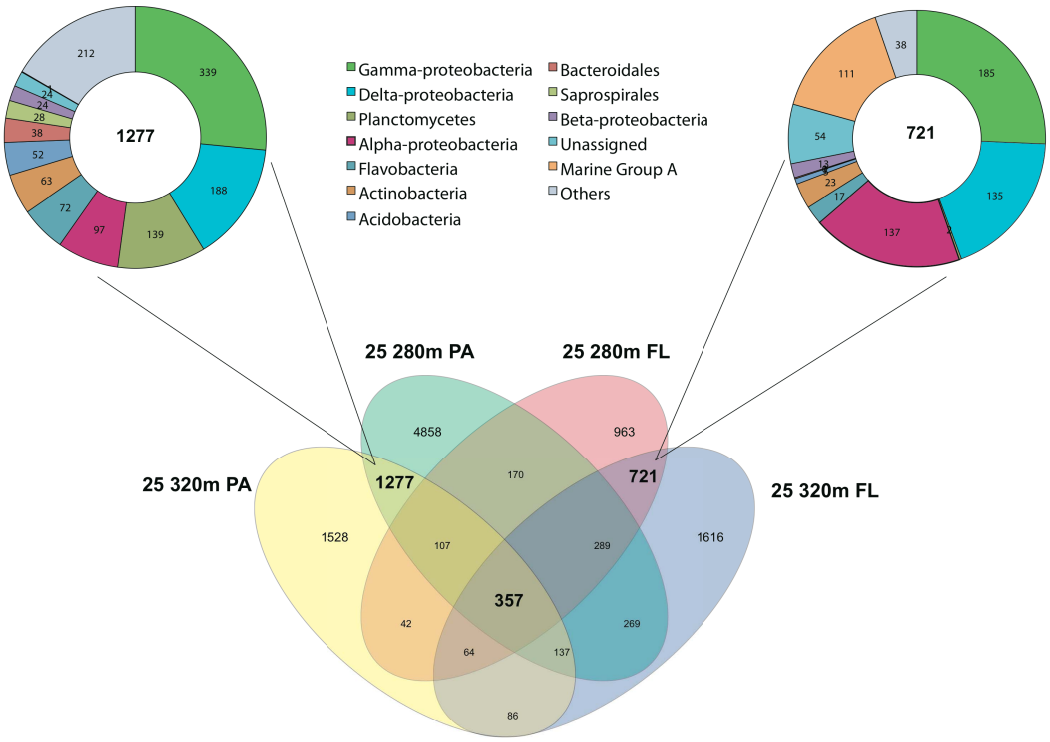

Figure 23) Comparison of phylotype distributions among free-living (FL) and particle-associated (PA) groups at Station 25 and their assigned taxonomy. The 1277 phylotypes are distributed among various taxonomic groups all assigned using the MOTHUR program and are only shared between PA groups. The other 721 phylotypes are only distributed among the FL groups and are also taxonomically assigned using MOTHUR. The phylotype assignments, as well as the taxonomy assignments, were all performed using the 97% sequence similarity cutoff.

Table 1. Environmental parameters at 10 sampling stations along the St. Lawrence Estuary transect. Values were measured in May of 2011 from the 16th to the 21st.

Station	Depth (m)	Temperature (°C)	Conductivity (mS/cm)	Salinity (PSU)	Oxygen (μmol /Kg)	Fluorescence	Beam Transmission (%)	PAR/ Irradiance	Density (Kg/m^3)	Bacterial Production (μgC/l/d)	SPM[1] (mg/L)	Cell Abund (cells/ml)
B	5.93	10.59	0.13	0.08	339.59	1.05	29.87	0.00	-0.29	21.64	16.20	6.48 x10^5
DE	5.24	9.72	0.14	0.09	343.96	1.04	4.28	0.00	-0.20	14.32	31.34	4.89 x10^5
F	3.13	5.83	13.66	12.82	344.37	0.52	23.24	0.28	10.08	5.86	124.31	3.21 x10^5
F	45.20	3.47	20.08	20.90	322.96	0.61	0.63	0.00	16.62	8.85	20.87	2.42 x10^5
I	3.16	4.26	18.37	18.53	329.40	0.40	58.81	3.74	14.70	2.94	8.58	2.77 x10^5
I	40.13	1.87	24.19	26.93	314.17	0.37	77.19	0.00	21.52	2.20	13.69	2.47 x10^5
I	145.50	1.31	25.33	28.79	309.20	0.39	69.11	0.00	23.04	1.94	24.03	2.63 x10^5
K	3.02	3.05	22.13	23.57	326.37	0.03	76.11	15.62	18.77	4.73	10.55	2.55 x10^5
K	29.80	2.59	23.00	24.93	320.14	0.02	78.52	0.00	19.88	2.87	12.68	2.80 x10^5
K	90.68	1.15	26.84	30.86	279.65	0.26	88.28	0.00	24.71	1.05	8.39	1.88 x10^5
25	2.97	5.49	21.50	21.20	369.73	0.28	78.57	3.31	16.71	15.90	3.46	4.24 x10^5
25	20.55	2.24	24.14	26.57	322.77	0.02	90.71	0.00	21.21	5.35	-	2.44 x10^5
25	79.97	0.94	27.83	32.34	260.17	0.01	98.53	0.00	25.91	1.34	3.85	2.01 x10^5
25	120.44	1.87	29.03	32.88	206.98	0.01	97.63	0.00	26.29	1.19	-	1.40 x10^5
25	160.42	3.05	30.55	33.51	141.53	0.01	96.82	0.00	26.69	0.50	-	1.40 x10^5
25	221.14	4.24	32.13	34.12	84.48	0.01	92.24	0.00	27.06	0.41	-	1.60 x10^5
25	280.46	4.66	32.71	34.34	67.25	0.01	74.43	0.00	27.19	0.58	10.02	1.91 x10^5
25	325.46	4.71	32.79	34.36	66.10	0.06	74.68	0.00	27.20	0.42	21.35	1.46 x10^5

