Antonie van Leeuwenhoek (2015) 107:613–632
DOI 10.1007/s10482-014-0357-3

ORIGINAL PAPER

Influence of freshwater discharge on the microbial degradation processes of dissolved organic nitrogen in a subtropical estuary

Juan C. Garcia · Rheannon D. J. Ketover · Ai Ning Loh · Michael L. Parsons · Hidetoshi Urakawa

Received: 2 October 2014 / Accepted: 12 December 2014 / Published online: 27 December 2014

Abstract River bacterioplankton communities, influenced by watershed usage, are responsible for water purification. Bacterioplankton may be critical in the degradation of dissolved organic nitrogen (DON), the major nitrogen pool in the Caloosahatchee River, Florida. We investigated how freshwater discharge influences estuarine bacterioplankton and how the freshwater-originated DON is utilized by estuarine bacterioplankton. Microcosm experiments were conducted during low and high discharge using two upstream freshwater samples: one site primarily influenced by Lake Okeechobee and the other site moderately influenced by an agricultural watershed. These freshwater samples were filtered to eliminate indigenous microbial populations, then mixed with estuarine bacterioplankton. High-throughput sequencing revealed that bacterioplankton differed between low and high discharge and were influenced by salinity. Alphaproteobacteria and Bacteroidetes dominated in low discharge while Bacteroidetes and Cyanobacteria dominated during high discharge. In the microcosm experiment, DON concentration decreased with increasing cell densities, suggesting that the DON was utilized as a carbon and nitrogen source. Band signals in denaturing gradient gel electrophoresis corresponding to Alphaproteobacteria and Actinobacteria decreased while Gammaproteobacteria increased during the 1 month incubation. This data suggests that estuarine bacterioplankton communities are influenced by variations in discharge patterns and use freshwater-originated DON as demonstrated by a shift in community structure.

Keywords Estuary · Dissolved organic nitrogen · Bacterioplankton · Subtropical · 16S rRNA · High-throughput sequencing

Electronic supplementary material The online version of this article (doi:10.1007/s10482-014-0357-3) contains supplementary material, which is available to authorized users.

J. C. Garcia · R. D. J. Ketover · A. N. Loh · M. L. Parsons · H. Urakawa (✉)
Department of Marine and Ecological Sciences, Florida Gulf Coast University, 10501 FGCU Blvd South, Fort Myers, FL 33965-6565, USA
e-mail: hurakawa@fgcu.edu

J. C. Garcia
e-mail: Juangarcia152@gmail.com

Present Address:
A. N. Loh
Department of Geography and Geology, Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA

Introduction

Estuaries are partially enclosed, highly productive areas that harbor flora and fauna adapted to the wide range of salinity conditions. A wide range of habitats

can be found in estuaries that provide crucial resources for organisms. In recent years increased development, in addition to other anthropogenic perturbations such as pollution, has led to habitat loss/alterations and changes in community dynamics (Livingston et al. 1997; Livingston 2000). Estuaries are influenced in some way by a wide range of anthropogenic activities, posing a significant issue for coastal zone management (Carman et al. 1995; Kennish 1997; Botsford et al. 1997; Livingston 2000).

Among the most serious issues in estuarine environments are nutrient enrichment and excess carbon loading. Eutrophication has the potential to lead to trophic structure imbalances and heightened primary production by an increase in phytoplankton activity leading to nuisance algal blooms and hypoxia (Livingston 2000). This, in turn, leads to mortality of organisms, reduced diversity, reduction of recreational fisheries, perturbed food webs and destabilization of phytoplankton communities (Weston 1990; Costello and Read 1994). Changes in water regimes to estuaries, by freshwater inflow and diversions, have the potential to change the trophic level dynamics and production of the estuary by influencing nutrient input and salinity (Livingston et al. 1997; Livingston 2000).

Microbial communities in riverine and estuarine environments play a critical role in biogeochemical cycles. In aquatic ecosystems, bacteria transform and remineralize nutrients in order to maintain an energy flux. In estuarine, as in many other environments, the nitrogen cycle plays a central role (Veuger et al. 2004). Microbial flora play important roles in degrading dissolved organic nitrogen (DON) which constitutes a major component of the nitrogen pool. Bacterial assimilation of nitrogen has been reported to account for a significant but variable fraction of nitrogen uptake in estuarine and marine systems (Middelburg and Nieuwenhuize 2000). Estuarine DON may be derived either from allochthonous sources, such as urban and agricultural runoff and atmospheric deposition, or from autochthonous sources derived from the primary production in the river and associated degradation processes (Berman and Bronk 2003). Freshwater inflow creates salinity and nutrient gradients in estuaries. These gradients, along with seasonality, influence the composition of bacterioplankton communities (Crump et al. 1999; Del Giorgio and Bouvier 2002; Smith and Kemp 2003; Apple et al. 2004; Bernhard et al. 2005; Hitchcock et al. 2010; Rodrigues et al. 2013). Factors such as salinity have been observed to lead to changes in dominant bacterioplankton communities in several estuaries: Chesapeake Bay (Bouvier and Del Giorgio 2002), Delaware Bay (Cottrell and Kirchman 2003; Castle and Kirchman 2004), Pearl River Estuary (Zhang et al. 2006) and the Baltic Sea (Herlemann et al. 2011). Generally in these estuaries, members of the Gammaproteobacteria and Alphaproteobacteria dominate in high salinity while members of the Betaproteobacteria and Actinobacteria have been found to thrive in lower salinities. Several studies have also shown a relationship between water source, dissolved organic matter (DOM) and shifts in bacterioplankton community structures (Crump et al. 2003; Kirchman et al. 2004; Judd et al. 2006).

The Caloosahatchee River located in southwest Florida is part of the Charlotte Harbor Estuary Basin. The Caloosahatchee watershed extends 110 km and drains from Lake Okeechobee into the Gulf of Mexico (Flaig and Capece 1997). Urban land development in the watershed has occurred since the 1870s with cattle, citrus and sugar cane fields present in the basin. The extensive urban and agricultural expansion has led to high demands for water resources and extensive strain to the estuary. Watershed development has changed the system through the addition of canals and through discharges from Lake Okeechobee (Doering and Chamberlain 1999). A growing concern in the system is the algal blooms occurring in the estuary and coastal shelf (Heil et al. 2007). Nutrient input brought by controlled discharges coupled with increased development in the watershed could be leading to an increase in nutrients and intensification of red tides in the system (Lapointe and Bedford 2007). In addition, varying rates of freshwater inflow to the estuary may lead to changes in important bacterioplankton communities via the oscillations of organic nutrients and salinity. Loh et al. (2011) have demonstrated that about 20 % of the nutrient load entering the Caloosahatchee River and its estuary was inorganic form while the remainder 80 % was organic form. Loh (2008) showed that the DON pool is being remineralized in the Caloosahatchee estuary and may provide inorganic nutrients for the phytoplankton community downstream. This may be crucial during the low discharge season when inorganic nutrients may be limited in the river system. Studies have shown that the transformation of riverine derived organic matter

is impacted by salinity and that bacterial degradation of allochthonous DOM is enhanced in estuarine environments (Wikner and Hagström 1999; Stepanauskas et al. 2002; Smith and Benner 2005).

Here we report how freshwater discharge influences estuarine bacterioplankton communities during the low discharge and high discharge periods through high-throughput sequencing and how the freshwater originated DON is potentially degradable by estuarine bacterioplankton communities through microcosm experiments.

Materials and methods

Water sampling and measurement of environmental parameters

Utilization of riverine DON by estuarine bacterioplankton was studied using water samples from the Caloosahatchee estuary and two upstream dams, the Franklin Lock and Dam (S-79; 26°43′21.7194″N, 81°41′34.4394″W) and the Moore Haven Lock and Dam (S-77; 26°50′21.48″N, 81°5′7.7994″W) (Fig. 1). These two upstream sites were chosen because they are the areas of discharge for the nutrient rich water of Lake Okeechobee (S-77) and source of organic matter from the watershed on to the system (S-79). A site (Site 3; 26°31′50.1594″N, 81°57′56.1594″W) from near the mouth of the Caloosahatchee estuary was selected to collect estuarine bacterioplankton biomass as a source of inoculum bacteria for further microcosm experiments. This site has been used in previous Caloosahatchee River studies (Loh 2008; Urakawa et al. 2013) and is proximal to an environmental monitoring point called Shell Point which is monitored in real-time by the River, Estuary and Coastal Observing Network (RECON), Sanibel-Captiva Conservation Foundation (http://recon.sccf.org/). Additionally, water samples from the Gulf of Mexico (GOM; 26°22′26.3994″N, 82°0′48.24″W) in June and Tennessee Reef, Florida Keys (24°44′42″N, 80°46′51.9594″W) in October (Fig. 1) were used as controls to examine marine DOM degradation by indigenous marine bacterioplankton communities that are not directly influenced by riverine DOM. In October the control was sampled from Tennessee Reef due to low salinity in the Gulf of Mexico. Surface water samples were collected during the low discharge period (June 8, 2011) and high discharge period (October 14, 2011). All water samples were collected into clean 19 L Culligan jugs using a submersible pump (Wildco. Yulee, Florida, USA). Physicochemical data (water temperature, salinity, dissolved oxygen [DO] concentration, and pH) were measured using a YSI 650 sonde (YSI Inc., Yellow Springs, Ohio, USA) in the GOM samples. RECON provided chlorophyll *a* data for the estuary. United States Army Corp of Engineers (USACE) provided the flow rates of S-79 (Fig. S1). Residence time was calculated based on the flow rates as previously described (Wan et al. 2013).