[1]SPM: Suspended Particulate Matter

Table 1 (cont'd). Environmental parameters at 10 sampling stations along the St. Lawrence Estuary transect. Values were measured in May of 2011 from the 16th to the 21st.

Station	Depth (m)	Temperature (°C)	Conductivity (mS/cm)	Salinity (PSU)	Oxygen (μmol /Kg)	Fluorescence	Beam Transmission (%)	PAR/ Irradiance	Density (Kg/m^3)	Bacterial Production (μgC/l/d)	SPM[1] (mg/L)	Cell Abund (cells/ml)
23	2.83	4.74	23.81	24.25	412.61	12.31	69.48	0.00	19.19	42.41	5.80	5.71 x10^5
23	39.74	1.07	26.09	30.02	330.94	0.21	96.58	0.00	24.04	2.62	-	2.90 x10^5
23	80.56	-0.17	26.50	31.78	331.49	0.21	99.30	0.00	25.51	2.80	4.48	2.58 x10^5
23	121.13	0.86	27.82	32.39	264.20	0.08	98.86	0.00	25.96	1.12	2.71	1.35 x10^5
23	161.07	2.64	30.09	33.37	158.64	0.01	98.53	0.00	26.61	0.23	3.12	8.30 x10^5
23	199.69	3.66	31.40	33.87	104.92	0.12	99.11	0.00	26.92	0.17	2.64	1.18 x10^5
23	240.19	4.19	32.08	34.11	83.45	0.01	97.12	0.00	27.06	0.15	6.50	1.97 x10^5
23	337.83	4.88	33.02	34.44	62.19	0.08	95.17	0.00	27.25	0.17	6.54	1.27 x10^5
22	3.06	5.43	22.35	22.16	416.06	0.85	73.03	2.88	17.48	59.12	9.82	6.92 x10^5
22	80.37	0.15	26.75	31.77	323.30	0.02	97.21	0.00	25.49	2.98	2.77	3.73 x10^5
22	180.54	2.93	30.50	33.55	138.63	0.01	99.47	0.00	26.74	0.90	-	2.10 x10^5
22	306.48	4.76	32.85	34.38	69.31	0.01	92.84	0.00	27.21	0.57	5.80	2.65 x10^5
21	2.97	5.98	27.29	27.14	369.04	0.03	98.53	3.83	21.35	26.03	2.69	3.64 x10^5
21	80.49	0.47	27.03	31.81	336.80	0.07	100.15	0.00	25.51	5.27	2.65	2.13 x10^5
21	180.28	2.49	29.96	33.36	166.34	0.01	100.27	0.00	26.62	1.68	-	2.64 x10^5
21	314.18	4.98	33.15	34.50	69.65	0.01	92.29	0.00	27.28	0.70	6.27	2.00 x10^5
20	3.10	6.16	28.82	28.67	371.80	0.01	100.25	23.70	22.54	21.22	2.67	3.36 x10^5
20	20.18	0.03	26.41	31.48	362.88	0.05	98.45	5.72	25.26	13.67	-	1.78 x10^5
20	80.12	1.21	28.25	32.60	240.02	0.04	99.58	0.01	26.11	6.69	-	5.51 x10^5
20	120.03	2.35	29.76	33.29	172.44	0.01	99.25	0.00	26.57	1.49	-	2.02 x10^5
20	159.93	3.27	30.93	33.73	122.03	0.01	100.33	0.00	26.85	1.43	-	2.03 x10^5
20	220.26	4.49	32.45	34.24	82.05	0.01	100.96	0.00	27.13	0.76	-	2.05 x10^5
20	280.05	5.03	33.20	34.52	77.06	0.01	99.57	0.00	27.29	0.41	-	1.97 x10^5
20	316.16	5.10	33.32	34.56	79.23	0.01	97.68	0.00	27.32	0.67	3.35	2.44 x10^5