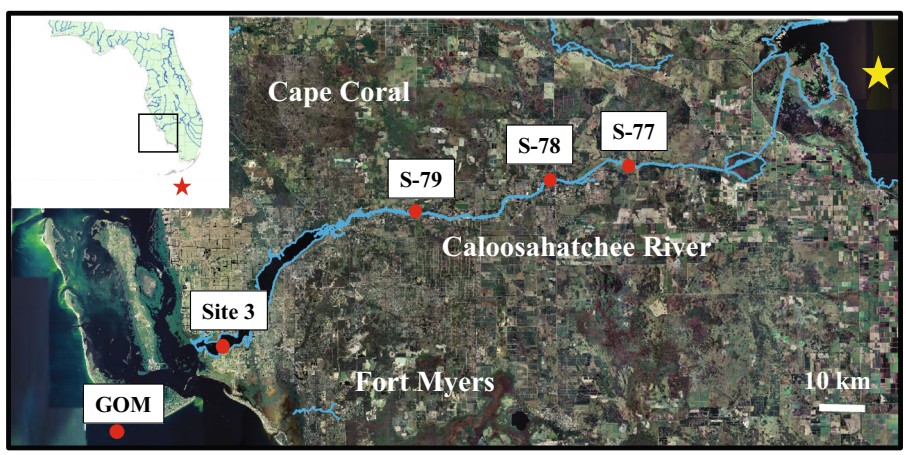


Fig. 1 The Caloosahatchee River and estuary. *Yellow star* represents Lake Okeechobee. *Red star* within an *inset map* represents Tennessee Reef, a marine sampling site during high discharge period (Oct). S-77, S-78 and S-79 indicate locks. Water samples were collected from S-77 and S-79. Site 3 is the estuary sampling point. *GOM* indicates a marine sampling site during low discharge period (Jun)

High-throughput sequencing analysis

The inoculum bacterioplankton communities collected from the estuary site (Site 3, Fig. 1) were analyzed using high-throughput sequencing to determine natural bacterioplankton flora present in the estuary. DNA was extracted using the bead beating and phenol–chloroform methods as described previously (Urakawa et al. 2010). The DNA purity was determined using a NanoDrop Spectrophotometer (Thermo Scientific, Hudson, New Hampshire, USA). DNA was sequenced using the 28F and 519R primer set for the V1–V3 region of 16S rRNA gene and a Roche 454 FLX/FLX + platform with titanium chemistry (Research and Testing Laboratory, Lubbock, Texas, USA). Denoising was performed by USEARCH. Chimera checking was performed by using the de novo built in method in UCHIME. Individual 454 reads were further annotated using BLAST, RDP pipeline (Cole et al. 2009) and MG-RAST (Meyer et al. 2008). For phylogenetic analysis, the MEGA 5 program was used (Tamura et al. 2011). Subsets of sequences were aligned by CLUSTAL W and manual inspection. Evolutionary distances were calculated by maximum composite likelihood pairwise distance method. Phylogenetic trees were constructed using the neighbor joining method using partial deletion with a 95 % site coverage. High-throughput sequences were performed twice: the first sequencing effort resulted in small data sets (small libraries) and the second attempt resulted in larger data sets (large libraries). The confidence interval (95 %) was calculated to determine the reproducibility of two data sets.

Microcosm design and experiments

Two microcosm experiments were conducted to examine the estuarine bacterioplankton utilization of riverine DON originating from Lake Okeechobee (S-77) and agriculturally derived DON (S-79) (Fig. 1). The initial experiment was in June 2011 during the low freshwater discharge period and a second experiment was in October 2011 during the high freshwater discharge period (Fig. S1). The overall concept in the design is to add concentrated estuarine bacterioplankton to sterile S-77 and S-79 water that had been adjusted to the estuarine salinity at time of collection. This elimination of native bacterioplankton allowed the nutrients present in the water of each site to act as the sole source of organic matter for estuarine bacterioplankton growth. Fluctuations in bacterioplankton biomass, inorganic and organic nitrogen and dissolved organic carbon concentrations were measured over a period of 28 days via sub-sampling of the microcosm at time periods 0, 1, 4, 7, 14, 21 and 28 days. In addition, DNA extractions were conducted for the sub-sampled material to decipher any changes occurring to the bacterioplankton communities during the time course experiments. Water collected from Site 3 was filtered through a 0.7 μm polycarbonate filter and then concentrated from 12 l to 6 ml using a 0.2 μm membrane bottle top filter. An additional estuarine sample was prepared and passed through a 0.2 μm filter for bacterial DNA. Water collected from S-77 and S-79 was filtered through a Whatman GF/F filter followed by a 0.2 μm membrane Acropak filter to eliminate microbial predators and the natural microbial flora present in the water. The water was filtered into triplicate 2 l polycarbonate bottles at a volume of 1,500 ml; salinity was adjusted to the Site 3 salinity condition using sterilized sodium chloride (31 ‰ low discharge; 13 ‰ during high discharge). Each treatment bottle was then inoculated with bacteria concentrated from Site 3 (low discharge, 1.15 ml added to each bottle; high discharge, 1.2 ml added to each bottle). The controls were collected and filtered through Whatman GF/F glass fiber filters to eliminate microbial predators. All samples (3×3 treatments $= 9$ bottles per season) were stored in a cool, dark cabinet and allowed to incubate for 28 days with sub-sampling from the microcosm occurring at 0, 1, 4, 7, 14, 21 and 28 days post-incubation. During each sub-sampling period, 10 ml were collected for bacterial cell counts and 75 ml were passed through a 0.2 μm membrane filter for bacterial DNA. Filters for DNA extraction were stored in Fast Prep-24 lysing matrix tubes (MP Bio, Santa Ana, California). Bacterial abundance water samples were preserved using formaldehyde (2 % final concentration). All samples were frozen at −80 °C until further analysis.

Nutrient analysis

Water samples were passed through Whatman GF/F filters prior to analysis. Phosphate, nitrogen species (nitrate, nitrite and ammonium) and total dissolved nitrogen (TDN) were analyzed in triplicate on a SEAL Auto Analyzer III (SEAL Analytical). Total dissolved nitrogen samples were digested via the persulfate oxidation method prior to analysis (Koroleff 1983). DON was

calculated by the subtraction of DIN (nitrate, nitrite and ammonium) from TDN. Dissolved organic carbon (DOC) was measured by Shimadzu TOC analyzer as described previously (Sugimura and Suzuki 1988).

Microscopic bacterial cell counting

Microbial biomass was determined using epifluorescence microscopy (Hobbie et al. 1977). Fixed water samples (3 ml) were stained with SYBR Green II for 10 min (5× as final concentration) and collected by low pressure filtration (<100 mbar) onto 0.2 μm pore size Nuclepore black polycarbonate filters (25 mm) (Millipore). The filter was rinsed with 3 ml of sterilized pure water. The anti-bleaching agent (AF1; Citifluor Ltd.) was used as a mounting medium. Cells were viewed with an Olympus BX-51 epifluorescence microscope. Samples were analyzed by viewing ten random fields on each filter.

Denaturing gradient gel electrophoresis analysis

DNA of each sample was extracted by using a phenol–chloroform method as described previously (Urakawa et al. 2010). Polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene using the extracted DNA. EconoTaq Plus Green 2× Master Mix (Lucigen) was used to amplify a 250 bp fragment with the GC clamp-containing DGGE primers 357F-GC and 518R (Muyzer et al. 1993). DNA was amplified using a PTC 200 Peltier ThermoCycler (MJ Research). PCR conditions were as follows: initial 94 °C for 5 min, with the next 25 cycles carried out for 1 min at 94 °C, annealing for 1 min at 65 °C and then decreased by 0.5 °C every cycle until 55 °C, followed by primer extension for 1 min at 72 °C and a final elongation step at 72 °C for 5 min. PCR amplification was confirmed by 1 % agarose gel electrophoresis. Denaturing gradient gel electrophoresis (DGGE) was performed using the D-Code Universal Mutation Detection System (BioRad). An 8 % acrylamide gel was made with a denaturant gradient range of 25–70 %. The gel was then run at 70 V for 16 h at 65 °C. After electrophoresis, the gel was stained with Gel Red (Biotium). Image was visualized and captured by using a BioDoc-It Imaging System (UVP). DGGE bands that were strong and well isolated were excised for each of the treatments. Excised DGGE gel bands were placed in sterile 1.5 ml centrifuge tubes containing 1 ml of sterilized pure water. DNA was eluted at 4 °C overnight. The supernatant solution was used as a template for a second PCR amplification using the same primer pair without a GC clamp. Amplicons were purified using the GeneJet PCR purification Kit (Fermentas). Products were confirmed via 1 % agarose gel electrophoresis. Amplified products were sequenced using an ABI 3730 analyzer with Big Dye 3.0 protocol (Genscript Sequencing Services).

In order to determine percent abundance of the different classes represented by the DGGE bands across treatments and discharge periods, total number of bands (unidentified + identified) per each time point was manually counted. Sequenced bands from DGGE were manually inspected and compared against 16S rRNA gene sequences in the GenBank using BLAST search to determine closest relatives. The identified bands were then binned into their respective classes. Percent abundance for each class was calculated for each subsampling point, site and discharge period by dividing the number of identified bands by total bands in each time point. Cluster analysis was done using the UPGMA algorithm. Principal component analysis (PCA) was performed by GelComparII (Applied Maths, Kortrijk, Belgium).

Nucleotide accession number

Sequence data for all DGGE bands can be found under supplemental materials (Online Resource 1). High-throughput sequencing data have been deposited in the Sequence Read Archive (SRA) under the study accession number SRP044706.

Results

Hydrological settings

Physical and chemical parameters for sampling sites are summarized in Table 1. The surface water temperature ranged from 27.8 to 30.4 °C between sampling periods. Dissolved oxygen was saturated in GOM and Tennessee Reef samples and ranged from 105 to 127 %, respectively. The dissolved oxygen in the two upper freshwater sites ranged from 55 to 76 %. High concentration of nitrate was detected in the

Table 1 Environmental parameters of water samples used for microcosm experiments

Sampling site	Date month/day/year	Temp (°C)	Salinity	pH	DO %	NH_4^+ μmol l^{-1}	NO_2^- μmol l^{-1}	NO_3^- μmol l^{-1}	PO_4^{3-} μmol l^{-1}
GOM	June 8, 2011	28.1	34.4	7.8	127.5	4.87	0.38	0.12	0.47
77	June 8, 2011	29.7	0	8.1	62.1	0.33	0.02	0.44	0.02
79	June 8, 2011	30.4	0	8.9	76	7.21	0.27	1.15	0.42
Tennessee reef	October 14, 2011	28.9	36.3	7.7	105.3	1.37	0*	0*	0.003
77	October 14, 2011	28.1	0	7.8	70.9	1.59	0.23	15.4	0.93
79	October 14, 2011	27.8	0	7.4	55	2.62	1.11	15.7	1.31

GOM Gulf of Mexico, *nd* not detected

* Below the detection limit

freshwater samples in the high discharge period and this observation was consistent with our previous report (Urakawa et al. 2013). The nutrient level was higher at S-79 than that of S-77 in both discharge conditions, which suggests a nutrient loading from the watershed. Particularly, the level of ammonium was high at S-79 in the low discharge condition and this result agreed with our previous report (Urakawa et al. 2013). pH ranged between 7.4 and 8.9 in the freshwater samples throughout both discharge periods. Flow rate for the sampling day was calculated based on the 30 days prior the sampling date to be an average of 5.8 m^3 s^{-1} during the low discharge period and 26.0 m^3 s^{-1} during the high discharge period. Residence time was calculated based on the flow rate yielding 38 days during the low discharge period and 25 days during the high discharge period. During high discharge, the S-77 lock remains open while the middle lock, S-78, is closed. This causes a negative flow at S-77 (Army Corps of Engineers) for the months following the June sampling and extending towards the October sampling. During the rainy summer months, high flow rate was recorded at S-79 as a consequence of watershed input. Additionally, near the end of the high discharge period, the water from S-78 was released down the river to S-79. During the low discharge period, a lesser amount of water is released on a schedule from S-77, subsequently passing through the downstream locks. Thus, during this low discharge period, the estuary is primarily influenced by water originating from S-77, while during high discharge, it is influenced by water from S-79 and (to a lesser extent) S-78.

pH measured at Site 3 ranged from 7.5 to 8 between seasons. Dissolved oxygen decreased from the low discharge to high discharge season from 85.3 to 33.4 %. Chlorophyll *a* levels were an average of 0.79 μg l^{-1} during the low discharge period and an average of 1.64 μg l^{-1} during the high discharge period. Nitrate, nitrite, and phosphate levels were lower during the low discharge period.