[1]SPM: Suspended Particulate Matter

Table 2. Number of phylotypes (OTUs)[1] of sequences assigned to samples from two distinct habitats (PA[2] and FL[2]) at unique, 97 and 90 percent sequence similarity.

			Sequences assigned to original dataset			
Station	**Depth (m)**	**Habitat[2]**	**Sequences**	**Unique**	**97%**	**90%**
B	5	FL	32168	5516	2978	1037
B	5	PA	37146	10717	6474	2848
DE	5	FL	23596	5020	3020	1191
DE	5	PA	31309	10030	6516	2870
I	3	FL	7697	1972	1689	716
I	3	PA	34722	8707	6413	3013
I	40	FL	18506	3489	3029	1002
I	40	PA	20580	5441	4095	1872
I	145	FL	24814	4835	4041	1213
I	145	PA	18276	5919	4730	2525
25	3	FL	42693	8085	6112	1648
25	3	PA	32887	8137	5897	2326
25	280	FL	18693	3644	2713	935
25	280	PA	42634	12573	7464	3096
25	320	FL	23956	4836	3539	1142
25	320	PA	10391	4458	3598	2141
23	3	FL	7810	1536	1386	455
23	3	PA	7792	1485	1264	649
23	80	FL	18925	2643	2278	628
23	80	PA	17599	2311	1865	675
23	200	FL	13069	3076	2442	757
23	200	PA	25215	6241	4565	1959
23	320	FL	24811	4870	3283	914
23	320	PA	NA	NA	NA	NA
22	3	FL	37731	6297	4817	1004
22	3	PA	16164	3685	2811	1196
21	3	FL	NA	NA	NA	NA
21	3	PA	23372	3073	2434	824
21	90	FL	30123	3649	2952	663
21	90	PA	89775	9256	6158	1785
21	180	FL	16500	3669	2895	857
21	180	PA	24530	4557	3136	1145
21	314	FL	24277	5222	3787	1227
21	314	PA	18852	4917	3473	1561
20	3	FL	57289	4746	3485	639
20	3	PA	35794	4164	3259	966
20	280	FL	26628	5255	3618	1072
20	280	PA	25736	5195	3597	1524
20	315	FL	17872	4091	3060	1003
20	315	PA	33009	6242	4081	1535
Total	**-**	**-**	**1080991**	**121127**	**50718**	**12635**

[1]OTU: Operational Taxonomic Units, [2]PA, Particle associated bacteria; FL, free-living, [2]FL: Free living bacteria

Table 3. Alpha Diversity (Chao 1 estimator) analysis of St. Lawrence Estuary (SLE) bacterioplankton by 16S sequence analysis at 97% and 90% sequence similarity. The data has been normalized by rarefraction to 7500 sequences per sample.

Station	Depth (m)	Habitat[1]	Chao 97%	Chao 90%
B	5	FL	3048.78	1067.62
DE	5	FL	3842.39	1419.65
I	3	FL	5483.47	1373.42
I	40	FL	5570.05	1253.76
I	145	FL	6063.74	1315.10
25	320	FL	4579.84	1241.05
25	280	FL	3889.29	1135.00
25	3	FL	6333.41	1407.17
23	320	FL	3980.37	961.94
23	200	FL	4650.32	1071.47
23	80	FL	3837.43	730.95
23	3	FL	4345.12	864.43
22	3	FL	5153.77	874.57
21	314	FL	4807.51	1312.84
21	180	FL	4778.88	1011.47
21	90	FL	3401.80	627.64
21	3	FL	6252.00	2264.23
20	315	FL	4635.03	1236.34
20	280	FL	4146.68	1066.01
20	3	FL	2711.60	491.84
B	5	PA	6291.70	2492.90
DE	5	PA	7207.28	2735.99
I	3	PA	7090.79	2813.43
I	40	PA	5388.94	1989.76
I	145	PA	7711.56	3113.65
25	320	PA	7169.83	3244.05
25	280	PA	6243.70	2581.12
25	3	PA	6906.02	2254.47
23	320	PA	3628.35	755.44
23	200	PA	5492.49	1971.89
23	80	PA	2871.61	828.69
23	3	PA	3523.63	1392.98
22	3	PA	5170.40	1697.30
21	314	P	4314.56	1632.42
21	180	P	3809.99	1158.72
21	90	P	3739.93	1071.88
21	3	P	3076.88	910.52
20	315	P	4008.28	1396.07
20	280	P	4335.21	1576.94
20	3	P	3376.30	874.35