High-throughput sequencing for estuarine bacterioplankton communities in low and high freshwater discharge periods

A total of 20,787 high-throughput sequences were determined in four data sets representing 11 phyla; 24 classes were analyzed revealing a dominance of four phyla (Proteobacteria, Bacteroidetes, Cyanobacteria and Actinobacteria), seven major classes (>5 %) (Alphaproteobacteria, Sphingobacteria, Cyanobacteria, Gammaproteobacteria, Flavobacteria, Cytophagia and Actinobacteria) in both the high discharge and low discharge periods. Mean G+C content for the data sets were 50 ± 2 and 51 ± 3 for the small low discharge and high discharge data sets, respectively and 50 ± 2 and 52 ± 3 for the large low discharge and high discharge data sets (Table S1). Average percent of predicted ribosomal RNAs in the four data sets was 99.8 %. The reproducibility of duplicated high-throughput DNA sequencing data sets were evaluated using four major phyla (Fig. 2). The calculated differences for four major phyla between large and small libraries in each discharge period were within 95 % confidence intervals, suggesting that there were no significant difference between large and small libraries in low and high discharge data. Proteobacteria were the most abundant bacterial phylum during the low discharge period and the third most abundant during the high discharge period, accounting for 63

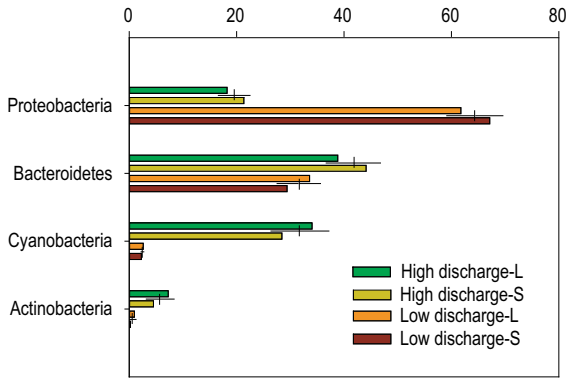


Fig. 2 Relative abundance of major estuarine bacterioplankton phyla. Replicated libraries are shown for both discharge periods. Mean and 95 % confidential values are shown as *cross*. *S* small library, *L* large library

and 19 %, respectively, of all the bacterial sequences (Fig. 2). During the low discharge period, 54.6 % of sequences belonged to the class Alphaproteobacteria which was dominated by a group of "*Candidatus* Pelagibacter" (Fig. S2). A substantial decrease of Alphaproteobacteria (80 % decrease) was noted in the high discharge period with moderate salinity condition (Table S2). Gammaproteobacteria accounted for 9.1 and 6.0 % of the sequences during the low and high discharge periods, respectively (Table S2). The remaining proteobacterial classes formed a small fraction of the sequences with Betaproteobacteria accounting for 0.55 % (low discharge) and 1.8 % (high discharge) of all sequences, Deltaproteobacteria representing 0.14 % (low discharge) and 0.78 % (high discharge) and Epsilonproteobacteria represented by one sequence that is closely related to the family Helicobacteraceae during the low discharge period (Fig. S2; Table S2).

In contrast to the Proteobacteria, Bacteroidetes was the most abundant phylum during the high discharge period, accounting for 41 % of the all sequences and 31 % in the low discharge period (Fig. 2). The phylum composition was moderately conserved in both salinity conditions, which was in contrast to the cases of Actinobacteria and Cyanobacteria (Fig. 3). The phylum Bacteroidetes composed of 26 genera was dominated by *Sphingobacterium* (44 %) and *Flavobacterium* (37 %) during the low discharge season and transitioned into *Sphingobacterium* (60 %) and *Cytophaga* (22 %) during the high discharge period (Figs. S3, 3). Actinobacteria was the fourth most abundant phylum in both low and high freshwater discharge periods representing 1.25 and 5.9 %, respectively (Fig. 2). A total of 30 genera were identified (Fig. S4). The most abundant genera during both periods included *Clavibacter* (24 %), *Actinomarina* (16 %), *Plantibacter* (15 %) and *Aquiluna* (13 %) (Fig. 3). Cyanobacteria was the second largest phylum during the high discharge period accounting for 33 % of the total sequences (Fig. 2). The genus *Synechococcus* (74.3 %) dominated followed by *Cyanobacterium* (15.7 %) and *Cyanobium* (8.6 %) during the high discharge period (Figs. S5, 3). During the low discharge period *Prochlorococcus* (90.6 %) was the largest group followed by *Cyanobacterium* (5.2 %) and *Synechococcus* (2.4 %) (Figs. S5, 3).

Denaturing gradient gel electrophoresis analysis for estuarine bacterioplankton communities

A total of 33 out of 50 bands were successfully sequenced from the low discharge samples and 17 out of 46 for the high discharge samples (Fig. 4). These bands, when compared to bacterial sequences in GenBank, showed 85–100 % similarity (Table S3). Phylogenetic analysis of the sequenced DGGE bands revealed an abundance of three major classes: Alphaproteobacteria, Gammaproteobacteria and Actinobacteria (Fig. 5).

DGGE was used to identify the original inoculum (Site 3) bacterioplankton communities for the microcosm experiments. Four bands were successfully amplified during the low discharge period identifying as members of the Actinobacteria and Alphaproteobacteria (Figs. 4, 5). Actinobacteria was represented by bands (L-42, L-44) which were identified as *Arthrobacter* spp. (Table S3). L-43 was identified as "*Candidatus* Planktophila limnetica". The band L-41 was the single Alphaproteobacteria hit identified as "*Candidatus* Pelagibacter" with 100 % similarity (Table S3). No bands from the Site 3 inoculum used for the high discharge condition experiment were successfully amplified; however, band H-34 from S-77 and bands H-11, H-14 and H-36 from S-79 were identical in position to bands in Site 3. These bands represented Alphaproteobacteria and Actinobacteria (Figs. 4, 5). This pattern was consistent with that found in the inoculum used for the low discharge experiment and high-throughput sequences (Figs. S2, S4).

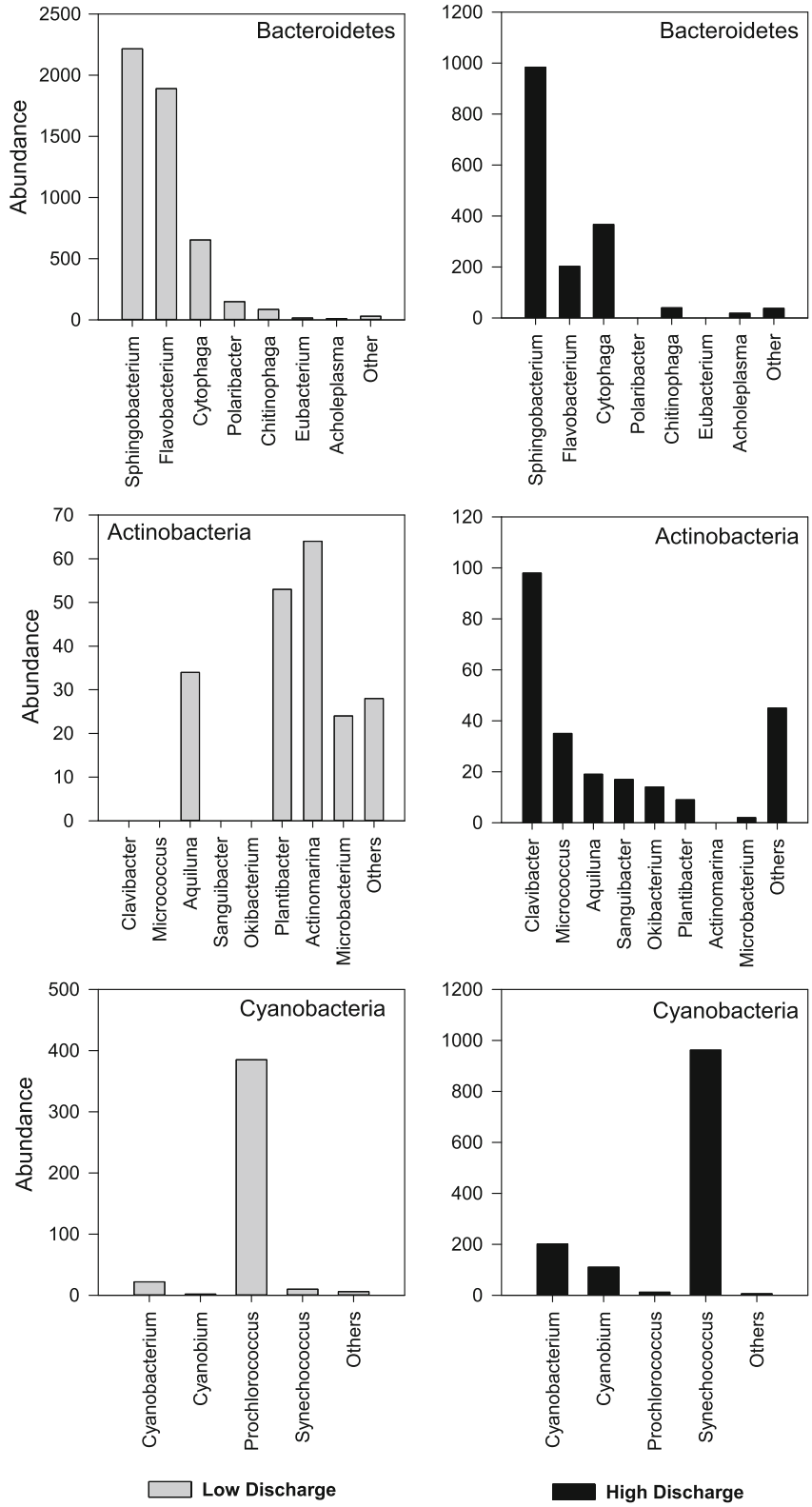


Fig. 3 Abundance of Bacteroidetes, Actinobacteria and Cyanobacteria genera during the low discharge and high discharge periods

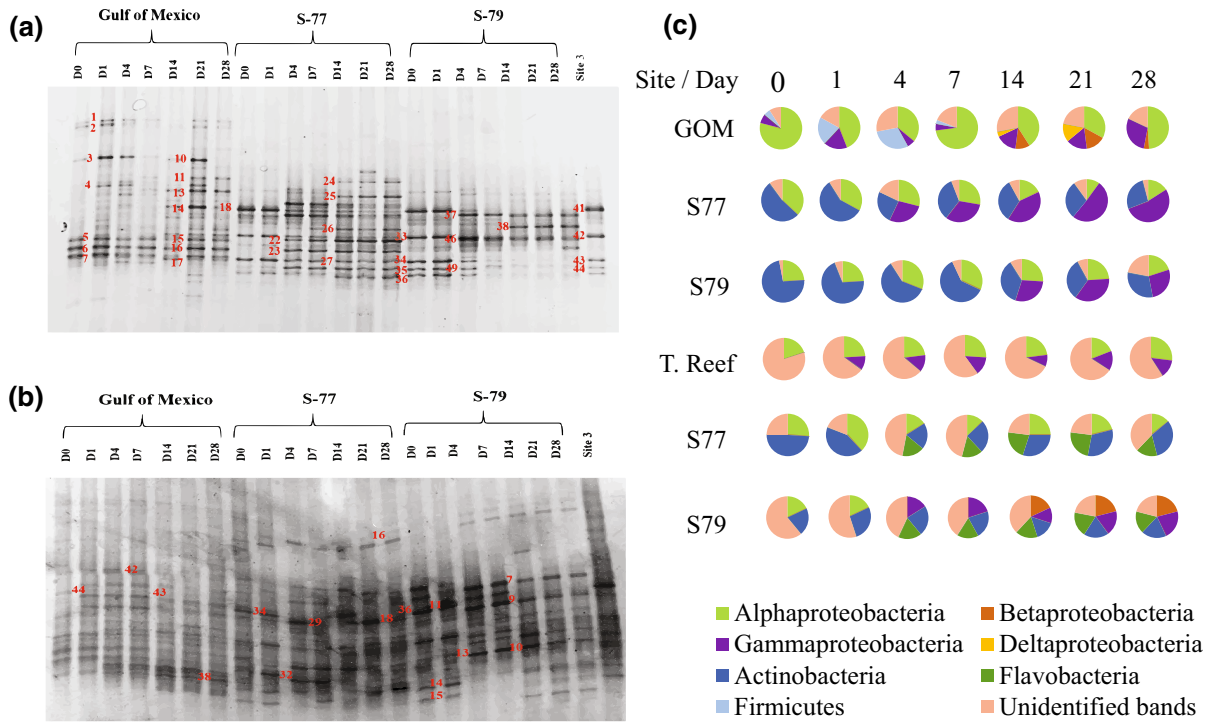


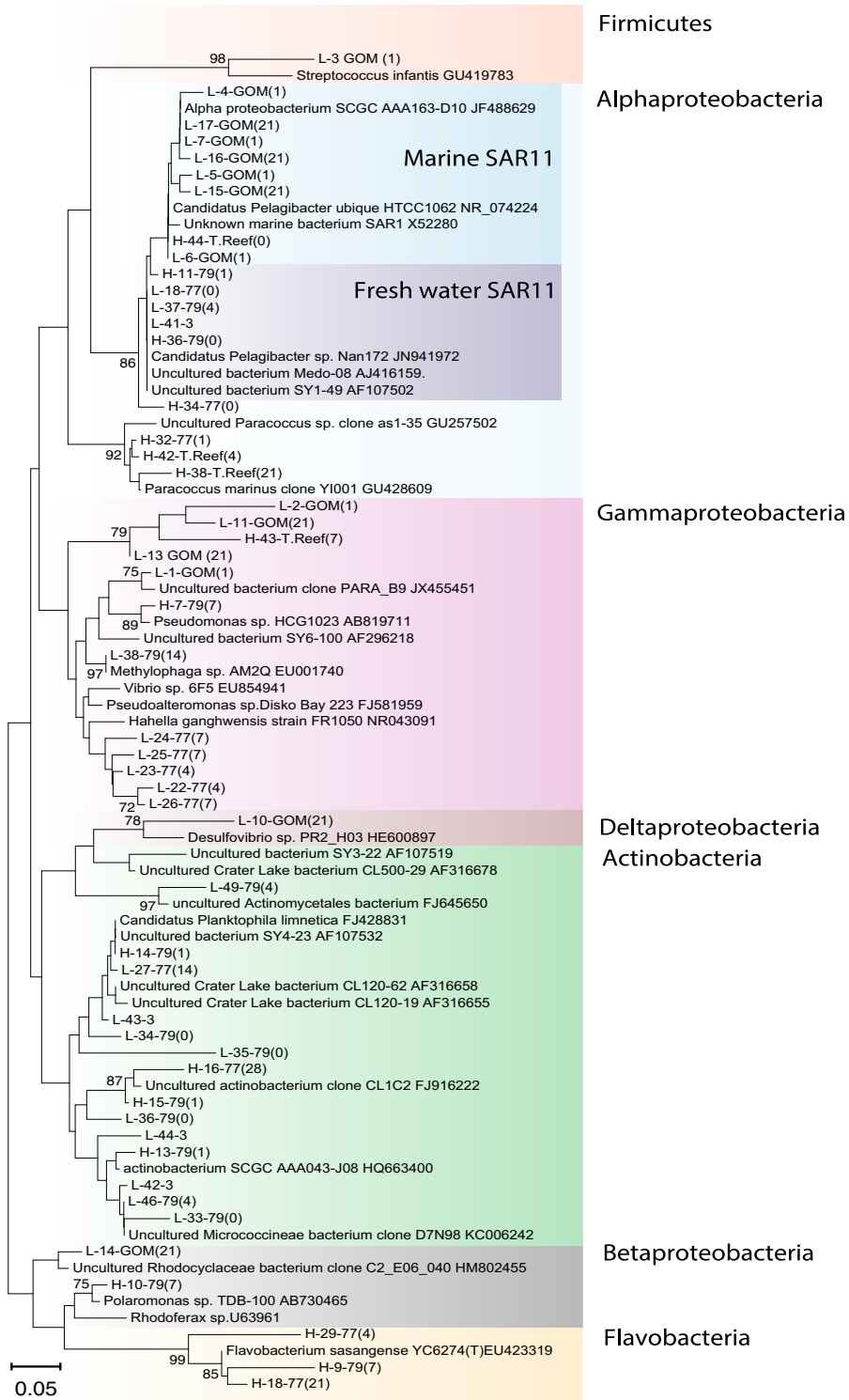
Fig. 4 Demonstration of successional DGGE patterns during the **a** low and **b** high freshwater discharge periods and **c** relative abundance of bacterioplankton classes based on the DGGE band sequencing and densitogram analysis in the time course experiments. *Upper* (GOM, S77 and S79) and *lower* (Tennessee Reef, S77 and S79) indicate the low and high discharge periods, respectively

DON degradation by estuarine bacterioplankton in low freshwater flow period

DGGE banding patterns were analyzed using clustering analysis (Fig. S6). The clustering analysis demonstrates the formation of phases during the incubation period via the similarity in successional and banding patterns. The succession patterns of the bacterial flora in S-77 and S-79 water samples were commonly categorized into three phases, phase I (days 0 and 1), phase II (days 4 and 7) and phase III (days 14, 21 and 28) based on the gel images and dendrograms (Fig. S6). A similarity in microbial flora during both discharge periods via the banding patterns between Site 3 and phase I samples (S-77-0, 77-1, 79-0 and 79-1) were observed. A similar pattern is shown during the low discharge period for the GOM cluster.

During the low discharge period DON concentrations in S-79 increased during the first 4 days then remained stable through day 14 (Fig. 6e). DON concentration rapidly decreased by 29 % from day 21 to the end of the incubation period (utilization rate of 2.96 μmol l^{-1}). DIN concentrations remained relatively constant throughout the time course experiment for all sites (Fig. 6c). Concentrations of DOC stayed constant for all sites during the low discharge period (Fig. 6g). Bacterial abundance in S-79 increased rapidly during the first 7 days (phase I and II) to a maximum biomass of 3.0×10^6 cells ml^{-1}. Biomass proceeded to decrease through day 21 with a rebound by day 28 (Fig. 6a) (phase III). This change in biomass correlates with the change seen in average DON concentration for the site and with what is observed in DGGE profiles (Fig. 4).

A total of eight bands were isolated from the S-79 incubation representing members in the Alphaproteobacteria, Gammaproteobacteria and Actinobacteria (Fig. 5). The estuary inoculum bacteria are present the first 2 days of incubation; however, the Actinobacteria present in the first 24 h decrease gradually throughout the remaining incubation periods (L-34, 35, 36) (Fig. 4). The recovered Actinobacteria includes six bands characterizing four different actinobacterial groups (Table S3). The DGGE bands L-33 and L-46


Firmicutes
98
L-3 GOM (1)
Streptococcus infantis GU419783
Alphaproteobacteria
L-4-GOM(1)
Alpha proteobacterium SCGC AAA163-D10 JF488629
L-17-GOM(21)
L-7-GOM(1)
L-16-GOM(21)
Marine SAR11
L-5-GOM(1)
L-15-GOM(21)
Candidatus Pelagibacter ubique HTCC1062 NR_074224
Unknown marine bacterium SAR1 X52280
H-44-T.Reef(0)
L-6-GOM(1)
H-11-79(1)
L-18-77(0)
Fresh water SAR11
L-37-79(4)
L-41-3
H-36-79(0)
86
Candidatus Pelagibacter sp. Nan172 JN941972
Uncultured bacterium Medo-08 AJ416159.
Uncultured bacterium SY1-49 AF107502
H-34-77(0)
Uncultured Paracoccus sp. clone as1-35 GU257502
H-32-77(1)
92
H-42-T.Reef(4)
H-38-T.Reef(21)
Paracoccus marinus clone YI001 GU428609
Gammaproteobacteria
L-2-GOM(1)
L-11-GOM(21)
79
H-43-T.Reef(7)
L-13 GOM (21)
75
L-1-GOM(1)
Uncultured bacterium clone PARA_B9 JX455451
H-7-79(7)
89
Pseudomonas sp. HCG1023 AB819711
Uncultured bacterium SY6-100 AF296218
L-38-79(14)
97
Methylophaga sp. AM2Q EU001740
Vibrio sp. 6F5 EU854941
Pseudoalteromonas sp.Disko Bay 223 FJ581959
Hahella ganghwensis strain FR1050 NR043091
L-24-77(7)
L-25-77(7)
L-23-77(4)
L-22-77(4)
72
L-26-77(7)
78
L-10-GOM(21)
Deltaproteobacteria
Desulfovibrio sp. PR2_H03 HE600897
Actinobacteria
Uncultured bacterium SY3-22 AF107519
Uncultured Crater Lake bacterium CL500-29 AF316678
L-49-79(4)
97
uncultured Actinomycetales bacterium FJ645650
Candidatus Planktophila limnetica FJ428831
Uncultured bacterium SY4-23 AF107532
H-14-79(1)
L-27-77(14)
Uncultured Crater Lake bacterium CL120-62 AF316658
Uncultured Crater Lake bacterium CL120-19 AF316655
L-43-3
L-34-79(0)
L-35-79(0)
H-16-77(28)
87
Uncultured actinobacterium clone CL1C2 FJ916222
H-15-79(1)
L-36-79(0)
L-44-3
H-13-79(1)
actinobacterium SCGC AAA043-J08 HQ663400
L-42-3
L-46-79(4)
L-33-79(0)
Uncultured Micrococcineae bacterium clone D7N98 KC006242
L-14-GOM(21)
Betaproteobacteria
Uncultured Rhodocyclaceae bacterium clone C2_E06_040 HM802455
75
H-10-79(7)
Polaromonas sp. TDB-100 AB730465
Rhodoferax sp.U63961
H-29-77(4)
Flavobacteria
Flavobacterium sasangense YC6274(T)EU423319
99
H-9-79(7)
85
H-18-77(21)
0.05

◀**Fig. 5** Phylogenetic tree of sequenced DGGE bands detected in the time course experiments. DNA sequences are assigned by discharge period (H or L), position number on the DGGE gels (see Fig. 4), site of isolation (S77, S79, GOM or Tennessee Reef) and day of isolation during microcosm experiments (i.e. day 0–28). *Numbers* near nodes indicate bootstrap values over 70 % for neighbor-joining analysis

were identified as *Microbacterium oxydans* with a similarity of 97 and 99 % respectively. Three additional bands were identified as unclassified Actinobacteria (L-35, 36, 49). L-34 was identified as "*Candidatus* Planktophila limnetica" with a 96 % similarity. Gammaproteobacteria had one representative with L-38 identified as *Methylophaga* sp. with a 98 % similarity (Table S3). The single representative of the Alphaproteobacteria was identified as "*Candidatus* Pelagibacter" with a 99 % similarity.