[1]PA: Particle associated bacteria

[1]FL: Free living bacteria

Table 4. Pearson and Kendall's rank correlations with the Non-metric multidimensional scaling (NMS) ordination axes for environmental characteristics at 90 percent sequence similarity for both free-living and particle-associated bacterioplankton.

	Free-living						Particle-associated					
	Axis 1			Axis 2			Axis 1			Axis 2		
Parameters	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**	**r**	**r^2**	**tau**
Depth	0.11	0.01	-0.18	-0.97	0.94	-0.74	0.15	0.02	-0.05	-0.96	0.92	-0.84
Temperature	-0.70	0.49	-0.24	0.20	0.04	0.14	-0.58	0.34	-0.10	0.19	0.04	0.12
Conductance	0.75	0.56	0.15	-0.75	0.57	-0.87	0.74	0.54	0.29	-0.60	0.36	-0.60
Salinity	0.77	0.59	0.12	-0.74	0.55	-0.91	0.75	0.56	0.27	-0.61	0.37	-0.67
Oxygen	0.01	0.00	0.25	0.98	0.95	0.66	-0.06	0.00	0.10	0.98	0.96	0.75
Fluorescence	0.03	0.00	-0.17	0.31	0.09	0.67	-0.08	0.01	-0.44	0.38	0.15	0.48
Beam Transmission	0.84	0.71	0.49	-0.55	0.30	-0.36	0.90	0.81	0.70	-0.37	0.14	-0.24
PAR/ Irradiance	0.38	0.14	0.11	0.30	0.09	0.34	0.30	0.09	0.14	0.30	0.09	0.45
Density	0.77	0.59	0.13	-0.73	0.54	-0.91	0.75	0.56	0.27	-0.60	0.36	-0.69
Cell Abundance	-0.61	0.38	-0.24	0.49	0.24	0.51	-0.59	0.34	-0.35	0.38	0.14	0.33

Table 5. ANOSIM analysis on different comparisons from the LSLE (Lower St. Lawrence Estuary) and the USLE (Upper St. Lawrence Estuary) regions at 97% and 90% using the Bray-Curtis, Jclass and ThetaYC calculators.

			97%		90%	
Region	**Comparison**	**ANOSIM**	**R-value**	**P-value**	**R-value**	**P-value**
LSLE	Surface PA and surface FL	Bray Curtis	0.435	0.005	0.452	0.008
		Jclass	0.513	0.002	0.603	0.001
		Thetayc	0.631	<0.001	0.648	0.001
LSLE	Deep PA and deep FL	Bray Curtis	0.962	< 0.001	0.969	< 0.001
		Jclass	0.950	< 0.001	0.927	0.001
		Thetayc	0.821	< 0.001	0.819	< 0.001
LSLE	Surface and deep FL	Bray Curtis	0.993	0.001	0.984	< 0.001
		Jclass	0.992	< 0.001	0.968	0.001
		Thetayc	0.996	< 0.001	0.987	0.001
LSLE	Surface and deep PA	Bray Curtis	0.905	0.001	0.900	< 0.001
		Jclass	0.905	0.001	0.807	< 0.001
		Thetayc	0.877	< 0.001	0.874	< 0.001
USLE	PA and FL	Bray Curtis	0.232	0.134	0.236	0.1
		Jclass	0.528	0.020	0.664	0.008
		Thetayc	0.244	0.068	0.280	0.039
LSLE	Surface and deep	Bray Curtis	0.926	<0.001	0.893	<0.001
		Jclass	0.898	<0.001	0.769	<0.001
		Thetayc	0.902	<0.001	0.845	<0.001
USLE and LSLE	Surface and deep FL	Bray Curtis	0.670	<0.001	0.618	<0.001
		Jclass	0.682	<0.001	0.569	<0.001
		Thetayc	0.721	<0.001	0.652	<0.001
USLE and LSLE	Surface and deep PA	Bray Curtis	0.860	0.001	0.844	<0.001
		Jclass	0.835	<0.001	0.703	0.001
		Thetayc	0.839	0.001	0.840	<0.001

PA: Particle associated bacteria
FL: Free living bacteria