Average DON levels in S-77 demonstrated minimal fluctuations throughout the incubation period (Fig. 6e). Microbial abundance in S-77 water showed a stable population during the first 4 days of incubation (phase I and II) (Fig. 6a). Day 7 showed an increase in bacterioplankton followed by a gradual decrease in biomass throughout the later incubation periods (phase II to III). Initial DOC concentrations were 1,129 μmol l^{-1} in S-77 and the concentration of DOC stayed constant (Fig. 6g).

A total of seven bands were successfully amplified (L-18, 22, 23, 24, 25, 26 and 27 with a similarity of 93–100 %) (Fig. 4). All original estuary inoculum bacteria identified in the Site 3 sample were present in the first 2 days of incubation (L-18, 27) (phase I). Estuary inoculum bacterioplankton L-18 (identified as "*Candidatus* Pelagibacter" of the SAR11 clade) and L-27 (identified as "*Candidatus* Planktophila limnetica" of the Actinobacteria) persisted with a gradual decrease in band strength until the end of the incubation experiment (Fig. 4; Table S3). Incubation periods days 4–28 (phase II to III) marked a change of succession of the bacterioplankton (Fig. 4). Succession led to a shift of dominant groups from Alphaproteobacteria to Gammaproteobacteria (Figs. 4, 5).

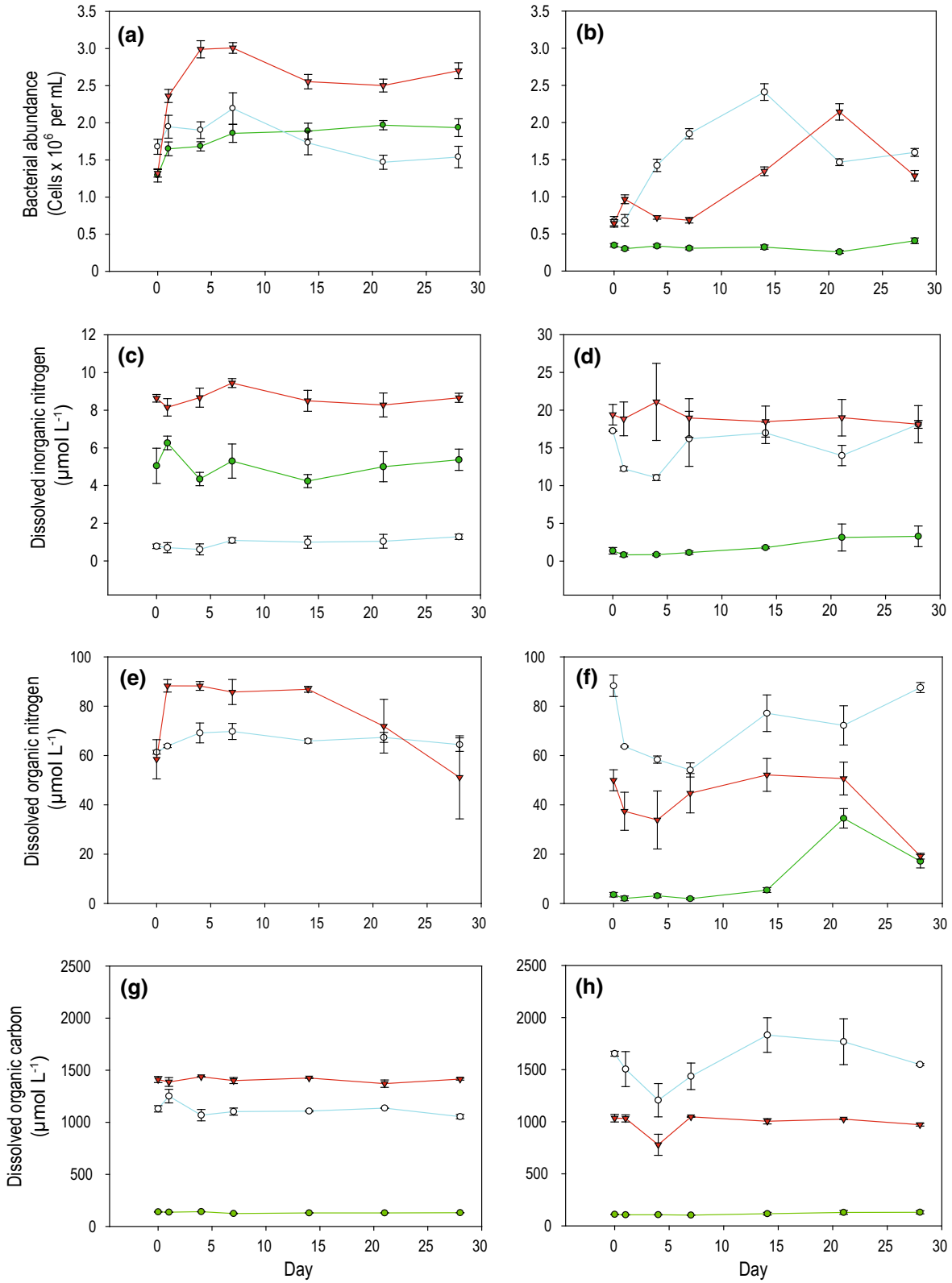
DON level was below the detection limit in the coastal water microcosm of Gulf of Mexico (Fig. 6e). The coastal water microcosm did not show marked variation in microbial biomass throughout the experiment (Fig. 6a). Initial DOC concentration was 139 μmol l^{-1} with no change in concentration throughout the incubation period. A total of 14 bands were sequenced from the GOM sampling representing the Proteobacteria and Firmicutes lineages (Fig. 5). "*Candidatus* Pelagibacter" (L-4, 5, 6, 7, 15, 16 and 17, 97–100 % similarity) and various members of the Gammproteobacteria (L-1, 2, 11 and 13, 88–100 % similarity) persist throughout the incubation (Fig. 5; Table S3). One band (L-3) isolated on day 7 was members of the Firmicutes (Figs. 4, 5; Table S3). A shift in community occurs on day 21 with the emergence of members of the Deltaproteobacteria (L-10, 90 % similarity) and Betaproteobacteria (L-14, 90 % similarity) (Figs. 4, 5).

The PCA provides a more subjective interpretation of results due to the lack of clustering of the samples (Fig. S7). Both treatments started with the same initial estuarine bacterioplankton, however both treatments evolved differently based on the DOM source provided. It also revealed a strong similarity between Site 3 and S-77-0, S-77-1 and S-79-0, S-79-1 communities, which was also confirmed in the dendrogram (Fig. S6). The PCA also shows a group consisting of GOM samples with high similarity and a group showing a strong similarity between S-79-14, 21 and 28 communities (Fig. S7a).

DON degradation by estuarine bacterioplankton in high water flow period

Average DON concentrations in S-79 decreased by 33 % during the first 4 days (utilization rate of 4.02 μmol l^{-1}) then increased through day 14 (phase I to III). From day 21 to day 28 (phase III), DON concentrations decreased by 62 % (utilization rate of 4.5 μmol l^{-1}). Microbial abundance in S-79 increased through the first 24 h of the time course experiment then went through a decrease and later a lag phase that lasted through day 7 (phase I to II). After day 7, the microbial population experienced a rapid exponential increase through day 21 (phase III) where it reached a maximum biomass of 2.1×10^6 cells ml^{-1}. This rapid period of growth was followed by a rapid decrease through the later part of the incubation period. DOC usage in S-79 showed a decrease in concentration during day 4; however, values stayed relatively constant throughout the incubation period (Fig. 6h).

Eight DGGE band samples representing five classes were identified from S-79 (Table S3; Fig. 5). As in the


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Bacterial abundance (Cells x 10^6 per mL)
Dissolved inorganic nitrogen (µmol L-1)
Dissolved organic nitrogen (µmol L-1)
Dissolved organic carbon (µmol L-1)
Day
Day

◀**Fig. 6** Changes in bacterial abundance (**a** and **b**), dissolved inorganic nitrogen (**c** and **d**), dissolved organic nitrogen (**e** and **f**) and dissolved organic carbon (**g** and **h**) in the time course experiments. *Left* (**a**, **c**, **e** and **g**) and *right* (**b**, **d**, **f** and **h**) panels show June and October samples, respectively. GOM/Tennessee Reef (*closed circle*), S-77 (*open circle*), S-79 (*closed triangle*). *Standard errors* are shown ($n = 3$). DON was below the detection limit in the GOM sample (**e**)

low discharge experiment, the estuary inoculum bacteria, consisting of Alphaproteobacteria and Actinobacteria, were present throughout the first 2 days of incubation (H-11, 14, 15 and 36) (phase I) (Fig. 4). These bands, however, disappeared in the later incubations. Days 4–28 (phase II to III) demonstrated succession occurring as an increase in bands leads to a shift in the bacterioplankton community from the estuary inoculum flora to a community of Actinobacteria, Betaproteobacteria, Flavobacteria and Gammaproteobacteria (H-7, 9 and 10) (Figs. 4, 5; Table S3).

Initial DON concentration in S-77 (88 μmol l^{-1}) decreased during the first 7 days (phase I to II) by 39 % (utilization rate of 8.5 μmol l^{-1}) then rebounded through the later incubation period to near initial DON concentrations (final concentration 87 μmol l^{-1}) (phase II to III) (Fig. 6f). Cell numbers in S-77 samples increased through day 14 to a maximum biomass of 2.4×10^6 cells ml^{-1} and then decreased through the later incubation period (Fig. 6b). DOC concentrations decreased by 27 % in S-77 during the first 4 days of incubation then increased with a spike during day 14, through the end of the incubation period to almost initial concentration (1,600 μmol l^{-1}) (Fig. 6h).

DGGE demonstrates the presence of Actinobacteria (H-16), Alphaproteobacteria (H-32 and 34) and Flavobacteria (H-18 and 29) in the S-77 incubation. Estuary inoculum bacteria dominated by Actinobacteria and Alphaproteobacteria were present in the first 2 days of the incubation period (phase I) (Fig. 4). A succession of flora was caused by Flavobacteria, which appeared on day 4 and were present through the end of the incubation.

The coastal water DON concentrations from Tennessee Reef remained constant through the first 14 days where a rapid increase in DON was observed in day 21 (94 % increase in DON from day 14 to 21) (Fig. 6f). Cell numbers and DOC concentrations remained constant throughout the incubation period (Fig. 6b, h). The Tennessee Reef incubation revealed a typical marine flora with members of the Alphaproteobacteria (H-38, 42 and 44) and Gammaproteobacteria (H-43) (Fig. 4). "*Candidatus* Pelagibacter" and *Paracoccus* sp. were identified as members within Alphaproteobacteria (Table S3; Fig. 5).

During the high discharge period, the PCA shows a strong similarity in banding patterns between the S-77 time periods 4–28 (Fig. S7b). PCA also showed succession in S-77 and S-79 leading to a change in the original inoculum microbial flora as the incubation period progressed (Fig. S7b). Tennessee Reef samples also form a cluster.

Discussion

Shifts in bacterioplankton communities may occur with variations in freshwater inflow and with river flushing events. Due to the impact that freshwater has on salinity and nutrient input, different bacterioplankton may become activated and be lost during the different periods of discharge that occur in the estuary. Our high-throughput sequencing revealed a succession in the original estuarine inoculums of a Proteobacteria–Bacteroidetes dominated flora during the high salinity period to a Cyanobacteria–Bacteroidetes dominated flora during the lower salinity period. Thus, the estuarine bacterioplankton abundance and diversity found in the Caloosahatchee estuary was strongly affected by the controlled freshwater releases that lead to high discharge and low discharge conditions. As we expected, an increase in typical freshwater Betaproteobacteria and Actinobacteria was found in low salinity condition while the Alphaproteobacteria and Gammaproteobacteria constituted a major portion of bacterioplankton found in a high salinity condition.

The most abundant member of Alphaproteobacteria was "*Candidatus* Pelagibacter" of the ocean dominating SAR 11 clade (Morris et al. 2002), which is a cosmopolitan marine bacterioplankton (Giovannoni et al. 2005) with a freshwater sister clade (Bahr et al. 1996; Zwart et al. 2002). Campbell and Kirchman (2013) found a dominance of SAR 11 in an estuarine environment with the presence of freshwater SAR 11. Our data suggests the Caloosahatchee estuary contains both a SAR 11 ocean ecotype and a freshwater ecotype. As demonstrated by DGGE cluster analysis, the SAR 11 identified in our samples were closely related to a marine ecotype ("*Candidatus* Pelagibacter" NR_074224) and a freshwater ecotype ("*Candidatus* Pelagibacter" JN941972). The dominance of this

group was also confirmed by high-throughput sequencing. Although the Gammaproteobacteria were found in both discharge conditions, the abundance was enhanced in the high salinity condition. A similar observation was reported previously by Jiang et al. (2007) where they looked at microbial succession patterns along a salinity gradient in an inland hypersaline lake.

Betaproteobacteria typically constitutes a major fraction of the bacterioplankton community in freshwater ecosystems (Hiorns et al. 1997; Zwart et al. 2002; Mueller-Spitz et al. 2009). Barberán and Casamayor (2010) compared the β-diversity patterns of major microbial groups from around the world and how the environment affected these. They concluded that Betaproteobacteria appears to be affected by salinity levels. Bouvier and Del Giorgio (2002) and Goni-Urriza et al. (2007) observed that the detection of Betaproteobacteria was limited to salinity lower than 10 and 13, respectively. It is likely that the salinity level of the high discharge condition (13 %) was sufficiently high and this salinity range negatively influenced the presence of Betaproteobacteria in the Caloosahatchee estuary. As suggested by our data we anticipate that Betaproteobacteria are more sensitive to salinity increase compare to other freshwater dominant groups, such as Actinobacteria and Bacteroidetes.

The members of Bacteroidetes are one of the major bacterioplankton in both freshwater and marine habitats (Kirchman 2002; Kirchman et al. 2005; Ordoñez et al. 2009). They are frequently found on suspended particles (DeLong et al. 1993) and are able to degrade DOM and particulate organic matter, making them crucial for biogeochemical cycles in aquatic ecosystems (Cottrell and Kirchman 2000; Kirchman 2002). In our study, Bacteroidetes were present throughout both discharge periods. Because the estuary inoculums samples for microcosm experiments were pre filtered through a GF/F filter (0.7 μm pore size) to eliminate microbial predators, it may have led to the elimination of particle-attached Bacteroidetes (DeLong et al. 1993; Rath et al. 1998) and therefore to an underestimation of these organisms in our microcosm experiments.

Actinobacteria are ubiquitous microorganisms that are found in freshwater and terrestrial but little is known about their marine groups (Jensen and Lauro 2008; Morris et al. 2010). In the present study, Actinobacteria accounted for the fourth largest phylum in both discharge periods in our high-throughput sequence data and accounted for 44 % of all the DGGE bands isolated for all treatments and discharge periods, suggesting their major contribution for river DON degradation. In freshwater environments, Actinobacteria can account for up to 70 % of bacteria and are major heterotrophs (Glöckner et al. 2000; Warnecke et al. 2005). They also have been observed to be favored by phytoplankton derived organic matter (Stepanauskas et al. 2003). During the microcosm experiments several bands were identified as the novel freshwater actinobacterial lineage "*Candidatus* Planktophila limnetica" (Jezbera et al. 2009). Their high abundance during the high discharge period may indicate that freshwater Actinobacteria may play an important role in the degradation of DOM. This is further confirmed with the abundance of actinobacterial high-throughput sequences. Interestingly, a fraction of actinobacterial sequences found in the high salinity condition matched the smallest free-living marine Actinobacteria lineage, "*Candidatus* Actinomarina" (Ghai et al. 2013). This novel lineage of low GC content Actinobacteria has preferential abundance in the photic zones from tropical to temperate areas (Ghai et al. 2013). They are more abundant in pelagic water and less abundant in estuaries. Our high-throughput sequencing data showed that "*Candidatus* Actinomarina" forms 0.3 % of total sequences, which may expand previously known habitats from pelagic to estuarine (Ghai et al. 2013). We anticipate the existence of an estuarine-adapted ecotype of this unique low GC content Actinobacteria in this estuary system. Our microcosm experiments and further DGGE analysis, however, could not detect the activity of this marine Actinobacteria in the degradation of river borne DON.

In aquatic ecosystems, dissolved organic carbon (DOC) is an important source of carbon for heterotrophic bacterioplankton (Moran et al. 1999; Wiegner and Seitzinger 2001) and influences bacterioplankton composition (Jones et al. 2009). DOC losses during the low discharge incubation period were negligible while DOC concentrations during the high discharge period were only substantial for S-77 during the first 4 days of incubation with a 27 % decrease. This suggests that the source and lability of DOC may vary seasonally in aquatic ecosystems (Wiegner and Seitzinger 2004; Holmes et al. 2008). Both the nitrogen and carbon components of the riverine DOM appear to have

different cycling times in the estuarine bacterioplankton, with the nitrogen fraction appearing to be cycled faster than the carbon fraction. Weigner et al. (2006) observed a higher consumption of DON versus DOC in several rivers in the eastern coast of the United States. This suggests that the bacterioplankton in the estuary preferentially use the nitrogen rich fraction in the DOM pool and that more extensively, during the low discharge compared to the high discharge period, the bacterioplankton cannot degrade the DOC present in the ecosystem due to low lability thus using DON as both a nitrogen source and carbon source.

In estuarine environments, DON contributes to nitrogen loading and is readily consumed by phytoplankton and bacteria (Yuan et al. 2012). DON consists of two fractions: the low molecular and high molecular nitrogen, of which the low molecular is composed of dissolved free amino acids, dissolved combined amino acids and urea. These low molecular fractions have been shown to be important nitrogen sources to microbes (Middelburg and Nieuwenhuize 2000). Literature also suggests that up to 70 % of DON is bioavailable to estuarine bacterioplankton (Seitzinger et al. 2002). In addition, DON availability is affected by residence time. It is expected that residence times in the order of weeks to months would allow riverine DON to be primarily used in the estuary while residence times shorter than that would lead to exportation of DON into coastal shelf waters (Seitzinger and Sanders 1997). Our study found that a change of bacterioplankton communities occurred as a result of salinity and DON source due to water discharge. Previous studies in other estuaries such as the Chesapeake Bay (Bouvier and Del Giorgio 2002), Delaware Bay (Cottrell and Kirchman 2003; Castle and Kirchman 2004), Pearl River estuary (Zhang et al. 2006) and the Baltic Sea (Herlemann et al. 2011) showed a change in bacterioplankton population with respect to salinity and studies looking at the effects of DOM on bacterioplankton communities showed a community shift in response to the source of DOM (Kirchman et al. 2004; Judd et al. 2006). Our results are consistent with those of other estuaries where a shift has been noted from typical marine Alphaproteobacteria–Gammaproteobacteria to typical freshwater bacteria such as Betaproteobacteria–Actinobacteria as salinity decreases (Crump et al. 1999). This suggests that water discharge changes induce salinity and DOM sources changes leading to a succession in important DOM degraders in the estuary. This is evident in our DGGE analysis, which revealed a difference in the bacterioplankton community per site and per discharge period, and in our high-throughput sequence data, which ultimately shows a difference in the important degraders in the system. Hopkinson et al. (1998) concluded that DOM quality accounts for 67–75 % of the variability in bacterial growth in estuarine environments. Evidence also suggests that bacterioplankton are capable of using different fractions of DOM (Cottrell and Kirchman 2000; Kirchman 2002; Elifantz et al. 2005). Fuchs et al. (2000) concluded that Gammaproteobacteria are able to outcompete other bacterioplankton when high DOM is present in the environment. Rocker et al. (2012) examined bacterial decomposition of humic acids at various salinities in marine and estuarine samples and observed a dominance of Alphaproteobacteria, Gammaproteobacteria and Actinobacteria in their estuarine samples.

Changes in the source of DOM, specifically DON, are driven in the Caloosahatchee River by two different water discharges: controlled input from Lake Okeechobee and by watershed discharges (Doering and Chamberlain 1999). As shown in Fig. 1, these discharges are controlled by the three locks found in the river. Liu et al. (2009) reported that Lake Okeechobee played a vital part on the water quality of the Caloosahatchee River specifically during the low discharge period. It accounted for 72 % of the water flow during the low discharge and 36 % during the high discharge period from the lake (Liu et al. 2009). During the low discharge period, low input is derived from the watershed while water that was being held in S-78 that originated in S-77, is released providing the sole source of discharge into the estuary. The vice versa was observed with the watershed runoff dominating during the high discharge with a lesser relative influence from S-77. USACE data confirm our observations and show a distinct flow pattern between the locks. During high discharge, water flow from S-77 is high but is truncated in the S-78 lock leading to flow rate in S-79 being dominated by watershed input. During low discharge, flow rate in S-79 increases as S-77 water is released from S-78 providing a new source of DOM to estuarine bacterioplankton. This pattern was reflected via the different biomass, banding patterns and DON changes seen dependent on the source of DOM water used (S-77 vs. S-79) in

our microcosm experiments. Both sources of water were inoculated with the same estuarine bacterioplankton for each period but differences in biomass and DON usage were observed dependent on DOM source. The observed bacterioplankton numbers were comparable to the Sarmento study (2012) and consistent with our previous study (Urakawa et al. 2013). During low discharge, however, bacterial biomass and nutrient abundance was greatest in S-79 water compared to S-77. DON usage in S-79 water during the low discharge period led to a community shift dominated by Actinobacteria (75 % of DGGE sequences), Alphaproteobacteria and Gammaproteobacteria (25 % of DGGE sequences), suggesting that these microorganisms were mainly responsible for the degradation of DON. S-77 showed a greater banding pattern dominated by members of the Gammaproteobacteria, but biomass, DIN and DON were below that of S-79. This suggests that the opportunistic Gammaproteobacteria may have dominated because of the high DOM present (Fuchs et al. 2000). This is further supported by the PCA analysis which clearly defines groups reacting to the DOM source and demonstrates successional patterns of the bacterioplankton inoculums. The evidence leads to the conclusion that estuarine bacterioplankton composition was impacted by DOM source.

Remineralization is an important source of nitrogen in estuarine systems (Alongi and Mckinnon 2005). The regenerated DIN from microbial degradation is readily available for phytoplankton usage (Bronk et al. 2002). This is particularly the case in the southwest Florida coast which is generally characterized as a nitrogen limited, oligotrophic area that suffers frequent and persistent algal blooms caused by *Karenia brevis* (Heil et al. 2007). Loh (2008) reported that about 80 % of nitrogen was in the organic form in the Caloosahatchee estuary. During low discharge when DIN level is low, the riverine DON may first be degraded by bacterioplankton into useable inorganic forms for algae growth. From the calculated residence time of 38 days, it may be possible that the bacterioplankton use the DON within the estuary and provide nutrients for algae blooms. In our previous study, a low substrate usage during the low discharge period (winter and spring) and a higher substrate usage during the high discharge period (summer and fall) were observed based on the carbon substrate utilization patterns of estuarine bacterioplankton (Urakawa et al. 2013). This observation was consistent with our microcosm experiment data in which the microbial degradation was more active in the high discharge condition. Moreover, our previous study showed that the number of utilized substrates was generally high in the upstream freshwater dominated zone and low in the downstream zone, suggesting a shift in metabolic profiles among bacterioplankton assemblages along the estuarine gradient (Urakawa et al. 2013). Our previous data, however, could not identify the cause of the observed substrate usage pattern changes which may be derived by the change of microbial metabolic activity or the shifts of microbial populations. Our high-throughput DNA sequence data demonstrated that the changes of substrate usage patterns were likely derived by the shift of bacterioplankton populations along the salinity gradients; the considerable fraction of bacterioplankton communities in the high salinity condition are occupied by SAR11 type Alphaproteobacteria, which do not grow on Biolog EcoPlates (Urakawa et al. 2013).

The flow rate strongly influences the species composition of phytoplankton species. The slow flow is favored by diatoms while the fast flow is preferred by cyanobacteria (Tolley et al. 2010). The difference of species composition is likely caused by the difference of doubling time between these two groups of autotrophs. Cyanobacteria *Prochlorococcus* and *Synechococcus* are major primary producers in the open ocean (Goericke and Welschmeyer 1993; Campbell et al. 1994) and their dominance varies spatially and seasonally (Johnson et al. 2006; Zwirglmaier et al. 2008; Tai and Palenik 2009). They have been described in both nutrient depleted and rich waters (Partensky et al. 1999; Vincent 2000). *Synechococcus* has also been reported to use various forms of organic and inorganic nitrogen (Glibert et al. 1986; Paerl 1991; Lindell and Post 1995; Collier et al. 1999). Urakawa et al. (2013) reported the annual abundance of autotrophic picoplankton in the river water that exceed the previously reported numbers of filamentous cyanobacteria and diatoms in the Caloosahatchee estuary (Tolley et al. 2010). In our study the autotrophic picoplankton, *Prochlorococcus* and *Synechococcus,* were strongly influenced by water discharge patterns: *Prochlorococcus* dominating during the low discharge while *Synechococcus* dominating during the high discharge. The target flow calculated from the residence time in the Caloosahatchee estuary is 31 m^3 s^{-1}

(Wan et al. 2013). With this flow rate, a community similar to our high discharge condition community would dominate. This condition would lead to lake derived DON as the dominant source of DON in the river. Increased water flow would flush the system leading to an increase in cyanobacterial populations such as *Synechococcus* via the addition of nutrients. Previous studies have suggested that *K. brevis* feeds on *Synechococcus* as a source of nitrogen (Jeong et al. 2005; Glibert et al. 2009). The abundance of *Synechococcus* during the high discharge period suggests that it may play a role in the nutrient dynamics of *K. brevis*. The freshwater discharge patterns found in the Caloosahatchee estuary are dynamic and vary from year to year with some years bringing in more freshwater into the system than others. Further studies will provide further understanding of this complex system.

Conclusion

The results presented here demonstrate the impact that artificial water releases have on the estuarine bacterioplankton via the change in salinity and DOM quality. The source of DOM is crucial and varies seasonally; DOM from the high discharge period was more labile than that of low discharge period, particularly the water from S-77. High-throughput sequencing demonstrates a succession in the original estuarine inoculums of a Proteobacteria–Bacteroidetes dominated flora during the high salinity period to a Cyanobacteria–Bacteroidetes dominated flora during the lower salinity period. Thus, the estuarine bacterioplankton abundance and diversity found in the Caloosahatchee estuary was strongly affected by the controlled freshwater releases. DGGE analysis reveals a dominance of Actinobacteria, Alphaproteobacteria and Gammaproteobacteria in the microcosm experiments suggesting these groups as major contributors in the degradation processes in the estuary. Further understanding of bacterioplankton communities and their sensitivity to freshwater inflow may be crucial in designing and implementing watershed usage plans.

Acknowledgments We would like to thank Ian Campbell, Christopher Lienhardt and Lacey Smith for sample collection. Dustin Chisum, Isaac Holowell and Spencer Talmage for processing of samples. We would also like to thank Eric C. Milbrandt at Sanibel-Captiva Conservation Foundation for providing RECON data. This research was supported by the internal grant of Florida Gulf Coast University Office of Research and Graduate Studies to Hidetoshi Urakawa and U. S. Department of Education Congressional Award P116Z090117 to Michael L. Parsons, Ai Ning Loh, and Hidetoshi Urakawa.

References

Alongi D, McKinnon A (2005) The cycling and fate of terrestrially-derived sediments and nutrients in the coastal zone of the Great Barrier Reef shelf. Mar Pollut Bull 51(1):239–252

Apple JK, Giorgio PA, Newell R (2004) The effects of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. J Coast Res, Spec Issue 45:110–133

Bahr M, Hobbie J, Sogin M (1996) Bacterial diversity in an arctic lake: a freshwater SAR 11 cluster. Aquat Microb Ecol 11(3):271–277

Barberán A, Casamayor EO (2010) Global phylogenetic community structure and β-diversity patterns in surface bacterioplankton metacommunities. Aquat Microb Ecol 59(1):1–10

Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat Microb Ecol 31(3):279–305

Bernhard AE, Donn T, Giblin AE, Stahl DA (2005) Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. Environ Microbiol 7(9):1289–1297

Botsford LW, Castilla JC, Peterson CH (1997) The management of fisheries and marine ecosystems. Science 277(5325):509–515

Bouvier TC, Del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47(2):453–470

Bronk DA, Sanderson MP, Mulholland MR, Heil CA, O'Neil JM (2002) Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. Harmful Algae 2004:80–82

Campbell BJ, Kirchman DL (2013) Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. ISME J 7(1):210–220

Campbell L, Nolla H, Vaulot D (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. Limnol and Oceanogr 39(4):954–961

Carman KR, Fleeger JW, Means JC, Pomarico SM, McMillin DJ (1995) Experimental investigation of the effects of polynuclear aromatic hydrocarbons on an estuarine sediment food web. Mar Environ Res 40(3):289–318

Castle D, Kirchman DL (2004) Composition of estuarine bacterial communities assessed by denaturing gradient gel electrophoresis and fluorescence in situ hybridization. Limnol Oceanogr Methods 2:303–314

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarell DM, Marsh T, Garrity GM, Tiedje T (2009) The ribosomal database project: improved

alignments and new tools for rRNA analysis. Nucleic Acids Res 37(Suppl 1):D141–D145

Collier JL, Brahamsha B, Palenik B (1999) The marine cyanobacterium *Synechococcus* sp. WH7805 requires urease (urea amiohydrolase, EC 3.5. 1.5) to utilize urea as a nitrogen source: molecular-genetic and biochemical analysis of the enzyme. Microbiology 145(2):447–459

Costello MJ, Read P (1994) Toxicity of sewage sludge to marine organisms: a review. Mar Environ Res 37(1):23–46

Cottrell MT, Kirchman DL (2000) Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low-and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66(4):1692–1697

Cottrell MT, Kirchman DL (2003) Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol Oceanogr 48(1):168–178

Crump BC, Armbrust EV, Baross JA (1999) Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl Environ Microbiol 65(7):3192–3204

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69(4):2253–2268

Del Giorgio PA, Bouvier TC (2002) Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnol Oceanogr 47(2):471–486

DeLong EF, Franks DG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38(5):924–934

Doering PH, Chamberlain RH (1999) Water quality and source of freshwater discharge to the Caloosahatchee Estuary, Florida. JAWRA J Am Water Resour Assoc 35(4):793–806

Elifantz H, Malmstrom RR, Cottrell MT, Kirchman DL (2005) Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware Estuary. Appl Environ Microbiol 71(12):7799–7805

Flaig EG, Capece J (1997) Water use and runoff in the Caloosahatchee watershed. Paper presented at the Proceedings Charlotte Harbor Public Conference and Technical Symposium

Fuchs BM, Zubkov MV, Sahm K, Burkill PH, Amann R (2000) Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. Environ Microbiol 2(2):191–201

Ghai R, Mizuno CM, Picazo A, Camacho A, Rodriguez-Valera F (2013) Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. Scientific reports, 3

Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, Bibbs L, Eads J, Richardson TH, Noordewier M, Rappe MS, Short JM, Carrington JC, Mathur EJ (2005) Genome streamlining in a cosmopolitan oceanic bacterium. Science 309(5738):1242–1245

Glibert P, Kana T, Olson R, Kirchman D, Alberte R (1986) Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. J Exp Mar Biol Ecol 101(1):199–208

Glibert PM, Burkholder JM, Kana TM, Alexander J, Skelton H, Shilling C (2009) Grazing by Karenia brevis on *Synechococcus* enhances its growth rate and may help to sustain blooms. Aquat Microb Ecol 55(1):17–30

Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. Appl Environ Microbiol 66(11):5053–5065

Goericke R, Welschmeyer NA (1993) The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. Deep Sea Res Part I 40(11):2283–2294

Goni-Urriza M, Point D, Amouroux D, Guyoneaud R, Donard O, Caumette P, Duran R (2007) Bacterial community structure along the Adour estuary (French Atlantic coast): influence of salinity gradient versus metal contamination. Aquat Microb Ecol 49(1):47–56

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnol Oceanogr 52(3):1067–1078

Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5(10):1571–1579

Hiorns WD, Methe BA, Nierzwicki-Bauer SA, Zehr JP (1997) Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. Appl Environ Microbiol 63(7):2957–2960

Hitchcock JN, Mitrovic SM, Kobayashi T, Westhorpe DP (2010) Responses of estuarine bacterioplankton, phytoplankton and zooplankton to dissolved organic carbon (DOC) and inorganic nutrient additions. Estuaries Coasts 33(1):78–91

Hobbie JE, Daley RJ, Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33(5):1225–1228

Holmes RM, McClelland JW, Raymond PA, Frazer BB, Peterson BJ, Stieglitz M (2008) Lability of DOC transported by Alaskan rivers to the Arctic Ocean. Geophys Res Lett 35(3), L03402. doi:10.1029/2007GL032837

Hopkinson CS, Buffam I, Hobbie J, Vallino J, Perdue M, Eversmeyer B, Fredrick P, Covert J, Hodson R, Moran MA, Smith E, Baross J, Crump B, Findlay S, Foreman K (1998) Terrestrial inputs of organic matter to coastal ecosystems: an intercomparison of chemical characteristics and bioavailability. Biogeochemistry 43(3):211–234

Jensen PR, Lauro FM (2008) An assessment of actinobacterial diversity in the marine environment. Antonie Van Leeuwenhoek 94(1):51–62

Jeong HJ, Park JY, Nho JH, Park MO, Ha JH, Seong KA, Chang J, Seong CN, Lee KY, Yih WH (2005) Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquat Microb Ecol 41(2):131–143

Jezbera J, Sharma AK, Brandt U, Doolittle WF, Hahn MW (2009) 'Candidatus Planktophila limnetica', an actinobacterium representing one of the most numerically important taxa in freshwater bacterioplankton. Int J Syst Evol Microbiol 59(11):2864–2869

Jiang H, Dong H, Yu B, Liu X, Li Y, Ji S, Zhang CL (2007) Microbial response to salinity change in Lake Chaka, a

hypersaline lake on Tibetan plateau. Environ Microbiol 9(10):2603–2621

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS, Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. Science 311(5768):1737–1740

Jones SE, Newton RJ, McMahon KD (2009) Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. Environ Microbiol 11(9):2463–2472

Judd KE, Crump BC, Kling GW (2006) Variation in dissolved organic matter controls bacterial production and community composition. Ecology 87(8):2068–2079

Kennish MJ (1997) Pollution impacts on marine biotic communities. CRC Marine Science series, CRC Press. Taylor and Francis Group, Boca Raton, p 336

Kirchman DL (2002) The ecology of Cytophaga-Flavobacteria in aquatic environments. FEMS Microbiol Ecol 39(2):91–100

Kirchman DL, Dittel AI, Findlay SE, Fischer D (2004) Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River New York. Aquat Microb Ecol 35(3):243–257

Kirchman DL, Dittel AI, Malmstrom RR, Cottrell MT (2005) Biogeography of major bacterial groups in the Delaware Estuary. Limnol Oceanogr 50(5):1697–1706

Koroleff F (1983) Total and organic nitrogen. In: Grasshoff K, Ehrhardt M, Kremling K (eds) Methods of seawater analysis (2nd ed). Verlag Chemie, Weinheim, pp 162–173

Lapointe BE, Bedford BJ (2007) Drift rhodophyte blooms emerge in Lee County, Florida, USA: evidence of escalating coastal eutrophication. Harmful Algae 6(3):421–437

Lindell D, Post AF (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat) Red Sea. Limnol Oceanogr 40(6):1130–1141

Liu Z, Choudhury SH, Xia M, Holt J, Wallen CM, Yuk S, Sanborn SC (2009) Water quality assessment of coastal Caloosahatchee River watershed, Florida. J Environ Sci Health, Part A 44(10):972–984

Livingston RJ (2000) Eutrophication processes in coastal systems: origin and succession of plankton blooms and effects on secondary production in Gulf Coast estuaries. CRC Marine Science series, CRC Press. Taylor and Francis Group, Boca Raton, p 352

Livingston RJ, Niu X, Lewis FG III, Woodsum GC (1997) Fresh water input to a gulf estuary: long-term control of trophic organization. Ecol Appl 7(1):277–299

Loh AN (2008) Technical report to South Florida Water Management District

Loh AN, Brand LE, Ceilley DW, Charette M, Coen L, Everham III EM, Fugate DC, Grizzle RE, Milbrandt EC, Riegl BM, Foster G, Provost K, Tomasello LL, Henderson P, Breier C, Liu Q, Watson T, Parsons ML (2011) Final executive and technical reports to the city of Sanibel and Lee County

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards RA (2008) The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinform 9(1):386

Middelburg JJ, Nieuwenhuize J (2000) Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar Ecol Prog Ser 203:13–21

Moran MA, Sheldon WM, Sheldon JE (1999) Biodegradation of riverine dissolved organic carbon in five estuaries of the southeastern United States. Estuaries 22(1):55–64

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA, Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420(6917):806–810

Morris RM, Nunn BL, Frazar C, Goodlett DR, Ting YS, Rocap G (2010) Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction. ISME J 4(5):673–685

Mueller-Spitz SR, Goetz GW, McLellan SL (2009) Temporal and spatial variability in nearshore bacterioplankton communities of Lake Michigan. FEMS Microbiol Ecol 67(3):511–522

Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59(3):695–700

Ordoñez OF, Flores MR, Dib JR, Paz A, Farías ME (2009) Extremophile culture collection from Andean lakes: extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. Microb Ecol 58(3):461–473

Paerl HW (1991) Ecophysiological and trophic implications of light-stimulated amino acid utilization in marine picoplankton. Appl Environ Microbiol 57(2):473–479

Partensky F, Blanchot J, Vaulot D (1999) Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review. Bulletin-Institut Oceanographique Monaco-numero special, 457–476

Rath J, Wu KY, Herndl GJ, DeLong EF (1998) High phylogenetic diversity in a marine-snow-associated bacterial assemblage. Aquat Microb Ecol 14(3):261–269

Rocker D, Brinkhoff T, Grüner N, Dogs M, Simon M (2012) Composition of humic acid-degrading estuarine and marine bacterial communities. FEMS Microbiol Ecol 80(1):45–63

Rodrigues CS, Souza SS, Rezende RP, Silva A, Andrioli JL, Costa H, Fontana R, Dias JC (2013) Application of denaturing gradient gel electrophoresis for detection of bacterial and yeast communities along a salinity gradient in the estuary of the Cachoeira River in Brazil. Genet Mol Res 12(2):1752–1760. doi:10.4238/2013.May.21.6

Sarmento H (2012) New paradigms in tropical limnology: the importance of the microbial food web. Hydrobiologia 686(1):1–14

Seitzinger SP, Sanders RW (1997) Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. Mar Ecol Prog Ser 159(1):1–12

Seitzinger SP, Sanders RW, Styles RV (2002) Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnol Oceanogr 47(2):353–366

Smith EM, Benner R (2005) Photochemical transformations of riverine dissolved organic matter: effects on estuarine bacterial metabolism and nutrient demand. Aquat Microb Ecol 40(1):37–50

Smith EM, Kemp WM (2003) Planktonic and bacterial respiration along an estuarine gradient: responses to carbon and nutrient enrichment. Aquat Microb Ecol 30(3):251–261

Stepanauskas R, JØrgensen NO, Eigaard OR, Žvikas A, Tranvik LJ, Leonardson L (2002) Summer inputs of riverine nutrients to the Baltic Sea: bioavailability and eutrophication relevance. Ecol Monogr 72(4):579–597

Stepanauskas R, Moran MA, Bergamaschi BA, Hollibaugh JT (2003) Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquat Microb Ecol 31(1):85–98

Sugimura Y, Suzuki Y (1988) A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar Chem 24(2):105–131

Tai V, Palenik B (2009) Temporal variation of *Synechococcus* clades at a coastal Pacific Ocean monitoring site. ISME J 3(8):903–915

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

Tolley SG, Fugate D, Parsons ML, Burghart SE, Peebles EB (2010) The responses of turbidity, CDOM, benthic microalgae, phytoplankton and zooplankton to variation in seasonal freshwater inflow to the Caloosahatchee Estuary. Final data analysis and interpretation Report to South Florida Water Management District

Urakawa H, Martens-Habbena W, Stahl DA (2010) High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. Appl Environ Microbiol 76(7):2129–2135

Urakawa H, Ali J, Ketover RD, Talmage SD, Garcia JC, Campbell IS, Loh AN, Parsons ML (2013) Shifts of bacterioplankton metabolic profiles along the salinity gradient in a subtropical estuary. ISRN Oceanogr 2013:410814. doi:10.5402/2013/410814

Veuger B, Middelburg JJ, Boschker HT, Nieuwenhuize J, van Rijswijk P, Rochelle-Newall EJ, Navarro N (2004) Microbial uptake of dissolved organic and inorganic nitrogen in Randers Fjord. Estuar Coast Shelf Sci 61(3):507–515

Vincent WF (2000) Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarct Sci 12(03):374–385

Wan Y, Qiu C, Doering P, Ashton M, Sun D, Coley T (2013) Modeling residence time with a three-dimensional hydrodynamic model: linkage with chlorophyll *a* in a subtropical estuary. Ecol Model 268:93–102

Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J (2005) Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. Appl Environ Microbiol 71(9):5551–5559

Weston DP (1990) Quantitative examination of macrobenthic community changes along an organic enrichment gradient. Mar Ecol Prog Ser 61(3):233–244

Wiegner TN, Seitzinger SP (2001) Photochemical and microbial degradation of external dissolved organic matter inputs to rivers. Aquat Microb Ecol 24(1):27–40

Wiegner TN, Seitzinger SP (2004) Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. Limnol Oceanogr 49(5):1703–1712

Wiegner TN, Seitzinger SP, Glibert PM, Bronk DA (2006) Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. Aquat Microb Ecol 43(3):277–287

Wikner J, Hagström A (1999) Bacterioplankton intra-annual variability: importance of hydrography and competition. Aquat Microb Ecol 20(3):245–260

Yuan X, Glibert PM, Xu J, Liu H, Chen M, Liu H, Yin K, Harrison PJ (2012) Inorganic and organic nitrogen uptake by phytoplankton and bacteria in Hong Kong waters. Estuaries Coasts 35(1):325–334

Zhang Y, Jiao N, Cottrell M, Kirchman D (2006) Contribution of major bacterial groups to bacterial biomass production along a salinity gradient in the South China Sea. Aquat Microb Ecol 43(3):233–241

Zwart G, Crump BC, Kamst-van Agterveld MP, Hagen F, Han S-K (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28(2):141–155

Zwirglmaier K, Jardillier L, Ostrowski M, Mazard S, Garczarek L, Vaulot D, Fabrice N, Massana R, Ulloa O, Scanlan DJ (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ Microbiol 10(1):147–161